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## Genetic Diversity of *Colletotrichum Sublineola* on Sweet Sorghum and Wild Sorghum Relatives in Kentucky and the Southeastern U.S.

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GENETIC DIVERSITY OF *COLLETOTRICHUM SUBLINEOLA* ON SWEET  
SORGHUM AND WILD SORGHUM RELATIVES IN KENTUCKY AND THE  
SOUTHEASTERN U.S.

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DISSERTATION

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A dissertation submitted in partial fulfillment  
of the requirements for the degree of Doctor of Philosophy in the College of Agriculture  
at the University of Kentucky

By

Katia Viana Xavier

Lexington, Kentucky

Director: Dr. Lisa J. Vaillancourt, Professor of Plant Pathology

Lexington, Kentucky

2016

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## ABSTRACT OF DISSERTATION

### GENETIC DIVERSITY OF *COLLETOTRICHUM SUBLINEOLA* ON SWEET SORGHUM AND WILD SORGHUM RELATIVES IN KENTUCKY AND THE SOUTHEASTERN U.S.

Anthracnose, caused by the fungus *Colletotrichum sublineola*, is one of the most important diseases on sorghum (*Sorghum bicolor*) in the United States (U.S.) and worldwide. The production of sweet sorghum for feedstock has been increasing in the Southeastern U.S. (SE), and anthracnose has emerged as a significant production constraint. Anthracnose is also common on the ubiquitous wild sorghum relative, johnsongrass (*Sorghum halepense*). The degree to which the pathogen population on johnsongrass contributes to disease epidemics on cultivated sorghum (*S. bicolor*) in the SE is unknown. Genetic and pathological diversity was characterized among a collection of *Colletotrichum* strains recovered from *S. bicolor* and *S. halepense* in Kentucky, Alabama, Georgia, and Florida. The pathogenicity of five *C. sublineola* isolates from sweet and grain sorghum and from johnsongrass was measured on the susceptible sweet sorghum inbred Sugar Drip in the field. Isolates from cultivated sorghum were more aggressive than isolates from *S. halepense*, which generally caused little or no disease. The disease levels observed in the field had no effect on the yields of sorghum biomass, grain, or juice, or on Brix levels. Removal of sorghum seed heads increased sugar levels in the plants, but this had no effect on susceptibility to anthracnose. Greenhouse and laboratory assays were developed that gave rankings of relative strain aggressiveness that were consistent with the field results. Marker analyses with repetitive fingerprinting probes were used to evaluate several

hundred *Colletotrichum* strains isolated from *S. bicolor* and *S. halepense* across the SE. Results revealed that, with a few exceptions, isolates from cultivated sorghum were genetically distinct from isolates from *S. halepense*. A restriction fragment length polymorphism (RFLP) analysis based on probes against individual sequences presumed to encode effectors and secondary metabolism enzymes confirmed that most isolates from johnsongrass grouped separately from most isolates from cultivated sorghum. The RFLP analysis revealed the presence of three distinct groups within the population that were distinguished by fixed allelic variations, or by presence-absence polymorphisms, of some of these putative pathogenicity genes. Phylogenetic trees were inferred based on a sampling of isolates from both host species and representing each of the three groups by using the internal transcribed spacer (ITS) sequence of the ribosomal DNA; portions of the DNA lyase gene (*Apn2*) and the manganese superoxide dismutase gene (*Sod2*); and a region between the *Mat1* and the *Apn2* genes (*Mat1/Apn2*). The trees were found to be congruent, and to identify three distinct species, including *C. sublineola* and two previously undescribed species. One of these novel species was found associated only with *S. halepense* throughout the SE. This new species was named *Colletotrichum halepense* (Xavier & Vaillancourt). There was evidence for cross-infection of *S. bicolor* and *S. halepense* by *C. sublineola* and the second new species, which was named *C. caselae* (Xavier & Vaillancourt). These findings have significant implications for the development and deployment of resistant sweet sorghum varieties in areas where johnsongrass is common. This information will help to evaluate the potential for *Colletotrichum* spp. to cause epidemics in sweet sorghum if acreages continue to expand in the SE in the future.

KEYWORDS: Anthracnose, johnsongrass, sweet sorghum, *Colletotrichum caselae*,  
*Colletotrichum halepense*.

Katia Viana Xavier  
December 1, 2016

GENETIC DIVERSITY OF *COLLETOTRICHUM SUBLINEOLA* ON SWEET  
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Date

To my mother, Maria Helena Viana da Silva, my father Jeronimo Xavier da Silva, my sister Karla Viana Xavier, my brothers Bruno Ferreira Xavier and Francisco F. S. Neto, and to her who has been always with me Juliana M. Soares.



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## Chapter 1 - Literature review

### Importance and history of sweet sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] ranks fifth in importance among cereal crops worldwide (56). Sorghum is an annual C<sub>4</sub> grass with chromosome number 2n=20 (56). It is characterized by its high photosynthetic efficiency, and also by its relative resistance to environmental stresses, including drought, salinity and high temperature (148). Sorghum can produce high yields in marginal soils (81).

The center of diversity for sorghum is in Africa. Early domestication was based on selection and hybridization to increase the number of branches within the inflorescence; decrease the length of the internode rachis; and increase the seed size (86). Sorghum varieties have been further improved by plant breeders by selection of traits such as photoperiod insensitivity, reduced height in order to reduce lodging, drought tolerance, and pest and disease resistance (140). Some of the newer sorghum varieties produce very high levels of green leaf area at flowering and high yields, and also resist insect pests (15).

Cultivated *S. bicolor* is divided into five major races: bicolor; guinea; caudatum; kafir; and durra. A comprehensive analysis of the diversity of sorghum collections worldwide, based mainly on molecular markers, demonstrated that the population is highly polymorphic (1, 4, 75). Different types of sorghum have been selected and optimized for production of grain, fiber, forage, or syrup (209). Sweet sorghum varieties grown for syrup production accumulate high levels of sugar in the stalk, and so they can also be used for the production of ethanol biofuel from cellulosic biomass or directly from sorghum sugar (12, 133, 158,

197, 208). The sweet sorghum plant can serve multiple purposes: the grain can be used for human and animal food, and the bagasse that remains after pressing the stalks, including the stripped leaves, makes a high-quality animal feed (6, 191). Thus, sweet sorghum is of significant and increasing interest as a potential multiuse biofuel crop.

Sweet sorghum is genetically very diverse (1, 142). It was taken from Africa to China (perhaps more than once) and cultivated there for more than 1000 years before being introduced to France in 1851. It was first brought to the United States (U.S.) in 1853 (160). In 1857, Leonard Wray brought 16 additional varieties of sweet sorghum to the U.S. from Africa, and since then the U.S.D.A has introduced more varieties from Australia, China, and Africa. The varieties that Wray introduced include Orange, Sumac, Planter, Gooseneck, Honey, Sapling, African Millet, and Sourless (166). The grain sorghum varieties Brown Durra and White Durra were introduced to the U.S. about 20 years after sweet sorghum. A forage type of sorghum called sudangrass [*S. sudanense* (Piper) Stapf)] was introduced in 1909 (166).

It is believed that almost all the varieties of sweet sorghum grown currently in the U.S. were developed from Wray's introductions, which were improved through selection and hybridization (Figure 1.1). The selection of sweet sorghum during the 1900s was done based mainly on agronomic traits, including sugar content (Brix), plant maturity, plant height, biomass yields, and environmental adaptation (166). Today there are approximately 4,000 varieties of sweet sorghum grown throughout the world (77). There are 2,163 accessions in the U.S. sweet sorghum collection (133). Sweet sorghum is grown in the U.S. primarily for the production of table syrup. Kentucky is one of the biggest producers of

sweet sorghum, and is among eight states responsible for 90% of the total U.S. output of sweet sorghum syrup (12, 188).

### **The potential of sweet sorghum as a biofuel**

Currently, sugarcane (*Saccharum officinarum* L.) and maize (*Zea mays* L.) are the primary crops used for the production of ethanol biofuel (32, 49, 155). Sugars in the extracted sugarcane juice can be fermented directly, while the starch in maize grains must first be enzymatically converted to sugar before fermentation (192, 197). The sugar content in juice extracted from the stalks of sweet sorghum is up to 20% Brix (compared with 20-24% in sugarcane) (139). Thus, the potential value of sweet sorghum for the direct production of ethanol is similar to that of sugarcane. In addition to the juice, sorghum grains could be used for production of biofuels after enzymatic conversion of starch to sugar, and sorghum feedstock (pressed stalks and stripped leaves) could be used for cellulosic biofuel production. Sweet sorghum is remarkably efficient in production of biomass per acre compared to other potential cellulosic biofuels crops such as switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus* sp.) (37, 110). A study was performed to evaluate the potential of sweet sorghum versus switchgrass for the production of biofuel. Over four years, sweet sorghum had an average production of 15-20t of dry matter ha<sup>-1</sup>. This was superior to switchgrass, the highest-yielding perennial crop, which produced only 8-15t of dry matter ha<sup>-1</sup> (81).

Sweet sorghum uses resources more efficiently than either maize or sugarcane. Thus, maize needs 20% more water and 35% more nitrogen than sweet sorghum to produce the same amount of sugar (9, 76, 123, 142, 204). The growing season for sorghum is shorter than

that for sugarcane, permitting two crops per year in warmer climates, while sugarcane can produce just one (9, 47).

Sweet sorghum is a seed-propagated annual plant and so, unlike perennial biofuel crops like switchgrass, it can easily be grown in rotations with food crops. This gives the farmer more flexibility and the ability to react to market forces. It also facilitates use of the same crop management practices and farm machinery as for other annual grain crops (134). There have been several studies of sweet sorghum in crop rotations and some advantages have been reported, including improvements in soil physical properties and fertility, soil moisture, increased biological activity, and increased content of organic matter (134). Significant increases in sorghum yield when it was planted in rotation with soybean or with maize, versus continuous cultivation, have been reported (34, 206, 85).

There are many potential advantages of sweet sorghum as a biofuel crop, but significant barriers remain to its widespread use in the U.S. One is a lack of appropriate equipment and infrastructure to harvest and move stalks or juice quickly from the farm to a processing plant. Furthermore, there are relatively few adapted cultivars for the U.S., and we have little information about yield parameters and agronomic qualities. Sweet sorghum is prone to lodging which interferes with machine harvest. We also lack data about disease and pest susceptibility. Rotation of sorghum with maize could increase the risk of losses from insects or disease, since many of the same pests and pathogens affect both crops (145).

### **Wild sorghum (johnsongrass and shattercane) in the United States**

*Sorghum bicolor* has a large number of wild relatives, and two of the most widespread and important of these are the weeds shattercane (*Sorghum bicolor*) and johnsongrass (*Sorghum halepense* L. Pers.).



The first appearance of shattercane has not been documented, but it is thought to have resulted from hybridization among different varieties of cultivated grain or forage sorghum and wild sorghum relatives (56). Shattercane is so similar to cultivated sorghum that is difficult to selectively control it when it is growing in sorghum fields (23).

The sorghum relative *Sorghum halepense*, thought to be native to the Mediterranean, was introduced into the U.S. before 1830 (5). It was planted and widely promoted as a forage by Colonel William Johnson near Selma, Alabama, when it came to be known as johnsongrass (5, 116). By 1900, johnsongrass had spread throughout the southern and eastern U.S. as a noxious weed. It has hybridized with various cultivated sorghum varieties, and the current population is comprised of numerous ecotypes containing various quantities of the *S. bicolor* genome (132). Johnsongrass is a perennial that reproduces by seeds and by the production of rhizomes, and it is considered to be one of the most destructive weeds in the U.S. and worldwide (84, 116). Johnsongrass could become an especially severe problem in a maize-sorghum-wheat rotation.

### **Anthracnose on sorghum**

The fungus *Colletotrichum* is the casual agent of anthracnose leaf blight and stalk rot of grasses (3, 76, 92, 130). Anthracnose is one of the most important diseases on both maize and sorghum in the U.S. and worldwide (193). Anthracnose on maize is caused by *Colletotrichum graminicola* (Ces.) Wils., while the closely related species *Colletotrichum sublineola* (Henn.) ex Sacc. & Trotter produces similar symptoms on sorghum. *Colletotrichum sublineola* is also reported to be a pathogen of the common related weeds johnsongrass and shattercane (118, 194). Morphological, molecular phylogenetic, and biochemical analyses have indicated that *C. graminicola* and *C. sublineola* diverged from

a common ancestor relatively recently (39, 40, 61, 157, 165, 167, 181). There are no reports of *C. graminicola* infecting sorghum, or of *C. sublineola* infecting maize, in the field, and inoculation of most healthy tissues of maize by *C. sublineola*, or of sorghum by *C. graminicola*, does not result in infection. Thus, these two species appear to be host-specific (45, 92, 105, 202). *Colletotrichum sublineola* can infect stalk epidermal cells of healthy maize plants, but the infection does not progress to the stalk interior (187). *Colletotrichum sublineola* can more extensively colonize the leaves and stalks of maize plants that are senescing, drought stressed, or light stressed (Vaillancourt unpublished data) and it can colonize maize leaf sheaths that have been killed by a localized application of liquid nitrogen (175). The factors that are responsible for host specificity of *C. graminicola* and *C. sublineola* are unknown. However, host specificity in plant-pathogen interactions seems in many cases to be related to the presence or absence of pathogen virulence factors, including specialized secondary metabolites (SSMs), and small secreted proteins (SSPs) (7, 36, 48, 59, 90, 103, 180).

Wherever grain sorghum is widely grown, anthracnose is the most important disease problem. It can reduce crop yields by 50% or more under warm and humid conditions in a susceptible variety (3, 83). *Colletotrichum sublineola* can infect all parts of the plant including the stem, peduncle, panicle, grain, and leaf blades and midrib (68). When it occurs in the stem, it produces a disease known as red stalk rot. The disease cycle of *C. sublineola* is shown in Figure 1.2. The pathogen overwinters on crop debris on the soil surface, as conidia and acervuli (193) (Figure 1.2 A). In the spring, when the weather conditions are favorable (i.e. high humidity and warm temperatures) the conidia are disseminated by water splash (Figure 1.2 B). Dried conidial masses can also be spread by

the wind to new plants. After a conidium lands on a healthy plant surface (Figure 1.2 C), it germinates to produce a germ tube, which eventually forms an appressorium, (195) (Figure 1.2 D). A penetration peg emerges from the appressorium and forces its way through the plant cell wall. Once inside the plant cell, the pathogen produces thick primary hyphae. Primary hyphae are surrounded by a membrane, and the plant cell stays alive during this initial biotrophic phase of development (176, 195) (Figure 1.2 E). Later, the colonized plant cells die and the pathogen switches to necrotrophic growth. During this phase, thin secondary hyphae are produced from the primary hyphae, and the plant cells are destroyed by the activity of lytic enzymes that are produced by these secondary hyphae (175, 176) (Figure 1.2 F). It is at this stage that visible lesions develop. Finally, the pathogen completes its life cycle by producing acervuli containing conidia and setae (195) (Figure 1.2 G). These spores can be disseminated to other plants or tillers during the same season as secondary inoculum, and at the end of the season they serve as overwintering structures (193) (Figure 1.2 H).

Symptoms of anthracnose on the leaf blades and midribs are similar on johnsongrass and on sweet sorghum (Figure 1.3). Symptoms begin as small, round to elliptical spots that vary in color from yellow to red or gray. The spots increase in number and size, eventually coalescing to cover large portions of the leaves. Signs (acervuli containing conidia and setae) are produced in the necrotic centers of the lesions and are visible to the naked eye as small black dots.

The occurrence of anthracnose in both maize and sorghum is associated with stresses during grain filling, including foliar diseases, drought, soil saturation, cold weather, and nutritional deficiencies (55). Reducing the stresses that predispose the plants, and using

resistant or tolerant varieties, helps to minimize the impact of the disease. However, no variety of maize or sorghum is immune to anthracnose. Leaf anthracnose has been reported to occur in sweet sorghum and to cause reductions in biomass yield in Kentucky (12). Red stalk rot is common in Kentucky sweet sorghum, as is stalk lodging. The relationship of these syndromes to *Colletotrichum* infection has not been established, although it is often assumed that they are connected.

Anthracnose has been studied most in grain sorghum. The primary control for anthracnose in grain sorghum is the use of resistant cultivars (28). However resistance frequently fails, which is thought to be related to the high degree of genetic variability in the population of *C. sublineola* affecting grain sorghum (25, 27).

Genetic diversity within the *C. sublineola* population on grain sorghum has been characterized by the use of molecular markers, including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP) and amplified fragment length polymorphisms (AFLP) (30, 184). The ability of *C. sublineola* to infect a series of different cultivars of sorghum has been used to identify races in this species (3, 14, 30, 143, 184). One study suggested that the population of isolates infecting johnsongrass did not contribute to the population infecting grain sorghum varieties grown in nearby fields in Texas (143). However, there have been no additional studies of this topic in the U.S., and there is no information in the literature about population or race structure of *C. sublineola* on sweet sorghum or weedy sorghum relatives in the southeastern U.S.

The interaction between sorghum and *C. sublineola* was suggested to follow the gene-for-gene pattern (26). Resistance of grain sorghum to *C. sublineola* is controlled mostly by dominant single genes (14, 35, 104). In 1993, Tenkouano reported that one source of

resistance in sorghum was controlled by a single genetic locus with multiple allelic forms (170). The value of these major gene sources of genetic resistance has been limited by the ability of the pathogen population to adapt in different locations, and also by variation in the effectiveness of resistance alleles in different genotypes (30, 135). Very little is currently known about the presence or basis for resistance to anthracnose in sweet sorghum.

Although sweet sorghum has been grown in the U.S. for more than 150 years, it has been on a limited scale, primarily for forage and for the production of syrup. If sweet sorghum is cultivated on a commercial scale in the U.S. in the future for biofuel, diseases, including anthracnose, may become a significant limiting factor for production.

### **Dissertation Goals and Predictions**

The goals of my dissertation research were: (1) to study the impact of anthracnose disease on sweet sorghum in the field in Kentucky; (2) to characterize the degree of genetic and pathological diversity among the population of *Colletotrichum* on cultivated and wild sorghum in Kentucky and the southeastern U.S.; and (3) to develop and test several greenhouse and laboratory assays for sorghum anthracnose disease.

In chapter 2 of this dissertation, I compared the pathogenicity of *C. sublineola* isolates from sweet and grain sorghum with that of isolates from the wild sorghum relative johnsongrass on Sugar Drip sweet sorghum in the field. The impact of the anthracnose disease on yields of biomass, seeds, juice, and Brix was determined. The same set of isolates was also tested in greenhouse and laboratory assays, to determine whether these protocols would be useful for screening sorghum germplasm for new sources of resistance or to characterize new isolates. The predictions for chapter 2 were: (1) *C. sublineola* from sweet sorghum will be more pathogenic and/or aggressive on sweet sorghum than isolates from johnsongrass or

grain sorghum; (2), anthracnose disease will have a negative impact on yield and Brix; (3) deheading sorghum will result in increased Brix and decreased levels of disease; and (4) the results of greenhouse and leaf sheath assays will be correlated with field inoculations.

In chapter 3, I investigated the genotypic and phenotypic diversity of a large population of *Colletotrichum* strains recovered from cultivated sorghum and johnsongrass, in Kentucky and the Southeastern U.S. The predictions for chapter 3 were: (1) isolates from sweet sorghum will resemble those on grain and forage sorghum more than they will resemble isolates from johnsongrass; (2) the population of strains recovered from johnsongrass will be more diverse genetically than the population recovered from cultivated sorghum; and (3) isolates from different hosts will vary in their virulence and aggressiveness to sweet sorghum varieties.

In chapter 4, I characterized and compared *C. sublineola* versus *C. graminicola* genes that were predicted to encode SSM proteins as part of a larger comparative genomic analysis of these two species. I utilized some of these genes, and also some that were predicted to encode SSPs, as probes for an RFLP analysis of a representative sample of isolates from the *Colletotrichum* population affecting cultivated sorghum and johnsongrass in the southeastern U.S. The predictions were: (1) that the sibling species *C. sublineola* and *C. graminicola* would differ in the presence of one or more genes predicted to encode SSPs and SSM-associated proteins (aka pathogenicity genes); (2) that pathogenicity genes found in *C. sublineola* but not in *C. graminicola* would be conserved among all members of the population infecting *Sorghum bicolor* in Kentucky and the southeastern U.S.; and (3) that populations affecting *S. bicolor* would be distinguishable from populations affecting *S. halepense* by allelic differences in individual pathogenicity genes.

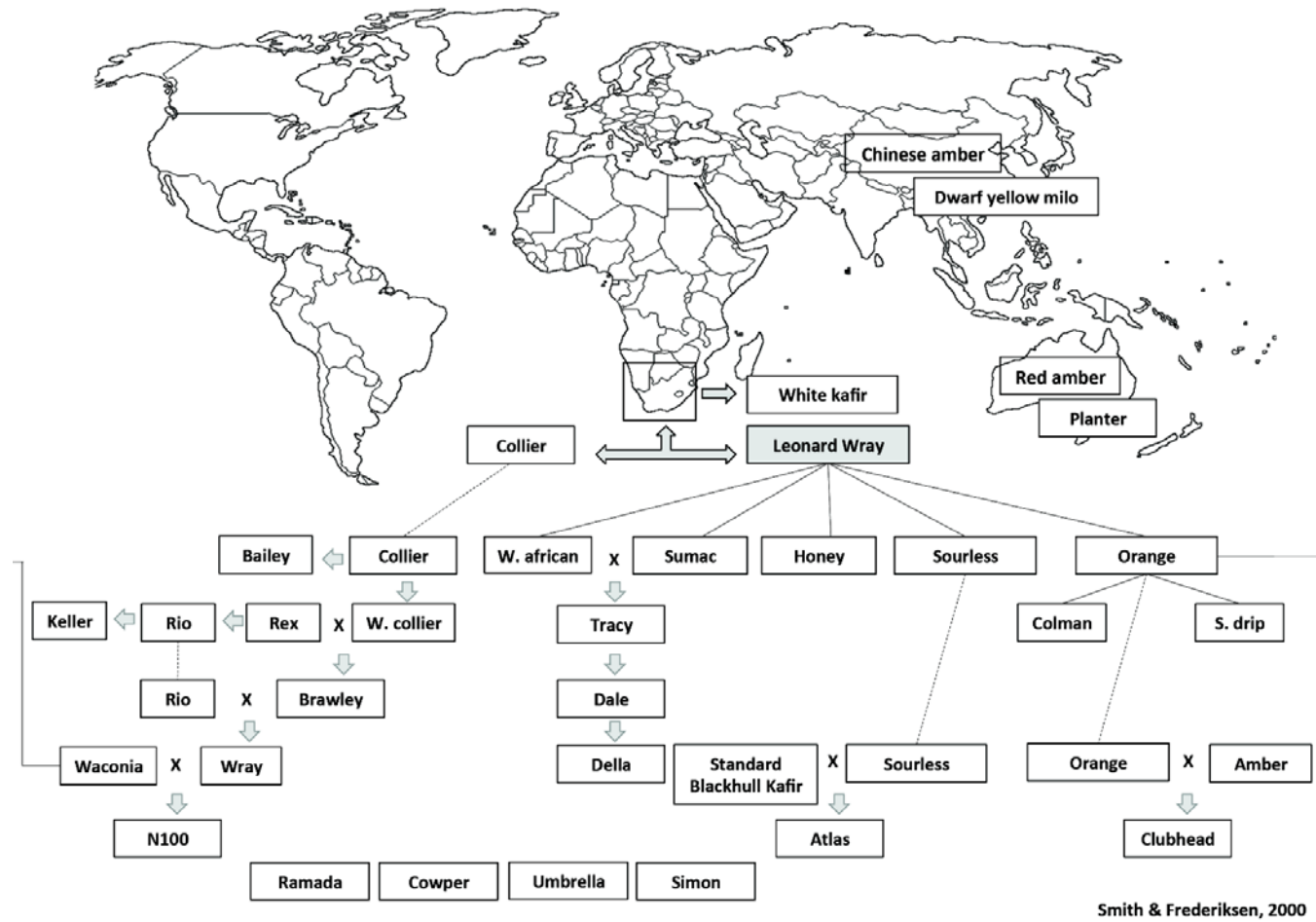
In chapter 5, I tested the prediction that genetically distinct groups that I had observed within the populations of *Colletotrichum* affecting cultivated sorghum and johnsongrass actually represent different species. Sequences from the *Sod2* gene, the *Apn2* gene, and from the intergenic region between *Apn2* and *Mat1*<sup>1</sup>, have been used previously to identify new species within the *C. graminicola* species complex that affects graminaceous hosts (40). To test my prediction, I analyzed these sequences together with the internal transcribed spacer (ITS) of the ribosomal DNA, as well as measuring various morphological traits in culture, for several representative *Colletotrichum* isolates.

In chapter 6, I present a summary of my dissertation findings and a discussion of their importance and relevance to sorghum cultivation in the region.

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*Colletotrichum* species contain only the HMG-domain MAT gene, which is by convention known as MAT2 (22). Thus, the gene referred to as MAT1 in (24) should really be called MAT2. Nonetheless, in this dissertation I will use the terminology of (24) to refer to the intergenic region adjacent to this gene (*Mat1*/*Apn2*).

Figure 1. 1. Pedigree of sweet sorghum varieties (Smith and Frederiksen, 2000).



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Smith & Frederiksen, 2000



Figure 1. 2. Disease cycle of *Colletotrichum sublineola*.

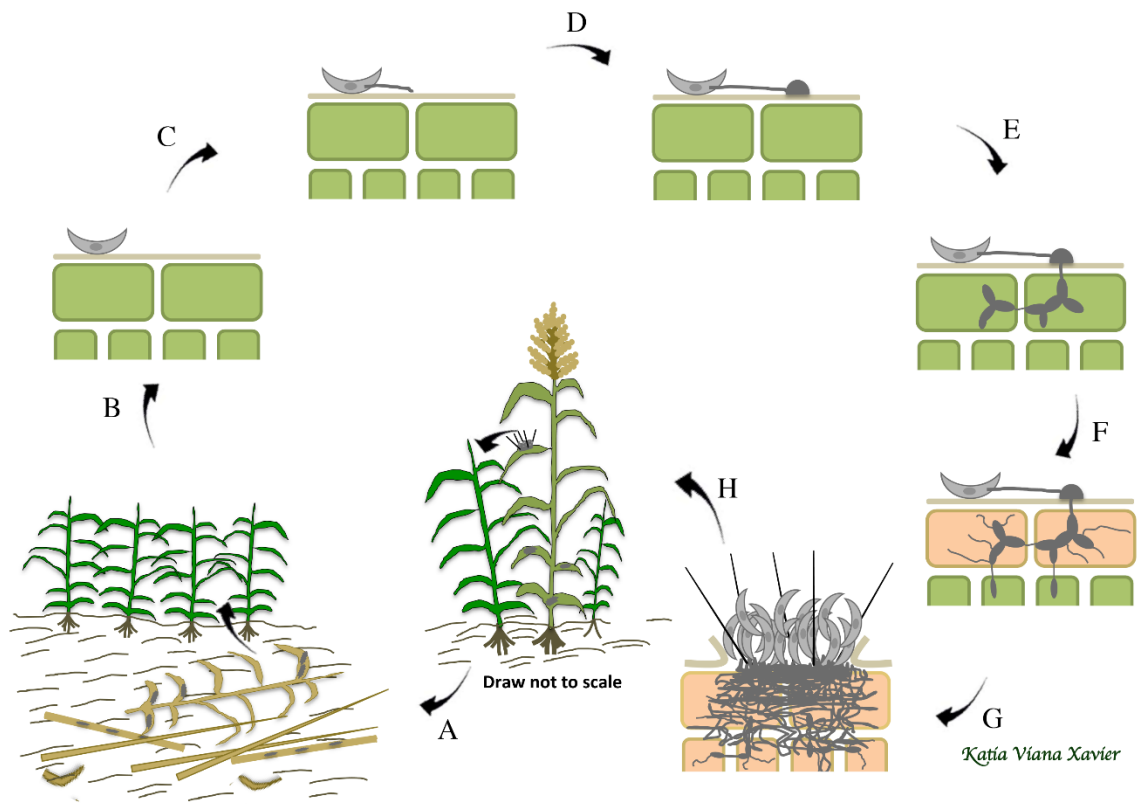
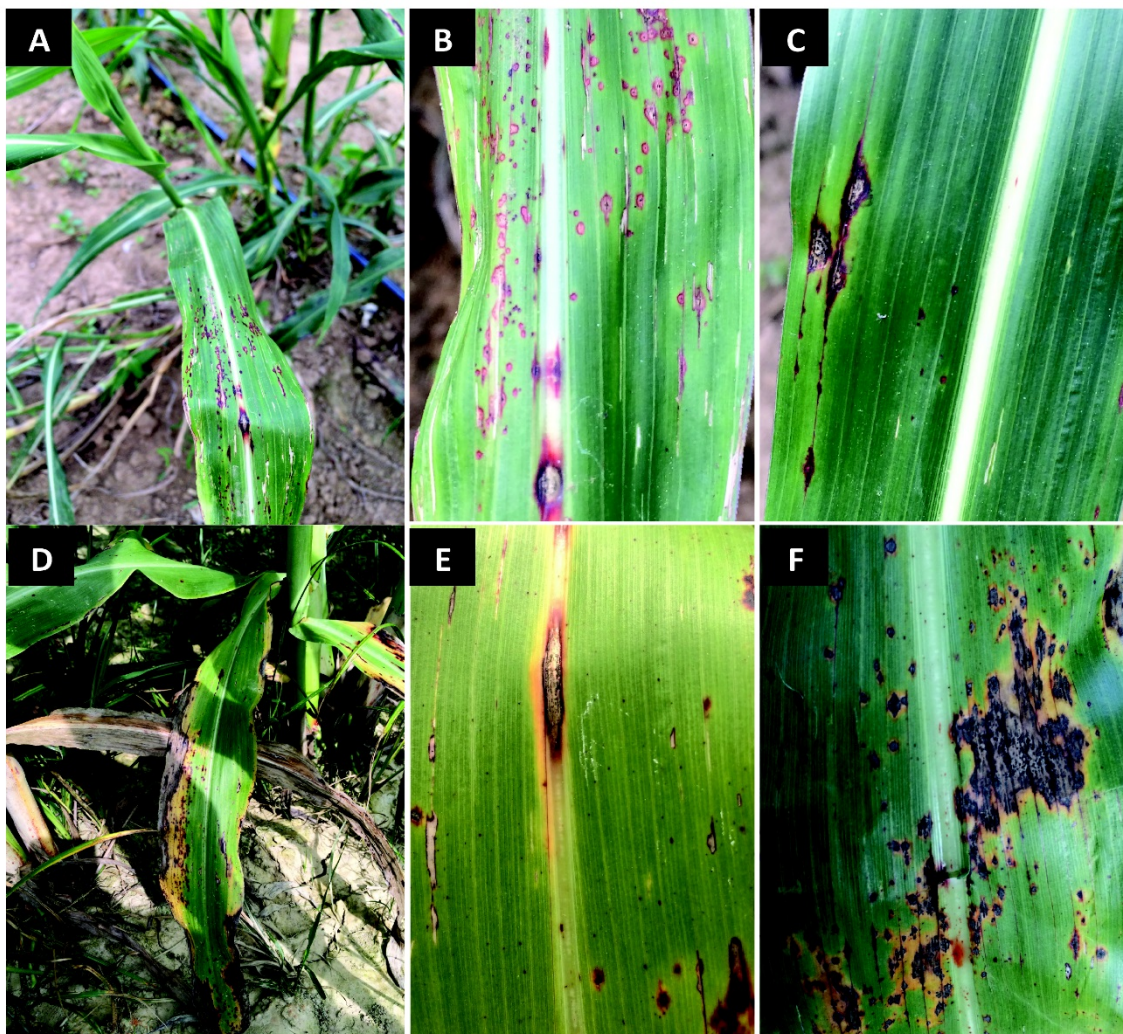


Figure 1. 3. Typical anthracnose symptoms on johnsongrass (A, B, C) and on sweet sorghum (D, E, F).



## **Chapter 2 - Impact of *Colletotrichum* strains from cultivated and wild Sorghum spp. on the sweet sorghum variety Sugar Drip in field, greenhouse, and laboratory assays**

### **Abstract**

Sweet sorghum (*Sorghum bicolor*) has been grown in the southeastern United States for more than 150 years on a relatively limited scale, primarily for forage and for the production of table syrup. However, interest in the crop has increased recently due to its potential as a feedstock for biofuels. *Colletotrichum sublineola* is reported to be the causal agent of anthracnose on cultivated sorghum and on the wild sorghum relative johnsongrass (*S. halepense*). Anthracnose is the most important disease of grain sorghum worldwide, but comparatively little is known about its impact on sweet sorghum in the United States. The pathogenicity of several *C. sublineola* isolates from sweet and grain sorghum and from johnsongrass was measured as disease incidence and severity on the susceptible heirloom sweet sorghum inbred Sugar Drip in the field. The isolate from sweet sorghum was consistently the most aggressive, while the johnsongrass isolates caused only minimal disease symptoms. Disease incidences of up to 95%, and severities of up to 20% of leaf area affected, had no effect on the yield of biomass, grain, juice, or Brix levels in the affected tillers. Removal of sorghum seed heads increased sugar levels in the stalks and leaves, but this had no effect on susceptibility to anthracnose. The same group of fungal isolates was evaluated for pathogenicity in greenhouse assays on juvenile plants, and in the laboratory on seedlings and detached leaf sheaths. Results of these assays were generally consistent with the field results; thus these protocols might be useful for pre-screening

sorghum germplasm for new sources of resistance or for characterizing the pathogenicity of new fungal isolates.

## **Introduction**

Sorghum [*Sorghum bicolor* (L.) Moench] is an annual C4 grass characterized by its high photosynthetic efficiency and its relative resistance to environmental stresses, including drought, salinity, marginal soils, and high temperatures (81, 148). Sorghum is grown primarily for grain, which is used mainly for animal feed and ethanol. Sorghum ranks fifth in importance among cereal crops worldwide (57).

In addition to grain sorghum varieties, which have been bred to produce a large, starchy seed head on a short stalk, there are also sweet sorghum types. These produce more biomass than the grain varieties, with many reaching heights of three meters or more, and they accumulate large amounts of soluble sugar in the stalks. Sweet sorghums are not as commonly grown as the grain varieties, and are used traditionally for forage and for the production of sweeteners (142). Recently however, interest in sweet sorghum has increased because of its potential for the production of ethanol biofuel from cellulosic biomass or directly from sorghum sugar (12, 133, 158, 197, 208). Sweet sorghum is well adapted for cultivation in the southeastern (SE) United States (U.S.), where the crop has been grown on a small scale for more than 150 years for the production of table syrup. Kentucky (KY) and its neighboring SE states are responsible for 90% of the total U.S. output of sweet sorghum syrup (12, 188).

Anthrachnose is the most important disease of grain sorghum worldwide, and has been reported to cause reductions of as much as 50% in grain yield (83, 173). *Colletotrichum sublineola* (Henn.) causes anthrachnose on all varieties of *S. bicolor*, and can infect all parts

of the plant including the stem, peduncle, panicle, grain, and leaf blades and midrib (67). Foliar anthracnose is the most common phase of the disease in grain sorghum. Foliar anthracnose occurs everywhere in the world where grain sorghum is produced, and is especially severe under conditions of high temperature and humidity, as are typical of the SE U.S. (3). Infection of the stem produces a disease called red stalk rot which, when severe, can result in lodging. The occurrence of anthracnose on grain sorghum has been associated with stresses during grain filling including drought, soil saturation, cold weather, and nutritional deficiencies (54).

During World War II, epidemics of foliar anthracnose severely limited the productivity of sweet sorghum that was widely grown in the Gulf region to provide a substitute for cane sugar (106). Anthracnose is still common on sweet sorghum in the SE U.S. (12, 79), but relatively little is known about the impact of this disease currently, or its potential for damage if sweet sorghum is grown more widely for biofuel in the future.

The main strategy for the management of foliar anthracnose in grain sorghum is the use of resistant cultivars (28, 124). Resistance fails frequently, and this is thought to be related to the high degree of genetic variability in the population of *C. sublineola* (25, 27). New sources of resistance must constantly be developed and deployed in order to manage the pathogen effectively. Another strategy recommended for the management of anthracnose on sorghum is to use crop rotation with other plant genera. However, *C. sublineola* also reportedly causes foliar lesions on the ubiquitous weed johnsongrass (194), and johnsongrass could serve as a refuge for *C. sublineola* during crop rotation schemes. Furthermore, johnsongrass could be a source of novel pathogenic strains, since this weed is genetically highly diverse (98). A prior study suggested that johnsongrass isolates did

not significantly contribute to the populations of *C. sublineola* on nearby grain sorghum in Texas (73, 144). However, the pathogenicity of johnsongrass strains to sweet sorghum has not been directly tested.

The primary goal of the work described here was to compare the pathogenicity of KY *C. sublineola* isolates from different hosts, including johnsongrass, on the susceptible sweet sorghum variety Sugar Drip. A second goal was to evaluate the impact of anthracnose on yields of biomass, juice, and Brix levels. Prior studies have suggested that removing the seed head from sorghum plants increases the amount of sugar in the stalks (63), and other studies have reported a correlation between increased levels of stalk sugar and decreased susceptibility of maize to anthracnose stalk rot (121). Thus a third goal of this study was to evaluate the effect of seed head removal on levels of sugar and disease in Sugar Drip.

Sorghum breeders have developed improved anthracnose-resistant varieties of sweet sorghum for the SE U.S., and this work has increased in importance as the potential of sorghum for biofuels production has become more widely recognized (18, 43, 46, 188). Breeders typically test the performance of new varieties in field trials (64, 156). However, field screening is resource-intensive, and results are complicated by environmental variables including temperature, humidity, and the presence of other abiotic and biotic stresses. A rapid, high throughput resistance assay that could be performed in the greenhouse or laboratory would allow pre-screening of a larger number of germplasms against a wider range of pathogen populations under controlled conditions, thus speeding the process of development of resistant sweet sorghum germplasm. To contribute to this goal, I also measured the pathogenicity of the *Colletotrichum* isolates to Sugar Drip in

several greenhouse and laboratory assays in order to test the ability of these assays to predict results in the field.

## **Materials and Methods**

**Fungal strains:** Six *Colletotrichum* isolates were used in this study (Table 2.1). The isolates were recovered from small pieces of plant tissue cut from the edges of foliar lesions, soaked in 70% ethanol for 30 seconds, transferred to 10% sodium hypochlorite (Clorox®) for 2 minutes, and then washed 3 times with sterile water. Disinfested pieces were blotted on sterile paper towels and then placed on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) containing ampicillin (0.1 mg/ml). All the isolates were genetically purified by single-sporing and preserved on silica gel at -80°C (179). Strains were identified based on their morphology, including the characteristic falcate spore shape and presence of acervuli with setae, and on blastn matches of the ribosomal DNA internal transcribed spacer (ITS) sequences (>99% identity) to the *C. sublineola* species type (accession NR\_111191) or the *C. graminicola* species type (accession NR\_111190) (Figure 2.1). All fungal strains were routinely cultured on PDA at 23°C under continuous fluorescent light.

**Plants:** The heirloom sweet sorghum inbred Sugar Drip was used for all experiments. Seeds were obtained from Dr. Todd Pfeiffer at the University of Kentucky (UK), and increased in 2012 at the UK Agronomy Farm in Lexington, KY. Twelve additional genetically diverse sorghum varieties (Honey, Simon, Chinese Amber, Dwarf Yellow Milo, Cowper, Orange, Planter, Bailey, Dale, Della, N100, and Keller) were used for some experiments (Table 2.2). Seeds of these varieties were obtained from Dr. Gary Pederson at

the Plant Genetic Resources Conservation Unit in Griffin GA, and increased in 2012 at the UK Agronomy Farm.

**Preparation of fungal inoculum for field and greenhouse experiments:** Fungal inoculum was produced by using a modified protocol of Prom and colleagues (136). Forty grams of autoclaved Sugar Drip sorghum grains were placed aseptically in a Petri dish. Falcate spores were harvested from two-week-old fungal PDA cultures by adding 10 mL of sterile water to each plate and scraping the surface gently with a mini-pestle. The spores were collected and washed three times with sterile water to remove the mucilage. A volume of spore suspension was added to achieve a final concentration of  $5 \times 10^6$  spores per gram of sorghum grains. The plates were incubated for 14 days at 23°C in the dark to allow the fungus to colonize the grains.

**Field experiment:** The field experiment was repeated in two sequential years (2014 and 2015) at the UK South Farm in Lexington, KY. Sorghum seeds were sown on 4 May 2014, and 12 May 2015, in 72 cell-flats (each cell 1/2" L x 1 1/2" W and 2 1/4" deep) containing Pro-Mix BX (Premiere Horticulture, Ltd, Riviere du Loup, PQ, Canada) in the greenhouse. After 4-6 weeks, the seedlings were transplanted into the field with a between-row spacing of 1.4m, and a within-row spacing of 0.7m. A randomized complete block design with four blocks was used. Each experimental unit consisted of two rows of five plants each. Each experimental unit was separated from the others by a row of maize to prevent the dissemination of inoculum between treatments.

Two different experimental plots, both with Maury silt loam soils, were used in the sequential years. Fertilizer (19-19-9 NPK) was applied at a rate of 56 kg/ha in 2014, and 89 kg/ha in 2015, prior to transplanting. The rate in 2015 was higher because the plot used



that year had previously been in pasture. Weeds were controlled mechanically: no herbicides were used. Drip irrigation was applied as necessary.

Approximately five weeks after transplanting, the main stem of each plant was cut back to the crown to promote the development of multiple tillers per plant. Tillers were inoculated by using a modified protocol of (136). Tillers were inoculated at the V5 or V6 growth stage by placing 20 colonized sorghum grains (see above) into the whorl. Non-colonized grains, which otherwise had been treated identically to the colonized grains, were used as a water control treatment. The optimal growth stage for inoculation was determined in preliminary experiments demonstrating that maximal levels of disease resulted when plants were inoculated at this stage (See Appendix 1 of this dissertation). Two randomly selected tillers per plant were inoculated, for a total of 20 inoculated tillers per experimental unit.

The distribution and amount of rainfall differed in 2014 and 2015. The amount of rainfall between transplanting and inoculation was much less in 2014 (56.6 mm) than in 2015 (233.2 mm). However, after inoculation, rainfall was much higher in 2014 (218.7 mm) versus 2015 (81.8 mm) ([http://www.kymesonet.org/historical\\_data.php](http://www.kymesonet.org/historical_data.php)).

Seed head removal (de-heading) was accomplished by removing all the developing panicles continuously from all of the plant tillers as they appeared, from boot stage until harvest. Control treatments (heads intact) retained the seed heads until harvest. The treatments were as follows: (1) SS1.001, heads intact; (2) GS.1.001, heads intact; (3) JG9.001, heads intact; (4) JG18.001, heads intact; (5) water control, heads intact; (6) M30.001, negative control, heads intact; (7) SS1.001, de-headed; (8) GS1.001, de-headed; (9) JG9.001, de-headed; (10) JG18.001, de-headed; (11) water control, de-headed; (12) M30.001, negative control, de-headed.

Inoculated tillers were harvested by cutting them at the crown at physiological maturity, 45 days after flowering. Five inoculated tillers were randomly selected from each treatment for yield measurements. In 2014, seed heads were removed from the tillers, but leaves were retained, while in 2015, both leaves and seed heads were removed prior to analysis. The tillers were weighed, and then pressed at 12.4 MPa of pressure in a grooved, two-roller mill powered by a 1.5-HP electric motor. The juice was collected, weighed, and the Brix was measured immediately with a hand-held refractometer (ATAGO, Tokyo, Japan). One degree Brix corresponds to one kilo of fructose per 100 kilos of solution. Mature seed heads were collected from the stems and pooled for each replication of the head-intact treatments, and the seeds were threshed and weighed.

**Greenhouse experiment:** The ability of the KY *Colletotrichum* strains to cause disease symptoms on the sweet sorghum variety Sugar Drip was evaluated in the UK Department of Plant Pathology greenhouse in Lexington, KY. Two replicate experiments were conducted. Four sorghum seeds were sown per 8-inch pot, in a mixture of three parts Pro-Mix BX (Premiere Horticulture, Ltd, Riviere du Loup, PQ, Canada) and two parts sterile topsoil. The pots were maintained on the bench with 14 hours of light, watered every other day to saturation by using an automated overhead watering system, and fertilized with a solution of 150 ppm of Peters 20-10-20 (Scotts-Sierra Horticultural Product Co., Marysville, OH) once a month, beginning one week after seedling emergence. The pots, each containing four plants, were arranged in a randomized complete block design with four blocks. Each experimental unit was comprised of three pots containing 12 plants. Plants were grown to the V5 or V6 stage, and then inoculated by placing 15 colonized sorghum grains (see above) into the whorl of each plant. Non-colonized grains, which

otherwise had been treated identically to the colonized grains, were used as a negative control treatment. Treatments were as follows: (1) SS1.001; (2) GS.1.001; (3) JG9.001; (4) JG18.001; (5) water control; (6) M30.001, negative control.

**Disease evaluation for field and greenhouse experiments:** Anthracnose disease symptoms were evaluated on a weekly basis for the field and greenhouse experiments beginning at 14 days after inoculation, for a period of four weeks. A standardized rating scale was used, with values ranging from 0-5, based on the one described and used in (22, 25, 184). On this scale, a “0” indicates no symptoms or very minor red flecks; a “1” is defined as small red dots on the leaves; a “2” indicates small-to-large red spots on the leaves; a “3” is lesions with necrotic centers but without acervuli; a “4” specifies necrotic lesions with acervuli in the centers of the lesions; and a “5” indicates the presence of coalescent necrotic lesions and abundant production of acervuli (Figure 2.2). This scale divides samples into two classes: resistant, including values of 0-3; and susceptible, including values of 4 and 5. Disease incidence was calculated as the number of infected (susceptible, ratings of 4 or 5) plants at each time point for the field and greenhouse experiments.

Disease severity was also evaluated in the field experiments at a single time point, six weeks after inoculation. Leaves were collected from the 6<sup>th</sup> and 7<sup>th</sup> internodes from five randomly chosen inoculated tillers per treatment, brought back to the laboratory, and scanned (by using an Epson GT-1500 scanner). The percentage of unhealthy (necrotic or chlorotic) tissue was evaluated for each leaf by using the software Quant (182).

**Laboratory Assays:**

Seedlings: Sugar Drip sorghum seeds were sown in 72 cell-flats (each cell 1/2" L x 1 1/2" W and 2 1/4" deep) containing Pro-Mix BX (Premiere Horticulture, Ltd, Riviere du Loup, PQ, Canada) in the greenhouse. Seedlings were maintained in the greenhouse with 14 hours of light, and watered every other day to saturation using an automated overhead irrigation system. After seven days, seedlings were brought to the laboratory and inoculated by pipetting 10 µl of a spore suspension ( $10^7$  spores per mL) into the whorl. Spore suspensions were prepared from 2-week-old PDA cultures and used immediately for inoculations. An experimental unit was a group of 11 seedlings. The experimental units were arranged in a completely randomized design. The inoculated seedlings were maintained in a humidity chamber at 23 °C under continuous light for 10 days. Disease evaluation was performed by using a dissecting stereoscope. Disease incidence was recorded as the number of seedlings with visible acervuli and pathogen sporulation.

Detached leaf sheath: Seeds of Sugar Drip sweet sorghum were sown in 3.8 x 21 cm containers (Super SC-10 UV stabilized, Stuewe & Sons, Inc. Oregon, USA) containing a mixture of two parts sterile topsoil and three parts Pro-Mix BX (Premiere Horticulture, Ltd, Riviere du Loup, PQ, Canada). Seedlings were maintained in the greenhouse with 14 hours of light, watered every other day to saturation using an automated overhead irrigation system, and fertilized beginning one week after emergence two or three times per month as needed with a solution of 150 ppm of Peters 20-10-20 (Scotts-Sierra Horticultural Product Co., Marysville, OH).

The detached sheath assay was based on the protocol described by Kankanala et al. (97), as modified by Torres et al. (97, 175). Briefly, sorghum seedlings at the V5 stage were cut at the soil line and brought back to the laboratory (see Appendix 2 of this dissertation for

details regarding the optimization of this assay). Leaf sheaths of the youngest fully expanded leaves were removed and cut into three-inch segments. A pipette was used to fill the rolled segments with 150  $\mu$ L of a fungal spore suspension ( $5 \times 10^5$  spores per mL). Water was used as a negative control. The filled sheaths were supported, with the midrib at the bottom, inside a Petri dish containing moistened Whatman paper (Whatman No. 1, Whatman, Hillsboro, OR, USA) to maintain high humidity, and incubated at 23 °C under continuous light for periods of up to 96 hours. Treatments were as follows: (1) SS1.001; (2) GS.1.001; (3) JG9.001; (4) JG18.001; (5) CgS11; and (6) M30.001, negative control. The treated sheaths were arranged in a completely randomized design.

After incubation the leaf blades were removed from each sheath segment, leaving only the midrib section. This was inverted on a wet microscope slide, and a single-edged razor blade was used to remove excess tissue from the abaxial surface until only a thin layer of mostly epidermal cells remained. For evaluation of disease incidence, a total of 50 fungal infection sites (consisting of appressoria and associated structures, if any) on each of 20 leaf sheaths were observed with the light microscope for each treatment at 60 hpi, and the number that had invaded more than one host cell was recorded.

### **Statistical analysis:**

Field and Greenhouse Experiments: Analysis was performed with SAS PROC GLM (version 9.3, SAS Institute, Inc., Cary, NC) to test the effect of removing the seed head of the plants, and to evaluate the relationship between plant yield and BRIX and the aggressiveness of the fungal strains. The incidence and severity data from 2014 and 2015 experiments were analyzed using Least Squares Means PROC GLM, and multiple mean comparisons among the different treatments were performed with t-tests. General linear

model was used with post-hoc comparisons. P-values less than 0.01 were considered significant. Incidence values were used to calculate the area under the disease progress curve (AUDPC) by the trapezoidal integration method (20). Analyses among the treatments were evaluated by using the general linear models (GLM) procedure of SAS (version 9.4) (SAS Users Guide, SAS Institute, Cary, NC). Means were separated with least squares estimates of marginal means (LSMEANS statement). P-values less than 0.05 were considered significant.

The analysis for the grain yield was performed using SAS version 9.3. Treatment means were compared using the Tukey's multiple comparison test procedure of the SAS software package (SAS Users Guide, SAS Institute, Cary, NC). P-values of less than 0.05 were considered significant.

Whole seedling and detached sheath assays: The analyses for the detached leaf sheath and whole seedlings assays were performed using SAS version 9.3. The data were subjected to analysis of variance (ANOVA). Treatment means were compared using the Tukey's multiple comparison test procedure of the SAS software package (SAS Users Guide, SAS Institute, Cary, NC). P-values of less than 0.05 were considered significant.

## **Results**

### **Field experiments**

Effects of De-heading: Brix values for the intact versus de-headed treatments were significantly different ( $P < 0.01$ ) in both years of the study (Table 2.3). The year-by-treatment interaction was not significant. There was no statistical effect of the removal of the seed heads on the yields of plant biomass or juice in either year (Table 2.3). Yields of

both biomass and juice were statistically reduced in 2015 in comparison with the year before.

Development of Disease in the Field: In both years, typical anthracnose disease symptoms developed, within two days after inoculation (dpi), on the midrib and on the leaf blades of the inoculated plants. Symptoms were first visible as small, elliptical reddish spots that increased in number and size, eventually coalescing to cover large portions of the leaves (Figure 2.3 A). Signs (acervuli containing conidia and setae) were produced in the necrotic centers of the lesions within 5 dpi. The pathogen spread from the inoculated tillers, resulting in the development of symptoms and signs on the leaves of neighboring, uninoculated tillers by the end of the experiment in the most heavily affected treatments. However, typical disease symptoms and signs were not observed in the water or non-pathogen control treatments, indicating that there was no natural infection, and no inter-plot pathogen movement.

De-heading the plants had no effect on the disease incidence, or on disease severity with the exception of SS1.001 in 2014, which produced more severe symptoms on the de-headed treatment (Table 2.4). Since de-heading had no effect on disease incidence or yield, the control and de-headed treatments were pooled for subsequent analyses of these parameters.

Disease Incidence and Severity: Generally, disease incidences were higher in the field in 2014 versus 2015 (Table 2.5, Figure 2.4). All of the strains from *Sorghum* spp. resulted in incidences of disease that were statistically greater than the negative controls (water and the strain from maize) in 2014. However in 2015, only the isolates from *S. bicolor* and johnsongrass isolate JG18.001 were different from the negative controls (Table 2.5).

The amount of leaf damage was also higher in 2014 than in 2015 (Table 2.5, Figure 2.5). Disease severity did not exceed 20% of the leaf area damaged on the inoculated tillers for any treatment in either year of the study. The sweet sorghum and grain sorghum isolates were the most damaging, while the johnsongrass isolates were not statistically different from the negative controls in either year ( $p$  value  $> 0.01$ ) (Table 2.5).

Although the overall amount of disease was different between the two years, the relative rankings of isolate aggressiveness were consistent. In both years of the study SS1.001, which was isolated from sweet sorghum, was the most aggressive, while the two strains isolated from johnsongrass (JG18.001 and JG9.001) produced much lower levels of disease.

Effect of anthracnose on plant yield and Brix: Pooled data from intact and de-headed sub-treatments by year revealed that yields of plant biomass and juice and Brix levels in all but one case, did not differ from the two controls for any level of disease that was achieved during the two years of this study (Table 2.6). Brix for the water control in 2014 was lower than for the treated samples (Table 2.6). Grain yields were also unaffected by any of the disease treatments (Table 2.6).

### **Greenhouse experiments**

Two repetitions of the greenhouse experiment were performed. Symptoms started to appear within 2 dpi as red spots on the leaf blade and midrib. Within 5 dpi, necrotic lesions containing *Colletotrichum* fruiting structures were visible. Symptoms produced in the greenhouse were similar to those in the field, although they were generally much milder (Figure 2.3B). Incidences of disease resulting from inoculation with the strains isolated from cultivated sorghum (SS1.001 and GS1.001) were statistically higher than the control treatments ( $p$  value  $> 0.01$ ) in both replications of the experiment. In the first repetition,



SS1.001 was the most aggressive while in the second replication, SS1.001 and GS1.001 were equally aggressive ( $p$  value = 0.001). The strains from johnsongrass did not differ statistically from the water or non-pathogen controls in either replication ( $p$  value > 0.01) (Table 2.5). Both of the johnsongrass isolates caused typical foliar anthracnose lesions, including acervuli with setae, when the spores were applied to leaves of johnsongrass plants grown from seed collected in Fayette County KY, confirming that they were pathogenic to *S. halepense* (data not shown).

### **Seedling assays**

Due to the necessity for containment, the Indiana grain sorghum isolate CgS11 was not used for field assays. All three isolates from cultivated sorghum (CgS11, SS1.001, and GS1.001) produced typical dark, sporulating anthracnose lesions on the seedlings within 10 dpi (Figure 2.3C). In the first replication of the study, these three isolates could be statistically separated based on incidence, with CgS11 being the most aggressive of the three, and GS1.001 the least ( $p$  value < 0.05). In the second replication, the isolates were not statistically different from each other ( $p$  value < 0.05) (Figure 2.3C, Table 2.5). The isolates from johnsongrass caused only relatively mild symptoms, and did not differ in incidence from the negative controls in either replication (Figure 2.3C, Table 2.5).

### **Detached leaf sheath assays**

The process of infection of sweet sorghum sheaths by *C. sublineola* was evaluated over a time course from 0 to 108 hours after inoculation (hpi) (Figure 2.5). At 0 hpi, only ungerminated spores were visible on the surface (Figure 2.5 A). The pathogen had formed appressoria by 12 hpi (Figure 2.5 B), and small primary hyphae could be seen within the host epidermal cells by 24 to 48 dpi (Figure 2.5 C, D). By 60 hpi, the hyphae in a compatible interaction had progressed to colonize adjacent cells (Figure 2.5 E).

Colonization continued to expand into additional cells by 72 hpi (Figure 2.5 F), and narrower secondary hyphae had begun to develop as branches from the hyphae behind the colonization front (not shown). By 108 hpi, tissue collapse was evident, and sporulation had occurred (Figure 2.5 G).

Based on observations of hundreds of individual inoculation sites, I found that whenever the pathogen was able to colonize more than one plant cell within 60 hpi, it almost always progressed to sporulation. On the other hand, if the pathogen had not colonized beyond one cell in that time, its growth was arrested and it almost always failed to complete its life cycle even up to 6 days after inoculation. Thus, I considered that a successful infection had occurred if the pathogen had progressed beyond one plant cell within 60 hpi. Isolate CgS11, from grain sorghum in Indiana, was the most aggressive on Sugar Drip in this detached leaf sheath assay (Figure 2.3D, Figure 2.6). SS1.001 and GS1.001 did not differ from one another, but both were significantly more aggressive than the johnsongrass isolates, which did not differ from the negative control (the maize isolate M30.001) (Figure 2.3D, Figure 2.6).

Sheaths of 12 additional sorghum lines were inoculated with the six fungal strains, and the johnsongrass isolates did not differ from the negative control on any of these lines (Figure 2.7). The ability of the three isolates from *S. bicolor* to colonize each of the lines varied. The grain sorghum isolates CgS11 and GS1.001 were each able to colonize five of these lines, whereas SS1.001 could only colonize one (Chinese Amber). None of the isolates successfully infected Cowper or Keller.

## Discussion

Anthrachnose is listed as one of the three most important diseases of sweet sorghum in KY (12). I made multiple visits to KY sweet sorghum growers in Clay, Russell, and Morgan Counties during the summers of 2013, 2014, and 2015. Foliar anthracnose lesions could be found on sweet sorghum (varieties Dale and M81E) and johnsongrass on all of these farms each year, but overall disease levels were very low. Red rot and lodging in sweet sorghum are often attributed to *C. sublineola*, however red rot was not observed during the years of this study. Lodged plants were relatively common, but I did not recover *Colletotrichum* from any of the lodged plants.

The results of the field study demonstrated that *C. sublineola* has significant potential to cause anthracnose disease on a susceptible variety of sweet sorghum in KY. Sugar Drip is an heirloom variety of unknown origin that was very popular in KY up through the 1980s. It is fast-growing, thus well adapted to the state and suited for late planting, and it produces very high quality syrup (12). However, it is highly susceptible to many sorghum diseases, including anthracnose, and for this reason it has been largely displaced by more resistant varieties.

Incidences of foliar anthracnose on inoculated tillers of up to 95%, and severities of close to 20% of the leaf area affected, were achieved in the field experiment, and I also observed frequent secondary spread of the disease to neighboring uninfected tillers in the most heavily affected plots. No red rot or lodging was observed, suggesting that the pathogen did not significantly colonize the stalk from the foliage when it was inoculated at this stage of development. Disease levels achieved in my experiments had no negative impact on the yield of biomass, grain, juice, or Brix. Given that the levels of disease in my experimental

fields were much higher than I observed at the collection sites, I conclude that the economic impact of this disease in KY on yield at present is probably minimal. It is important to point out that I inoculated two tillers per plant. Most of the plants eventually produced up to 10 additional tillers, and these may have compensated for the tissue loss by remobilizing photosynthate to the diseased tillers (159).

Removal of seed heads has been recommended as a way to increase yields of sugar for syrup (13). Maize anthracnose is known to be negatively affected by increases in stalk sugars (121), and so I wanted to see if removal of seed heads would also increase resistance of sweet sorghum to anthracnose. In the current study, removal of seed heads did increase the Brix, but this had no significant effect on the levels of foliar anthracnose.

Although I followed the same experimental protocol in both years of the study, disease levels varied significantly between years. This was probably due to environmental factors, especially the amount of rainfall. In 2014, rainfall levels were unusually high throughout the season. It rained on the day of inoculation, and it continued to rain frequently up through harvest. In 2015, there was torrential rainfall and flooding at the beginning of the season, but it stopped before the inoculation, and after that the weather became hot and dry. The higher levels of leaf moisture during and subsequent to inoculation probably favored the development of the pathogen in 2014, leading to more disease. One of the johnsongrass isolates did not differ from controls in 2015, but did in 2014. This suggests that high levels of leaf wetness, and low light intensities resulting from overcast conditions, may be important determinants of infectivity for less aggressive isolates. Similar results have been reported for anthracnose on grain sorghum (135).

Despite higher levels of disease, yields of biomass, grain, and juice were significantly higher in 2014 than in 2015. The difference in biomass may be due in part to the removal of leaves from the stalk samples in 2015 prior to pressing, whereas they were included in 2014. Additionally, the plants were noticeably damaged and stunted early in the season in 2015, probably as a result of the unusually high levels of rainfall and flooding that occurred just after transplanting (Figure 2.8).

Control plots (mock-inoculated or inoculated with the maize *Colletotrichum* isolate) were disease-free, indicating that there was little or no natural inoculum in the fields. This is in spite of the fact that johnsongrass was common in the grassy areas surrounding the fields, and that *Colletotrichum* could be readily recovered from these plants. My observations are consistent with a prior study that reported that johnsongrass isolates did not contribute to the population of *C. sublineola* infecting nearby grain sorghum in Texas (73, 144). Even though they were significantly less aggressive, the KY johnsongrass isolates in the current study were nonetheless both able to infect inoculated Sugar Drip plants in the field and complete their life cycle. This suggests that, if conditions for infection are ideal, johnsongrass could serve as a reservoir for the pathogen during crop rotation schemes, and as a source for new races.

The sweet sorghum strain SS1.001 used in my study was isolated from a foliar lesion on the sorghum variety Sourless on the UK Agronomy Farm in Fayette County. This isolate was the most aggressive to Sugar Drip in both years in the field study, but with so few isolates included in the experiment it would be premature to conclude that sweet sorghum isolates are generally more aggressive than isolates from grain sorghum to sweet sorghum. It is necessary to test more isolates in order to address this question. Field experiments are

limited by time and resources, and it is possible to investigate only relatively small numbers of local isolates. I developed and tested three alternative greenhouse and laboratory (controlled environment) assays for more rapid evaluation of larger numbers of strains, and exotic strains that require containment.

All three of the controlled-environment assays gave results that were consistent with the field results, in that they confirmed that the johnsongrass isolates were significantly less aggressive to Sugar Drip than the isolates from cultivated sorghum. Tests on 12 additional lines using the sheath assay revealed that the johnsongrass isolates were unable to infect any of them. In the greenhouse and laboratory assays, the two johnsongrass isolates were completely nonpathogenic, but the field assays demonstrated that these isolates did have some potential to complete their life cycles on Sugar Drip sweet sorghum, especially when environmental conditions were highly favorable to the development of disease, as occurred in 2014. Thus, a lack of pathogenicity in the controlled environment assays will not necessarily mean that the interaction will be incompatible in the field, but it does suggest that the disease severity will be relatively low. The ability of the three isolates from *S. bicolor* to colonize the sheaths varied depending on the sorghum variety, and this could suggest the presence of race interactions. *Colletotrichum sublineola* is known to exist as multiple races (25, 27). Two of the sorghum lines, Cowper and Keller, were resistant to all three of the *S. bicolor* isolates in the sheath assay. Tests involving whole plants in the greenhouse and in the field will be necessary to confirm these results.

There were higher levels of disease in the field in both years than in the greenhouse. Similar results were observed by Ferreira and colleagues when evaluating 23 grain sorghum cultivars for resistance to *C. sublineola* in the greenhouse and in the field (67). Plants in

the field are exposed to multiple stresses including insects; wounds that facilitate pathogen entry; abiotic stressors (wind, high temperature, drought or flooding, hail, etc.); and biotic disease (bacteria and other fungi). In contrast, the plants in the greenhouse are maintained at optimal conditions of temperature and moisture and protected from most other biotic and abiotic stressors. Stress is well-known to predispose grain sorghum to anthracnose (54). Disease incidence was higher in the second biological repetition of the greenhouse experiment than in the first. The second replication took place in the mid-winter months, whereas the first was done in the late summer, and thus light intensity may have played a role. It has been reported that higher light intensities result in lower anthracnose disease severity in maize, due to an increase in the ability of the plant to express resistance (82, 126, 150). Although supplemental lighting is provided in the greenhouse, it does not match natural summer light intensities. The sweet sorghum isolate (SS1.001) was significantly more aggressive than the grain sorghum isolate (GS1.001) in the first replication, similar to the field observations. However, it did not differ from the grain sorghum isolate in the second replication, which may indicate that a reduction in resistance caused by lower light intensities favors less aggressive pathogens more than highly aggressive ones.

The sheath assay provides a uniquely detailed view of the host-pathogen interaction in living tissues. Snyder et al. (1991) described the accumulation of red vesicles at the infection sites of the non-pathogen *C. graminicola* (161). Wharton and Julian (1996) compared the colonization of susceptible versus resistant grain sorghum leaf sheaths by *C. sublineola*. They described how the resistant plants responded by accumulating red vesicles at the sites of infection, which eventually burst and released their contents, filling the colonized cells and also often coating the fungal hyphae as well (195). In contrast, in

susceptible interactions the accumulation of pigmented vesicles was relatively slow, and the pigmentation was more orange than red (195). It has been shown that the dark red vesicles formed in incompatible interactions with *C. graminicola* contain anthocyanidin phytoalexins, which have also been strongly implicated in the resistance of sorghum to *C. sublineola* (89, 112, 129). My observations in sweet sorghum leaf sheaths (Figure 2.3D) were generally consistent with the published observations of grain sorghum. Thus, I observed a rapid and intense production of vesicles containing a bright red pigment in Sugar Drip responding to the non-pathogen *C. graminicola*. None of the strains from sorghum produced as intense a reaction in Sugar Drip sheaths. Strains SS1.001 and GS1.001 (both virulent) induced the production of a diffuse reddish pigment throughout the colonized cells, whereas CgSI1 (virulent) and JG9.001 (avirulent) didn't cause any change in color. Strain JG18.001 (avirulent) caused only a slight accumulation of red pigments surrounding the infection hyphae. Production of these more diffuse reddish compounds was not correlated with resistance, and it is possible that they are not related to the anthocyanidin phytoalexins that have been previously described. Biochemical assays will need to be performed to address the question of their identity and significance. The sheath assays are well-suited for this kind of detailed analysis of the host-pathogen interaction.

### **Summary**

My predictions for chapter 2 were: (1) *C. sublineola* from sweet sorghum will be more pathogenic and/or aggressive on sweet sorghum than isolates from johnsongrass or grain sorghum; (2), anthracnose disease will have a negative impact on yield and Brix; (3) de-heading sorghum will result in increased Brix and decreased levels of disease; and (4) the



results of greenhouse and leaf sheath assays will be correlated with field inoculations. Prediction 1 was supported, in that SS1.001 was the most aggressive isolate in the field study. However, it was not always the most aggressive in the other assays, and since I could include only one isolate from sweet sorghum, it seems premature to conclude that sweet sorghum isolates will always be more aggressive to sweet sorghum. Prediction 2 was not supported. The levels of disease achieved in my field study had no negative effect on yield parameters or BRIX. However, since I only inoculated only two tillers, and the plants eventually developed additional tillers, these uninoculated tillers may have been able to compensate for the diseased tissues. Prediction 3 was partially supported. De-heading the sorghum plants did increase BRIX. However, this increase had no effect on levels of anthracnose disease. Prediction 4 was supported. Greenhouse and laboratory assays generally agreed with the results of the field assays, in that they confirmed that the two johnsongrass isolates were much less aggressive than the isolates from cultivated sorghum.

Table 2.1. *Colletotrichum* isolates used in this study.

| Strain   | Host          | Species               | State | County     | Year Collected | ITS sequence Accession | Reference               |
|----------|---------------|-----------------------|-------|------------|----------------|------------------------|-------------------------|
| CgSI1    | Grain sorghum | <i>C. sublineola</i>  | IN    | Tippecanoe | 1982           | KY006070               | Jamil & Nicholson, 1987 |
| SS1.001  | Sweet sorghum | <i>C. sublineola</i>  | KY    | Fayette    | 2012           | KY006074               | This study              |
| GS1.001  | Grain sorghum | <i>C. sublineola</i>  | KY    | Hopkins    | 2013           | KY006071               | This study              |
| JG9.001  | Johnsongrass  | <i>C. sublineola</i>  | KY    | Russell    | 2012           | KY006073               | This study              |
| JG18.001 | Johnsongrass  | <i>C. sublineola</i>  | KY    | Fayette    | 2012           | KY006072               | This study              |
| M30.001  | Maize         | <i>C. graminicola</i> | KY    | Fayette    | 2012           | KY006075               | This study              |

Table 2.2. Pedigree and origin of sweet sorghum varieties used in the leaf sheath assay.

| Variety           | Origin             | Year Imported/Released | Pedigree (If Known)                      | References                  |
|-------------------|--------------------|------------------------|--|-----------------------------|
| Chinese Amber     | China              | 1853                   | Unknown                                  | Smith and Frederiksen 2000  |
| Honey             | South Africa       | 1857                   | Unknown                                  | Swanson & Laude, 1934       |
| Orange            | South Africa       | 1857                   | Unknown                                  | Swanson & Laude, 1934       |
| Planter           | South Africa       | 1857                   | Unknown                                  | Swanson & Laude, 1934       |
| Dwarf Yellow Milo | Texas and Oklahoma | 1900                   | From Standard Yellow Milo (Texas – 1890) | Swanson & Laude, 1934       |
| Dale              | Mississippi        | 1973                   | Tracy/MN 960 (PI 152857)                 | Ali, Rajewski et al., 2008  |
| Keller            | Mississippi        | 1982                   | Mer. 50-1/Rio                            | Ali, Rajewski et al., 2008  |
| Bailey            | Georgia            | 1984                   | Wiley (Collier/MN822/MN2046)/Tracy       | Ali, Rajewski et al., 2008  |
| N100              | Nebraska           | 1990                   | Waconia/Wray                             | Ali, Rajewski et al., 2008  |
| Della             | Virginia           | 1993                   | Dale*2/ATx622                            | Ali, Rajewski et al., 2008  |
| Cowper            | Unknown            | Before 1951            | Unknown                                  | LeBeau, Stokes et al., 1951 |
| Simon             | Unknown            | Unknown                | Unknown                                  | Ali, Rajewski et al., 2008  |

Table 2.3. Effect of removing the seed head of the plants on the plant yield (fresh biomass and juice content) and BRIX.

| Year | Plant top   | Brix <sup>¥</sup> ∂ | Fresh biomass <sup>∫</sup> | Juice content <sup>∫</sup> |
|------|-------------|---------------------|----------------------------|----------------------------|
| 2014 | Intact Head | 12 a <sup>β</sup>   | 4.74 a                     | 2.4 a                      |
|      | De-head     | 14.1 b              | 5.0 a                      | 2.5 a                      |
| 2015 | Intact Head | 15.2 c              | 1.17 b                     | 0.66 b                     |
|      | De-head     | 16.4 d              | 1.05 b                     | 0.62 b                     |

¥ One (1) degree Brix corresponds to 1 Kg of fructose in 100 Kg of solution

∫ Data average weight in Kg of five inoculated tillers

∂ Data average based on extraction of five inoculated tillers

β Means followed by the same letter, for each column, are not significantly different according to Least Square Means (t-test significant at P = 0.001)

Table 2.4. Effect of heading and de-heading on incidence (transformed in area under the disease progress curve) and severity (percentage damaged area) in the field.

| Treatments | 2014                    |         |          |         | 2015                    |         |          |          |
|------------|-------------------------|---------|----------|---------|-------------------------|---------|----------|----------|
|            | Incidence <sup>¥π</sup> |         | Severity |         | Incidence <sup>¥π</sup> |         | Severity |          |
|            | Head                    | De-head | Head     | De-head | Head                    | De-head | Head     | De-head  |
| GS1001     | 48.5 b                  | 51.8 b  | 8.2 c    | 9.5 bc  | 25 b                    | 20 b    | 7.9 bcd  | 11.4 abc |
| SS1001     | 66.8 a                  | 72.8 a  | 12.4 b   | 16.7 a  | 59.3 a                  | 59.5 a  | 16.8 a   | 12.8 ab  |
| JG18001    | 33 c                    | 27.8 cd | 2.8 ed   | 3.9 d   | 5.8 cd                  | 7.4 c   | 3.1 de   | 2.6 de   |
| JG9001     | 19 d                    | 20.5 d  | 3.2 ed   | 2.4 ed  | 1.6 cd                  | 5.7 cd  | 4.7 de   | 5.8 cde  |
| M30001     | 0 e                     | 0 e     | 0.8 e    | 2.2 ed  | 0 d                     | 0 d     | 2.0 e    | 2.0 e    |
| Water      | 0 e                     | 0 e     | 1.1 ed   | 1.6 ed  | 0 d                     | 0 d     | 2.9 de   | 1.2 e    |

¥ Incidence, number of infected tillers out of 20 tillers in the field.

π Incidence data was used to calculate the least-square means for area under the disease progress curve (AUDPC).

Means followed by the same letter, for each column, are not significantly different according to Least Square Means (t-test significant at P = 0.001).

Table 2.5. Field and greenhouse pathogenicity assay of five strains of *Colletotrichum* on sweet sorghum var. Sugar Drip.

| Treatments | Field               |        | Greenhouse          |        | Lab/seedlings          |       |
|------------|---------------------|--------|---------------------|--------|------------------------|-------|
|            | AUDPC <sup>¥π</sup> |        | AUDPC <sup>¥π</sup> |        | Incidence <sup>¥</sup> |       |
|            | 2014                | 2015   | 1                   | 2      | 1                      | 2     |
| CgS11      | NA                  | NA     | NA                  | NA     | 2.7 a                  | 6.5 a |
| GS1.001    | 50.1 b              | 22.5 b | 1.3 b               | 23.3 a | 1 c                    | 6 a   |
| SS1.001    | 69.8 a              | 59.4 a | 3.7 a               | 22 a   | 1.7 b                  | 6.5 a |
| JG18.001   | 30.4 c              | 6.6 c  | 0 c                 | 0.3 b  | 0 d                    | 0 b   |
| JG9.001    | 19.8 d              | 3.6 cd | 0 c                 | 0 b    | 0 d                    | 0 b   |
| M30.001    | 0 e                 | 0 d    | 0 c                 | 0 b    | 0 d                    | 0 b   |
| Water      | 0 e                 | 0 d    | 0 c                 | 0 b    | 0 d                    | 0 b   |

Incidence, number of infected plants out of 11 seedlings in the lab or 12 plants in the greenhouse, or number of infected tillers out of 20 tillers in the field (pooled for intact and de-head treatments).

π Least-square means for area under the disease progress curve (AUDPC) for the greenhouse and field pathogenicity assays of five strains of *Colletotrichum* on Sugar Drip. For the AUDPC means followed by the same letter, for each column, are not significantly different according to Least Square Means (t-test significant at P = 0.001)

For incidence means followed by the same letter, for each column, are not significantly different according Tukey's multiple comparison test procedure

Table 2.6. Effect of disease treatments on plant yield (fresh biomass, grain, and juice content) and BRIX.

| Treatments | 2014                |                    |                    |                     | 2015                |                    |                    |                     |
|------------|---------------------|--------------------|--------------------|---------------------|---------------------|--------------------|--------------------|---------------------|
|            | Weight <sup>∫</sup> | Juice <sup>∫</sup> | Brix <sup>¥∂</sup> | Grain* <sup>∨</sup> | Weight <sup>∫</sup> | Juice <sup>∫</sup> | Brix <sup>¥∂</sup> | Grain* <sup>∨</sup> |
| GS1001     | 4.9 ba <sup>β</sup> | 2.3 a              | 13.3 a             | 146.4 ba            | 1.1 ba              | 0.6 a              | 15.7 a             | 58.1 a              |
| JG18001    | 4.8 ba              | 2.5 a              | 13.0 a             | 113.3 ba            | 1.2 a               | 0.7 a              | 15.8 a             | 55.9 a              |
| JG9001     | 4.6 b               | 2.5 a              | 13.1 a             | 174.1 a             | 1.1 ba              | 0.6 a              | 16.2 a             | 83.5 a              |
| M30001     | 4.7 b               | 2.6 a              | 13.5 a             | 148.1 ba            | 0.99 b              | 0.6 a              | 15.6 a             | 109.1 a             |
| SS1001     | 4.9 ba              | 2.5 a              | 13.4 a             | 79.2 b              | 1.1 ba              | 0.6 a              | 16.1 a             | 52.7 a              |
| Water      | 5.4 a               | 2.4 a              | 12.1 b             | 134.8 ba            | 1.2 a               | 0.7 a              | 15.6 a             | 62.0 a              |

¥ One (1) degree Brix corresponds to 1 Kg of fructose in 100 Kg of solution

∫ Data average weight in Kg of biomass or juice from five inoculated tillers

∂ Data average from five inoculated tillers

\* Average grams of seeds from five seed heads from harvested inoculated tillers

β Means followed by the same letter, for each column, are not significantly different according to Least Square Means (t-test significant at P = 0.001). The exception is grains, in which means followed by the same letter, for each column, are not significantly different according Tukey's multiple comparison test procedure

Figure 2. 1. Alignments of ITS sequences of CgS11, SS1.001, GS1.001, JG9.001, JG18001 and M30.002 with *Colletotrichum sublineola*, species type S3.001\_NR, and *Colletotrichum graminicola*, species type M1.001\_NR. Alignments done with MUSCLE version 3.8.

```

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

M1.001 NR      CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
M30.001       CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
CgS11         CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGTAGGGTCCGGC
GS1.001       CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
JG18.001      CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
JG9.001       CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
S3.001 NR     CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
SS1.001       CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCCAAAGGAGGCTCCGGGAGGGTCCGGC
*****

M1.001 NR     ACTACCTTTAAGGGCTACGACGTACGCCGTAGGGCCCCAACACCAAGCGGAGCTTGAGG
M30.001       ACTACCTTTAAGGGCTACGACGTACGCCGTAGGGCCCCAACACCAAGCGGAGCTTGAGG
CgS11         ACTACTTTTGAGGGCTACGTCA---ACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGG
GS1.001       ACTACTTTTGAGGGCTACGTCA---ACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGG
JG18.001      ACTACTTTTGAGGGCTACGTCA---ACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGG
JG9.001       ACTACTTTTGAGGGCTACGTCA---ACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGG
S3.001 NR     ACTACTTTTGAGGGCTACGTCA---ACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGG
SS1.001       ACTACTTTTGAGGGCTACGTCA---ACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGG
*****

M1.001 NR     GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
M30.001       GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
CgS11         GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
GS1.001       GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
JG18.001      GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
JG9.001       GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
S3.001 NR     GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
SS1.001       GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTC
*****

M1.001 NR     AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
M30.001       AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
CgS11         AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
GS1.001       AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
JG18.001      AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
JG9.001       AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
S3.001 NR     AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
SS1.001       AAAGATTCGATGATTCACCTGAATTCCTGCAATTCACATTAATATCGCATTTCCGCTCGGTT
*****

M1.001 NR     CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
M30.001       CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
CgS11         CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
GS1.001       CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
JG18.001      CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
JG9.001       CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
S3.001 NR     CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
SS1.001       CTTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTAATTTGCTTGTGCC
*****

M1.001 NR     ACTCAGAAGAGACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC-
M30.001       ACTCAGAAGAGACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC-
CgS11         ACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC
GS1.001       ACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC
JG18.001      ACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC
JG9.001       ACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC
S3.001 NR     ACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC
SS1.001       ACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGAGCCCC
*****

M1.001 NR     -----GCAGTGGGGCCCGGCGGGAGGGCGTCCCGCGGAGAGGGGACCCCTAACCCG
M30.001       -----GCAGTGGGGCCCGGCGGGAGGGCGTCCCGCGGAGAGGGGACCCCTAACCCG
CgS11         TCCCGGAGGAGGAGGGCCCGGCGGGAGGGCGTCCCG-----GGGGGACCCCTAACCCG
GS1.001       TCCCGGAGGAGGAGGGCCCGGCGGGAGGGCGTCCCG-----GGGGGACCCCTAACCCG
JG18.001      TCCCGGAGGAGGAGGGCCCGGCGGGAGGGCGTCCCG-----GGGGGACCCCTAACCCG
JG9.001       TCCCGGAGGAGGAGGGCCCGGCGGGAGGGCGTCCCG-----GGGGGACCCCTAACCCG
S3.001 NR     TCCCGGAGGAGGAGGGCCCGGCGGGAGGGCGTCCCG-----GGGGGACCCCTAACCCG
SS1.001       TCCCGGAGGAGGAGGGCCCGGCGGGAGGGCGTCCCG-----GGAGGGACCCCTAACCCG
*****

M1.001 NR     CCGAAGCAACGTTAGGTATGTTCA
M30.001       CCGAAGCAACGTTAGGTATGTTCA
CgS11         CCGAAGCAACAGTTAGGTATGTTCA
GS1.001       CCGAAGCAACAGTTAGGTATGTTCA
JG18.001      CCGAAGCAACAGTTAGGTATGTTCA
JG9.001       CCGAAGCAACAGTTAGGTATGTTCA
S3.001 NR     CCGAAGCAACAGTTAGGTATGTTCA
SS1.001       CCGAAGCAACAGTTAGGTATGTTCA
*****

```



Figure 2. 2. Representative disease symptoms, illustrating the rating scale that was used for this work. (A) no symptoms=0; (B) small necrotic dots on the leaves=1; (C) small red spots on the leaves=2; (D) necrotic lesions without acervuli=3; (E) necrotic lesions with acervuli in the center of the lesions=4, and (F) coalescent necrotic lesions and abundant production of acervuli=5.

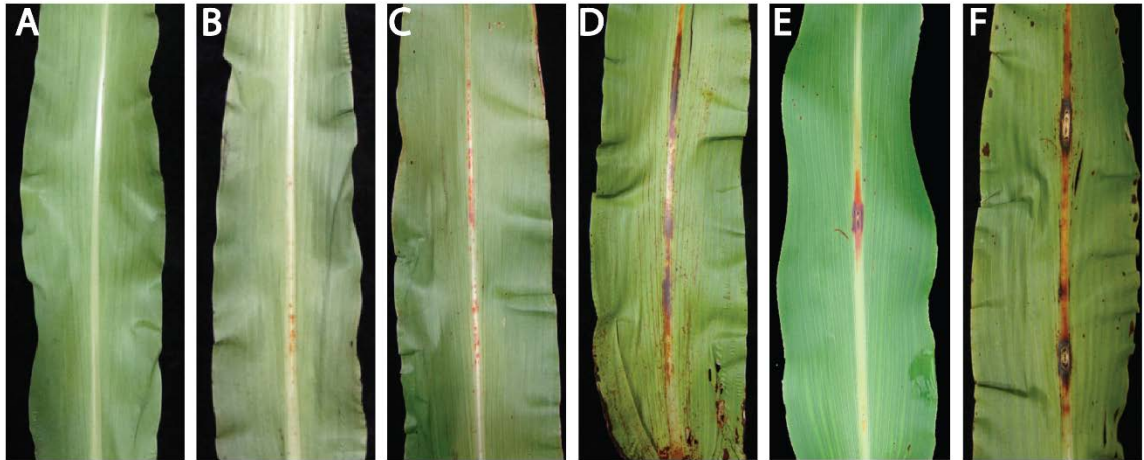


Figure 2. 3. Disease symptoms. (A) field experiment; (B) greenhouse experiment; (C) whole seedling inoculation in the lab; (D) detached leaf sheath assay, inoculation in the lab. Scale bars (C) 0.6 cm and (D) 50  $\mu$ m.

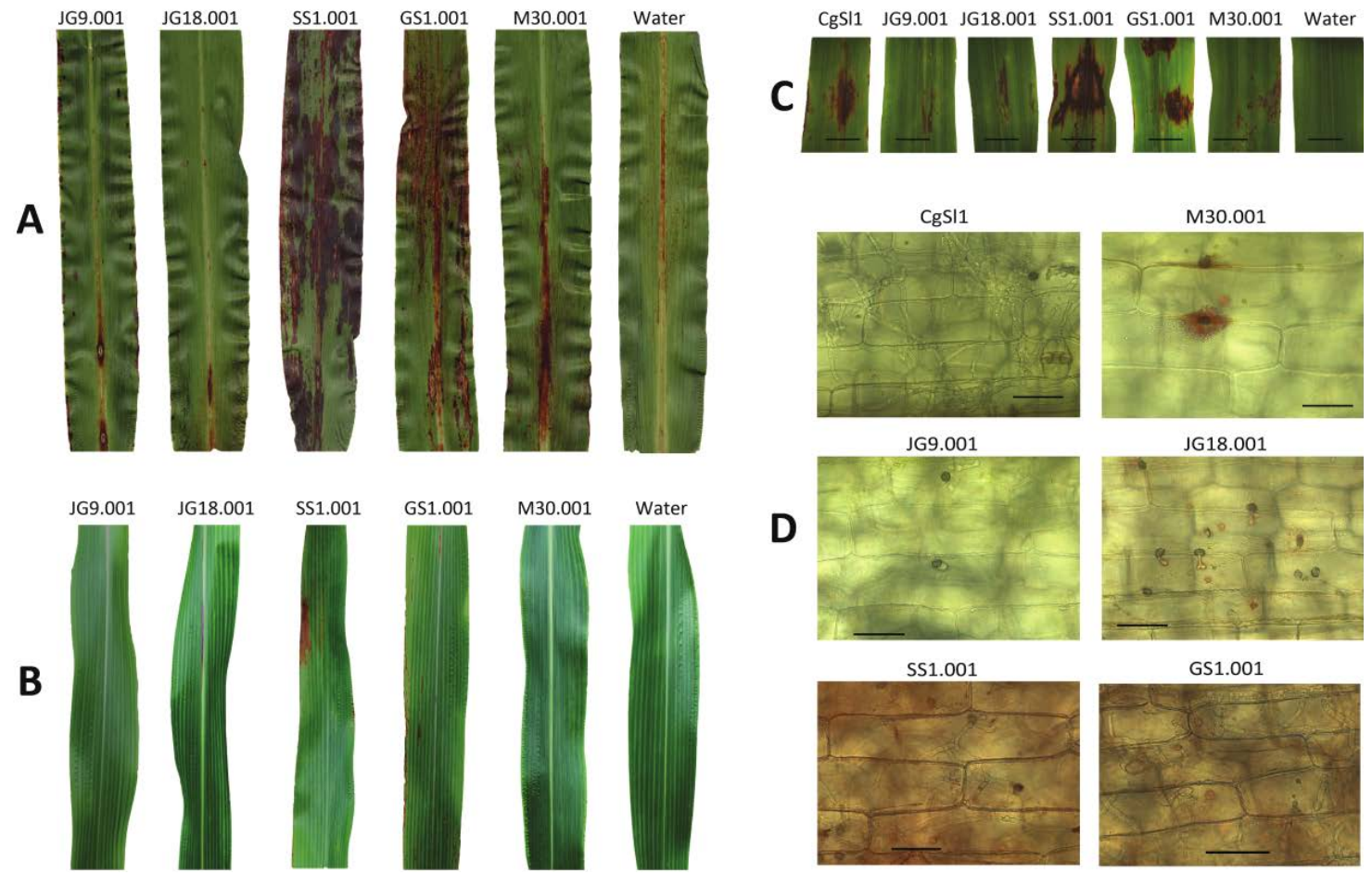


Figure 2.4. Average disease ratings (= the average number of plants rated 0-5) at 6 weeks post-inoculation in 2014 and in 2015.



Figure 2.5. Representative micrographs showing the infection process during the interaction between *C. sublineola* and sweet sorghum. A. Leaf sheath inoculation; (0) ungerminated spore, (1) Appressoria has been formed, (2) Hyphae smaller than appressoria, (3) Hyphae bigger than appressorium but restricted in one cell, (4) Hyphae growing in more than one cell, (5) Hyphae growing in more than three cells, and (6) Acervulus has been formed.

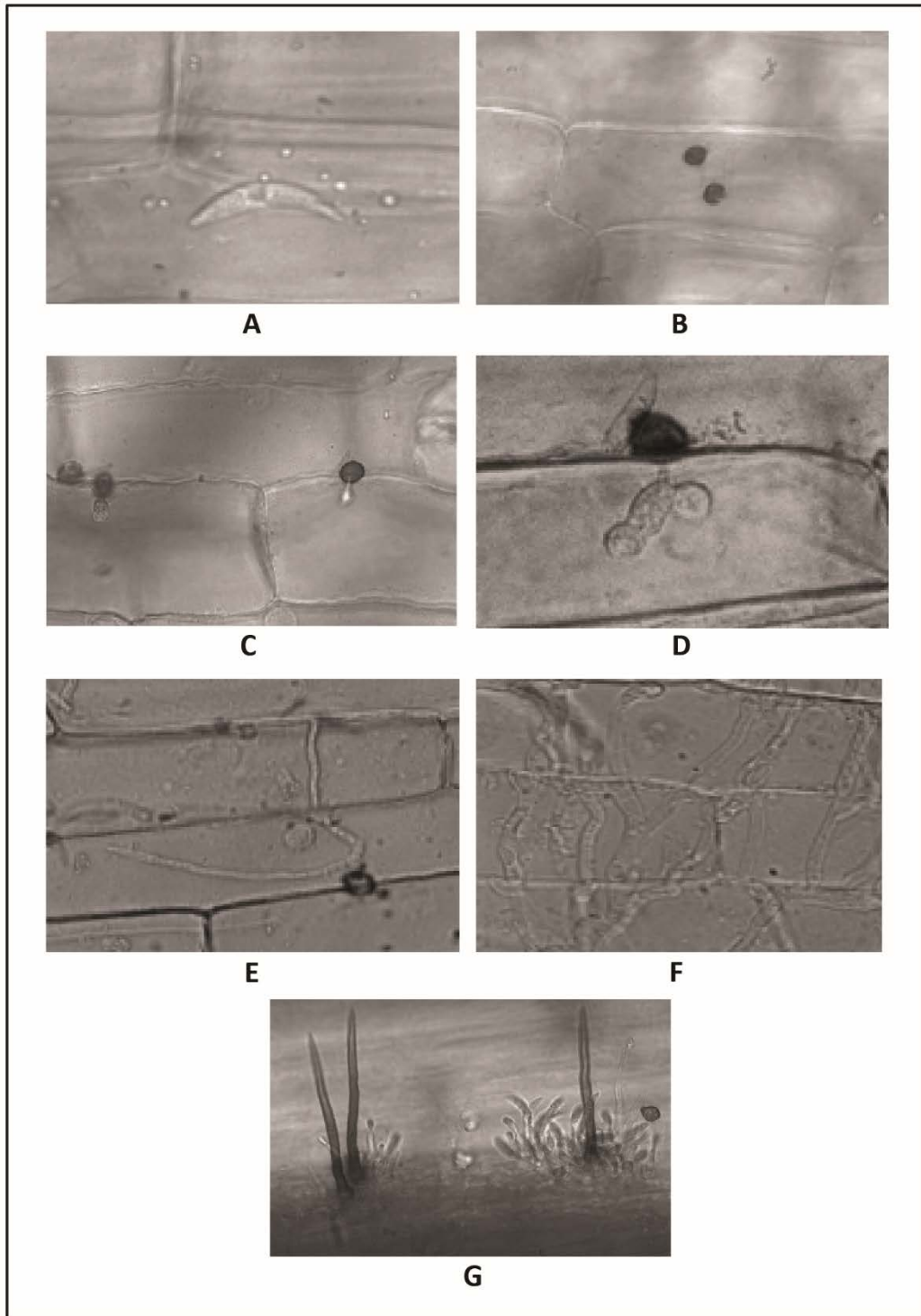


Figure 2.6. Leaf sheath assay of six strains of *Colletotrichum* on Sugar Drip. Disease evaluation was performed based on the number of infection sites in which the pathogen was able to grow beyond one plant cell at 60 hours after inoculation. Combined data from the evaluation of 50 infection sites on 20 leaf sheaths per treatment. The evaluation was performed under light microscope.

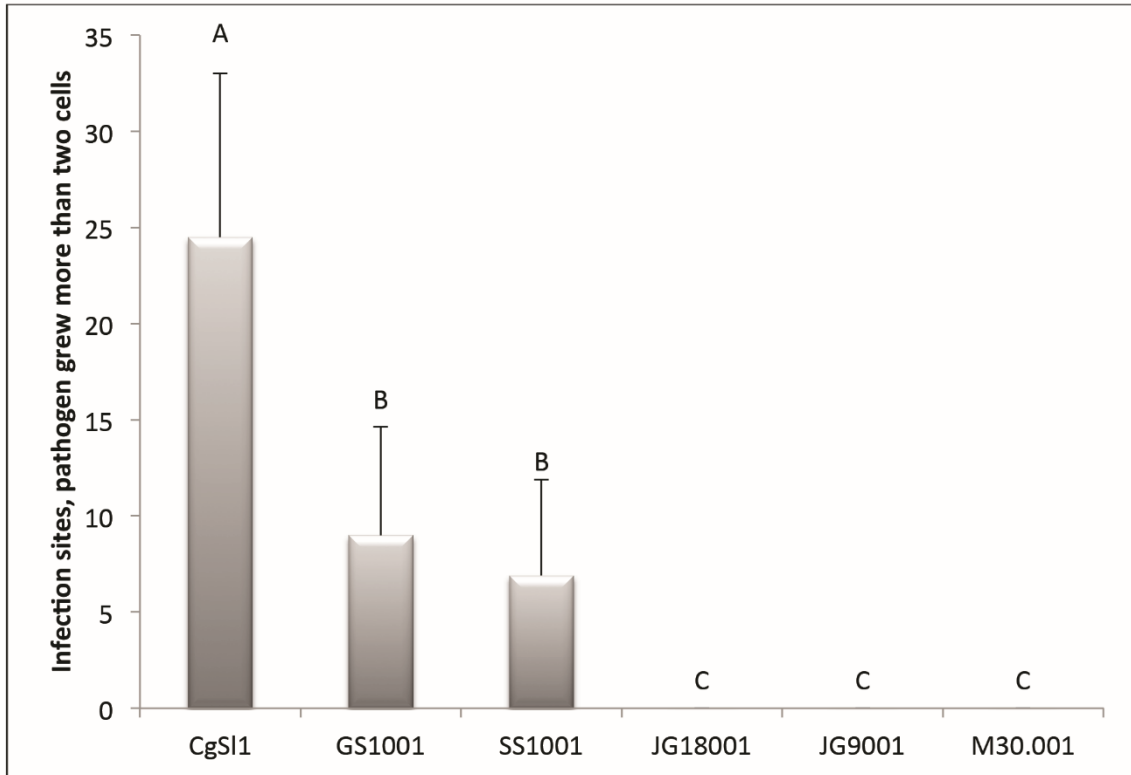


Figure 2.7. Leaf sheath assay comparing 6 fungal strains in each variety of Sorghum. Treatment means were compared among each other using Tukey's test.

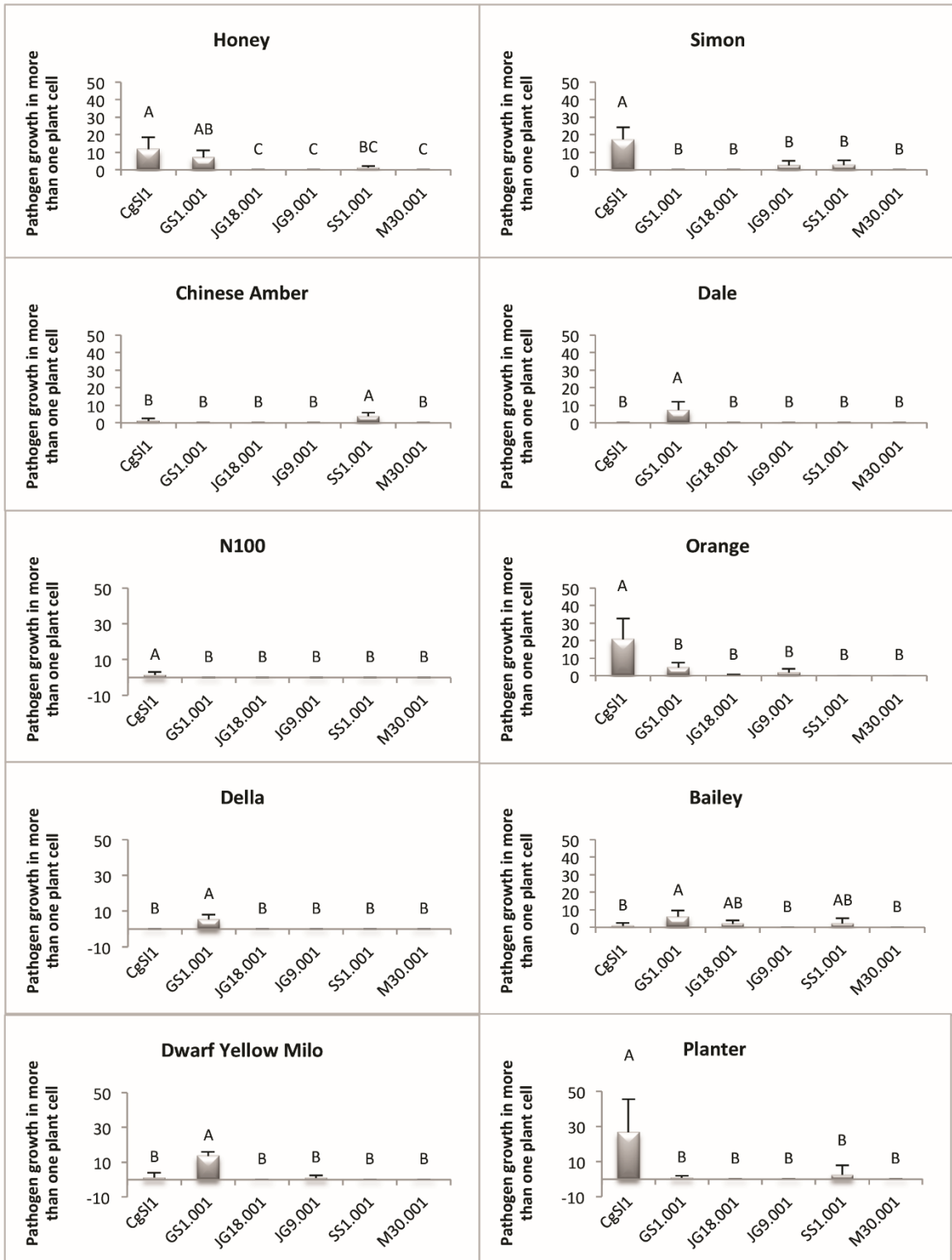


Figure 2.8. Pictures illustrating the plant damage caused by the weather (high temperature and excess of rain) at the beginning of the season in 2015.



### **Chapter 3 - Genetic and pathogenic diversity of *Colletotrichum* isolates from *Sorghum bicolor* and *S. halepense* in the Southeastern United States**

#### **Abstract**

Anthracnose caused by *Colletotrichum sublineola* is the most important disease of grain sorghum worldwide, and it is emerging as a significant production constraint for sweet sorghum grown in the southeastern United States. Anthracnose is also common on the wild sorghum relative, johnsongrass (*S. halepense*). Marker analysis using repetitive fingerprinting probes revealed that isolates from across the region were genetically highly diverse, with relatively few haplotypes that could be found in more than one location. With a few exceptions, the populations of strains recovered from cultivated sorghum were genetically distinct from the populations recovered from *S. halepense*. Nearly all of the isolates from cultivated sorghum were pathogenic to one or more of 13 genetically diverse lines of sorghum that were tested. A few of the isolates from *S. halepense* were also pathogenic to some of the lines of *S. bicolor*, while others caused no disease on any of the lines. The evidence suggested that cross-infection of sweet sorghum and johnsongrass by the same strains does occur, although relatively rarely, and this could be expected to complicate efforts to develop and deploy resistant sweet sorghum varieties in areas where johnsongrass is common.



## Introduction

Anthrachnose, caused by the fungus *Colletotrichum sublineola* Henn. ex Sacc. & Trotter (146), is the most important disease of sorghum (*Sorghum bicolor* (L.) Moench) worldwide (3, 29, 71, 92, 193). Anthracnose can reduce yields of grain sorghum by 50% or more under warm and humid conditions in a susceptible variety (3, 83, 171). *C. sublineola* can infect all parts of the plant including the stem, peduncle, panicle, grain, and leaf blades and midrib (68, 171). The primary control for anthracnose in grain sorghum is the use of resistant cultivars, although no cultivar is immune to the disease (28). Resistance frequently fails, and genotypes that provide high levels of resistance in one location often succumb to the disease in another. This is thought to be related to the high degree of genetic variability in the population of *C. sublineola* affecting grain sorghum (25, 27). Genetic diversity within the *C. sublineola* population on grain sorghum has been characterized by the use of various types of molecular fingerprinting markers, including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), and amplified fragment length polymorphisms (AFLP) (30, 135, 184).

Resistance to *C. sublineola* in sorghum is controlled mostly by single dominant genes, some with multiple allelic forms (14, 35, 104, 170). The ability of *C. sublineola* to infect a series of different cultivars of sorghum has been used to identify a large number of races in this species (3, 14, 30, 143, 184). The interaction between sorghum and *C. sublineola* was suggested to follow the gene-for-gene pattern (26). The durability of these major-gene sources of genetic resistance has been limited by the genetic and pathogenic diversity of the fungal population (30, 135).

Sweet sorghum has been grown in the southeastern U.S. for more than 150 years, but it has been on a limited scale, primarily for forage and for the production of syrup. During the Second World War, production was increased in the region to provide a substitute for imported cane sugar (87). Anthracnose disease became a serious problem in those plantations, which led to the development of a breeding program to produce resistant varieties (106). Although production of sweet sorghum in the southeast decreased dramatically after the war, it has continued to be an important specialty crop in Kentucky, which is presently one of the top producers of sorghum syrup in the U.S (12). Recently, interest in sweet sorghum as a potential source of biofuel has increased in the U.S., especially in the Gulf States, where infrastructure used for the sugar cane industry could be readily converted to the production of ethanol from sorghum juice and biomass (102). Anthracnose is listed as one of the most important diseases of sweet sorghum in Kentucky (13), and it is also common on the hybrids that are increasingly grown in the Gulf States for feedstock (80). If sweet sorghum acreages continue to increase in the southeastern U.S., anthracnose can be expected to grow in importance as a significant limiting factor.

*C. sublineola* is also reported to be a pathogen of the weed johnsongrass (*S. halepense*), which is related to *S. bicolor* (194), and which is ubiquitous across the southeastern U.S. Johnsongrass is known to be genetically highly diverse (120). This host diversity could lead to the selection of multiple *C. sublineola* pathotypes in the population, and thus johnsongrass could serve as a refuge and an incubator for genetic diversity in the pathogen. Rosewich found that isolates from johnsongrass belonged to a population that was distinct from the isolates from grain sorghum being grown in nearby fields in Texas, suggesting that cross-infection did not occur in that location (144). However, there is currently no

information about the population structure of *C. sublineola* on sweet sorghum and johnsongrass in the southeastern U.S.

My goal in this chapter was to characterize the degree of genetic and pathological diversity among the population of *Colletotrichum* on cultivated and wild sorghum in Kentucky and the southeastern U.S. This information will help to evaluate the potential for *Colletotrichum* to cause epidemics in sweet sorghum if it is more widely grown for biofuels in the region in the future. A second goal was to develop and evaluate different types of molecular markers for the evaluation of population diversity in *C. sublineola*.

## **Materials and Methods**

**Fungal strains:** A total of 400 fungal isolates were collected and archived in a permanent collection for this study (Supplemental Table 1). The isolates were recovered in 2011-2015 from sweet, forage, or grain sorghum (*S. bicolor*), as well as from johnsongrass (*S. halepense*), in Kentucky (KY), Georgia (GA), Alabama (AL), and Florida (FL). A few grain sorghum isolates from Indiana, Texas, Brazil, Burkina Faso, Zambia, South Africa, and Sudan, which were previously collected in the 1980s up through 2013, were also included in parts of the study (Supplemental Table 1).

Isolates were recovered from the margins of anthracnose lesions on leaves or stalks. Tissue pieces were soaked in 70% ethanol for 30 seconds, then 10% sodium hypochlorite (Clorox®) for two minutes, followed by three washes with sterile water. Disinfected tissue pieces were dried on sterile paper towels and placed on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) containing ampicillin (0.1 mg/ml). Cultures were incubated at 23°C under continuous fluorescent light. Isolates were purified by single-

sporling, and preserved on silica gel at  $-80^{\circ}\text{C}$  (179). Fungal strains were routinely cultured on PDA at  $23^{\circ}\text{C}$  under continuous fluorescent light.

**Genomic DNA extraction:** Fungal spores were harvested from 14-day-old PDA cultures and washed three times with sterile water. A volume of 10 ml of potato dextrose broth (PDB; Difco Laboratories, Detroit, MI, USA) was added to a 60 mm Petri plate, and inoculated with a spore suspension to the final concentration of  $5 \times 10^4$  spores/ml. Plates were incubated at  $23^{\circ}\text{C}$  under continuous fluorescent light for 7 days, after which mycelial mats were collected, frozen, and lyophilized. Genomic DNA was extracted following the protocol of (174).

**Random Amplified Polymorphic DNA (RAPD):** Genomic DNA of *Colletotrichum* strains was amplified by using the Random Amplified Polymorphic DNA (RAPD)-Polymerase Chain Reaction (PCR) method (201) with 10 different 10-base random primers (19, 181) (Table 3.1). Reactions were carried out in a final volume of  $25\mu\text{L}$  containing: 10 ng of genomic DNA, 10X PCR buffer, 50 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  dNTPs, 5  $\mu\text{M}$  of primer, and one unit (U) of *Taq* DNA polymerase. The PCR cycling protocol consisted of 3 cycles of; 1 min  $94^{\circ}\text{C}$  denaturation; 1 min annealing at  $35^{\circ}\text{C}$ ; 2 min for extension at  $72^{\circ}\text{C}$ : followed by 34 cycles of; 1 min denaturation at  $94^{\circ}\text{C}$ ; 20 sec annealing at  $40^{\circ}\text{C}$ ; and 2 min extension at  $72^{\circ}\text{C}$ : and with a final extension of  $72^{\circ}\text{C}$  for 10 min. Amplicons were resolved on 1.0% agarose gels, stained with ethidium bromide, and visualized under UV light.

**RFLP fingerprints with transposon probes:** Proteins containing predicted transposase domains were identified by using the Protein Family Database (Pfam) to screen the predicted proteome of *C. sublineola* strain CgS11 (see chapter 4 of this dissertation). A putative transposase sequence was identified that was very similar to JMSE01000036, a

predicted transposase sequence from the *C. sublineola* isolate CsTX430BB (Figure 1). The reverse complement of the CgS11 sequence was used to design the transposase primers CsTn1F and CsTn1R (Table 1, figure 1). Genomic DNA from strain CgS11 was used as a template for the PCR. PCR conditions consisted of 3 min of denaturation at 94°C; followed by 35 cycles of denaturation for 30 sec at 94°C, 60 sec of annealing at 57°C, and 40 seconds of extension at 72°C; and a final extension of 10 min at 72°C. The resulting amplicon was sequenced (Figure 1) and submitted to Genbank as CsTn1 (accession number KY006076). The amplicon was labeled with <sup>32</sup>P to produce a probe for Southern hybridization. The Southern blot was performed by using the protocol of (174). Briefly, one µg of the genomic DNA from each fungal strain was digested with the restriction enzyme *HindIII*. The digested DNA was separated on a 0.7% agarose gel and transferred to a positively charged nylon membrane. The membrane was cross-linked with UV light, and probed with the radioactively labeled probes.

**RFLP fingerprints with telomere probes:** Telomere probes were produced by using a published protocol (65). The resulting amplicons were gel-purified and labeled with <sup>32</sup>P. Conditions for doing the Southern blot were as described above.

**Identification and characterization of haplotypes:** Strains with 100% (RFLP), or at least 95% (RAPD) identical band patterns in each case were visually identified as members of the same haplotype (Supplemental table 1). Polymorphic RAPD bands of 84 representative isolates were scored as presence (1) or absence (0), then Jaccard's coefficients (91) were applied to the data by using the Statistical Analysis Software (SAS) package, in order to group the isolates based on the overall similarity of the band patterns.

**Pathogenicity assay:** Isolates representing 34 different haplotypes (based on the fingerprinting analyses) from a range of hosts and locations were tested for pathogenicity against the sweet sorghum variety Sugar Drip in the greenhouse (Table 3.2). The experiment consisted of two sets of experiments. The first set was repeated three times (summer and fall of 2014; and summer 2016) and the second one was repeated twice (July and August, 2016). One isolate from maize was used as a negative control: maize isolates do not infect healthy sorghum plants (175). A subset of these isolates, plus one additional representative, (Table 3.2) was also tested on 12 additional sorghum varieties including: Honey, Simon, Bailey, Chinese Amber, Dale, Dwarf Yellow Milo, Cowper, Orange, Planter, N100, Della, and Keller. Sugar Drip seeds were obtained from Dr. Todd Pfeiffer at the University of Kentucky. Seeds of the other varieties were obtained from Dr. Gary Pederson at the Plant Genetic Resources Conservation Unit in Griffin GA. All seed stocks were increased in 2012 at the UK Agronomy Farm.

The fungal strains were cultured on PDA at 23°C under continuous fluorescent light for two weeks. Production of inoculum and disease assessment methods were done according to a modified method of Prom and colleagues (136). Spores were harvested from two-week-old cultures by adding 10 mL of sterile water to each plate and loosening the spores with a mini-pestle. The spores were collected and washed three times with sterile water to remove the mucilage. Finally, a spore suspension ( $5 \times 10^6$  spores per mL) was used to inoculate 40 grams of autoclaved Sugar Drip sorghum grains in a Petri plate at a final concentration of  $5 \times 10^6$  spores per gram. Grains were previously prepared for inoculation by washing them, soaking them in water for 48 hours, and then autoclaving twice (121°C/30 minutes). The inoculated plates were incubated for 14 days at 23°C in the dark.

The pathogenicity experiments were performed in the greenhouse in pots containing four plants, which were arranged in a randomized complete block design (RCBD) with four blocks, each experimental unit or treatment including eight plants per block. Inoculations of Sugar Drip were repeated three times; inoculations of the other 12 lines were repeated twice. To inoculate the plants, ten colonized grains were dropped into the leaf whorl.

Disease symptoms were evaluated weekly, beginning 14 days after inoculation (dpi), for a period of four weeks. A rating scale with values ranging from 0-5 was used (22, 25, 184). On this scale, 0 indicates no symptoms; 1 is small necrotic dots on the leaves; 2 is small red spots on the leaves; 3 is necrotic lesions without acervuli; 4 is necrotic lesions with acervuli in the centers of the lesions; and 5 is coalescent necrotic lesions and abundant production of acervuli. Two types of interaction were considered, (i) incompatible, including values of 0, 1, 2, and 3; and (ii) compatible, including values of 4 and 5. The disease evaluation was performed based on incidence, expressed as the number of infected plants (compatible interactions).

Incidence values from the combined data sets from the repetitions of each experiment were used to calculate the area under the disease progress curve (AUDPC) by the trapezoidal integration method (20). The AUDPC data were subjected to analysis of variance (ANOVA) after square root transformation before further analysis, to stabilize the variance. Treatment means were compared with the negative control (M30.001) using the Dunnett's test procedure of the SAS software package (SAS Users Guide, SAS Institute, Cary, NC). In addition, the data for the isolates from *S. bicolor* versus *S. halepense* on Sugar Drip were grouped, and the group means were compared to one another by using the general linear models (GLM) procedure of SAS (SAS Users Guide, SAS Institute, Cary, NC). Means

were separated with least squares estimates of marginal means (LSMEANS statement). P-values less than 0.05 were considered significant. The analyses were performed using SAS version 9.4.

## **Results**

### **Molecular marker analysis**

RAPD analyses: Strains that shared the same band patterns for all ten primers were considered to belong to the same haplotype. A total of 286 haplotypes were identified among 371 strains that were evaluated by amplification with the RAPD primers (Supplemental table 1). These included 176 haplotypes among the 221 isolates from sweet sorghum; 80 haplotypes among the 91 isolates from johnsongrass; 11 haplotypes among the 16 grain sorghum isolates; and 22 haplotypes among the 42 forage sorghum isolates. Forty-one of the haplotypes encompassed more than one strain (Supplemental table 1); the rest were represented by only a single strain each. Only three of the haplotypes were found on more than one host type (RAPD haplotypes 14, 15, and 16, recovered from both sweet and forage sorghum in AL). With only one exception (RAPD haplotype 17, found in two counties in AL), each RAPD haplotype was recovered from only a single location.

RFLP analyses, transposon probe: A total of 386 isolates were analyzed with the transposon probe. The probe hybridized to multiple polymorphic restriction fragments in all of these isolates. However, hybridization to the johnsongrass and maize isolates was generally weaker, and fewer fragments were recognized, compared with the isolates from forage, sweet, or grain sorghum. Representative results are shown in Figure 3.2 A. A total of 61 different haplotypes were identified with this probe (Supplemental table 1). These included 32 haplotypes among the 229 isolates from sweet sorghum; 15 haplotypes among



the 89 isolates from johnsongrass; 15 haplotypes among the 18 grain sorghum isolates; and 3 haplotypes among the 49 forage sorghum isolates.

RFLP analyses, telomere probe: A total of 386 isolates were analyzed with the telomere probe. The telomere probe identified a total of 49 different haplotypes (Supplemental table 1). These included 5 haplotypes among the 229 isolates from sweet sorghum; 33 haplotypes among the 89 isolates from johnsongrass; 12 haplotypes among the 18 grain sorghum isolates; and 1 haplotype among the 49 forage sorghum isolates.

Most of the johnsongrass isolates had diverse patterns of hybridization, consisting of between 4 and 36 individual bands (Figure 3.2 B, Supplemental table 1). For the grain sorghum isolates, the probe hybridized to between one and 24 fragments. None of the patterns of multiple hybridizing fragments matched those from the johnsongrass isolates. Most of the isolates from forage and sweet sorghum had just one major band of similar size when hybridized with the telomere probe, (Figure 3.2 B, Supplemental table 1). For this reason, telomere fingerprinting was not as useful for differentiating among sweet and forage sorghum strains. However it could be used to distinguish between two groups, one mostly from johnsongrass and grain sorghum, and the other mostly from sweet and forage sorghum. There were exceptions to this rule including seven grain sorghum isolates, and three isolates from johnsongrass (JG35.001, JG86.001 and JG88.001), that had the single band, and seven isolates from sweet sorghum representing three haplotypes that had multiple telomere bands. Although these three sweet sorghum haplotypes were relatively rare, they were also cosmopolitan, with representatives from KY, FL, and GA (Supplemental table 1).

### **Comparisons among the marker types**

All three types of markers were evaluated, and the results were compared, for 360 representative isolates (Table 3.3). The RAPD fingerprints included the largest number of polymorphic bands, and as a result differentiated the largest number of haplotypes overall (Table 3.3). Most of these haplotypes were represented by only a single strain. Among the 37 RAPD haplotypes represented by multiple strains (a total of 114 strains that could be evaluated), most were consistent with the telomere and transposon haplotypes of the strains. Only 15 strains, in eight different RAPD haplotypes, were not consistent (Supplemental table 1).

Among the 48 telomere haplotypes, only two included more than one transposon haplotype. One of these was type 33, which had only a single major hybridizing band and included the majority of sweet sorghum and forage sorghum isolates. Among the 59 transposon haplotypes, only seven included more than one telomere haplotype, and only four strains within these seven had telomere haplotypes that were also found in combination with other transposon haplotypes. Thus, the vast majority of strains were consistent for the transposon and telomere haplotypes. Each of the 360 isolates was classified into a group based on the combined transposon-telomere haplotype (Supplemental table 1). The total number of combined haplotypes was 83.

Four cosmopolitan combined haplotypes were found in more than one state. The most common of these was 17:33 (Figure 3.3, Supplemental table 1), which included 80 isolates from sweet sorghum and 13 from forage sorghum (Supplemental table 1), and was found in all four states (KY, AL, GA, and FL) (Figure 3.3, Supplemental table 1). Haplotype 17:33 also included two isolates from KY johnsongrass, and two isolates from grain sorghum, one from Texas and one from South Africa (Figure 3.3, Supplemental table 1).

The two johnsongrass isolates represented the only cases in which the same combined haplotype was found on both *S. bicolor* and *S. halepense*. Two other haplotypes (19:33 and 21:33) were found on both forage and sweet sorghum in GA, FL, and AL. The remaining 80 haplotypes were confined to only a single host type, and 76 were found in only a single location.

Among the johnsongrass isolates, 35 different combined haplotypes could be discerned (Table 3.4, Supplemental table 1). There was a relatively high degree of diversity (richness) in most locations, with even isolates from same plant sometimes belonging to several different haplotypes. Nearly all of the haplotypes were found in only a single location (Figure 3.3). However, there were four combined haplotypes that were more widely dispersed in Kentucky, including haplotypes 42:60 and 44:62, found in both Fayette and Franklin counties (Figure 3.3, Supplemental table 1). None of these more widely dispersed haplotypes was found outside KY.

A cluster analysis of the RAPD fingerprint markers for 84 strains representing the 80 different combined haplotypes revealed that the clusters were mostly consistent with host (*S. bicolor*, highlighted in red in Figure 3.4, versus *S. halepense*, highlighted in gray) and with geographic origin. There were a few exceptions. Thus, strain SS1.001 from Kentucky sweet sorghum clustered with isolates from *S. halepense*, while two KY johnsongrass isolates (JG14.001 and JG16.001) were grouped together with isolates from *S. bicolor*. Several Brazilian grain sorghum isolates clustered together with a Sudanese grain sorghum isolate and several sweet sorghum isolates from Florida (Figure 3.4 G). Two other grain sorghum isolates (from Burkina Faso and Indiana) were grouped with the two atypical KY johnsongrass isolates (Figure 3.4 E). Forage sorghum isolates formed a discreet cluster

within the larger cluster of isolates from *S. bicolor* (Figure 3.4 F). A few isolates, e.g. JG1.001 from johnsongrass in Indiana and GS1.001 from grain sorghum in Kentucky, seemed to be relatively unique and didn't cluster closely with any other strains.

### **Pathogenicity assays**

Thirty four representative isolates from johnsongrass, sweet, grain, and forage sorghum were chosen for pathogenicity tests against the susceptible sweet sorghum inbred Sugar Drip (Table 3.2). All the isolates were compared with the negative control, the non-pathogenic maize isolate M30.001. Most of the isolates were able to cause disease (Figures 3.5-3.7). Six JG isolates (JG2.001, JG9.001, JG18.001, JG62.001, JG75.001 and JG102.001), one forage sorghum isolate (FS34.001), one grain sorghum isolate (TX430BB), and two sweet sorghum isolates (SS41.001 and SS100.001), did not differ statistically from the negative control (M30.001) (Figure 3.5-3.7). The other isolates caused moderate to high levels of disease on Sugar Drip in the greenhouse (Figures 3.5-3.7). Most of these isolates were from cultivated sorghum; however there were five johnsongrass isolates (JG7.001, JG34.001, JG42.001, JG86001 and JG88.001), all from Kentucky, that were also pathogenic to Sugar Drip (3.5-3.7).

A statistical analysis comparing aggressiveness of isolates from different hosts revealed that the johnsongrass isolates, as a group, were statistically less aggressive to Sugar Drip than isolates from sweet and forage sorghum ( $p=0.05$ ).

One additional isolate from sweet sorghum (Table 3.2) and a representative subset of the original 34 isolates were tested on 12 additional sorghum lines in greenhouse assays. Results revealed that most of the johnsongrass isolates could not cause disease on any of the sorghum lines (Figure 3.8-3.11). The exception was JG88.001, which was pathogenic

on Honey, Simon, Bailey, Chinese Amber, and Dale. The ability of the pathogenic isolates to cause disease depended on the sorghum line (Figure 3.12). Honey and Simon were susceptible to most of the isolates from *S. bicolor* that were tested, as well as to JG88.001. On the other hand, Della, N100, and Keller were resistant to all of the isolates. Similarly some isolates from *S. bicolor*, e.g. SS63.001, were pathogenic to most of the sorghum lines tested, while others were pathogenic to only a few (e.g. SS240.001). Based on the results of the inoculations performed on the 12 varieties listed above, and on 11 additional varieties inoculated only with SS1.001 and CgSI1 that I have not shown here, I have added colors to the pedigree map to identify the varieties that were susceptible to at least one isolate of *Colletotrichum* versus those that were resistant to all the isolates tested (Figure 3.12).

## **Discussion**

This work revealed that the population of *Colletotrichum* causing lesions on *Sorghum* spp. in the southeastern U.S. is genetically diverse, and comprised mostly of local, genetically distinct sub-populations. Previous studies have suggested that clonal reproduction is of primary importance in population structure of *C. sublineola* on grain sorghum (72, 144). However, results of RAPD analysis suggested that most strains in the current study were not clonal, even when they came from the same fields or the same plants. Although RAPD markers have been widely used to evaluate *C. sublineola* populations (24, 26, 69, 72, 78, 172, 181, 184), they have become less popular as they have developed a reputation for poor reproducibility (72, 144), and as other PCR-based methods (e.g. AFLP) have become available. I found that RAPD groupings based on ten different random primers correlated very well with groups identified by RFLP markers, but they detected about three times as many haplotypes, probably because they identified more polymorphic bands (Table 3.2).

The RAPDs were convenient because they required relatively little DNA and were PCR-based rather than hybridization based. However they were challenging to develop and validate because they had to be carefully screened for reproducibility. I found that only the major bands were reliable and should be included in the analysis. By their nature, PCR-based methods like RAPD and SSR are less stringent than RFLP because band size is the only criterion used for marker identification. It is possible that some bands of similar size actually represent different markers, thus genetic diversity may be underestimated. Use of multiple RAPD primers can alleviate this concern when identifying haplotypes, and given the high degree of reproducibility and correlation with other markers in this study, I conclude that the RAPD markers added useful information because of their greater power to differentiate among individual clones, and the fact that they were equally informative for all strains.

RFLP uses a combination of band size and sequence homology to identify markers, thus providing more reproducibility and reliability than PCR-based methods. I developed and used two repetitive primers, one based on transposon sequences and the other on subtelomeric sequences that often vary among fungal strains e.g. (2, 66, 109, 147). Comparing the RFLP marker types I saw that the telomere markers overall allowed identification of 49 haplotypes, as compared with 61 for the transposon marker. However, the telomere markers were less informative for a relatively large group of isolates, nearly all from sweet and forage sorghum, which had only a single major telomere band of a similar size. Use of several different restriction enzymes in the Southern blots did not change the result. Presumably this group (group 33) has very little diversity of different telomere ends within their genomes. Within the 275 members of group 33, 34 haplotypes

could be identified by the addition of the transposon probe. The transposon probe on the other hand was less useful for isolates from johnsongrass, which generally hybridized poorly to this probe, suggesting that they may contain a different related group of transposons. Both probes together provided the most reliable differentiation of all of the strains, and yielded a total of 80 combined haplotypes across the southeast. Most of these were confined to a single location, confirming the RAPD results suggesting high levels of local isolation. A few were more cosmopolitan, with the most common combined haplotypes being 17:33, mostly from sweet sorghum across the southeast, 19:33, from sweet and forage sorghum in AL, FL, and GA, and 21:33, mostly from forage sorghum in AL, but also including a few representatives from GA on sweet sorghum.

RAPD markers provided similar amounts of information for all isolates (in contrast with the telomere and transposon RFLPs, which were more informative for one group versus another). Thus, I used the RAPD markers for an analysis of similarity among strains that were selected to represent all of the combined haplotypes. Results of the cluster analysis suggested that populations from johnsongrass were mostly distinct from those on cultivated sorghum, with a few exceptions. Cluster analysis also indicated that isolates from sweet, forage, and grain sorghum were not separate, suggesting that cross-infection within the cultivated sorghum group is common. This conclusion was also supported by pathogenicity assays, in which most isolates from grain, forage, and sweet sorghum were able to cause disease in Sugar Drip. One grain sorghum isolate, TX430BB was not pathogenic. This isolate was recently subjected to genome sequencing (8). When I tested this isolate on the susceptible line Della, it was able to cause lesions (not shown). Results of this and my other inoculations of multiple strains with multiple isolates provided evidence for significant

race structure in the population on sweet sorghum in the SE, as is well known for this pathogen on grain sorghum (25, 27). The presence of races is expected to complicate the task of plant breeders as they develop and deploy new sweet sorghum germplasm in the region in the future.

Considering the two major host families, johnsongrass (*S. halepense*), and cultivated sorghum (*S. bicolor*), the haplotypes recovered from one did not usually appear among the isolates from the other, even when the isolations were made from the same locations. Two johnsongrass isolates (JG86.001 and JG88.001) were rare exceptions to this rule. Both belonged to haplotype 17:33, which was common on sweet and forage sorghum across the region, and was also represented by two grain-sorghum isolates. The two johnsongrass isolates belonging to haplotype 17:33 came from different plants that were in the rows between infected sweet sorghum in Morgan County KY. More than 50 isolates from two varieties of sweet sorghum in those fields also belonged to combined haplotype 17:33, showing that this population was capable of cross-infection of the isolated johnsongrass plants as well as the sweet sorghum. Although they shared the same combined haplotype, these isolates from johnsongrass represented unique RAPD haplotypes and this suggests they were not clonal with one another or with other strains from sweet sorghum plants nearby. Results from pathogenicity assays also suggested the possibility for cross-infection by some, but not all isolates. Four isolates from Kentucky, including JG88.001, showed significant potential for infection of Sugar Drip sorghum, although the majority of isolates from johnsongrass did not differ from the negative controls on Sugar Drip or on 12 other genetically diverse sorghum genotypes. Although cross-infection events seem to be



relatively rare, the potential exists nonetheless for johnsongrass to serve as a refuge and a source of inoculum for the pathogen in sweet sorghum fields under rotation conditions.

Some of the same strains that were tested in this chapter on whole plants were also tested in sheath assays on those same plant varieties in chapter 2. In general, results of the two assays were consistent for most of the strains. The notable exception was strain SS1.001, which was pathogenic on Honey, Simon, Bailey, and Planter in whole plant assays but didn't differ from the negative control in sheath assays involving those plant varieties. One possibility is that SS1.001, although overall a highly aggressive strain, may be a "slow starter". If I had waited longer than 60 hours to evaluate the sheath assay, it may have been able to colonize the sheaths effectively. Alternatively, it may react differently compared with the other strains to the different nutritional environments of the sheaths versus the leaf blades (sheaths are sink tissues while leaf blades are source tissues). Whatever the explanation, this demonstrates that the sheath assay should not be relied upon to give accurate predictions of field pathogenicity for all strains on all varieties.

Overall it appeared that there was a higher level of haplotype diversity (richness) among the isolates from johnsongrass than among the isolates from *S. bicolor*. This seems reasonable given the higher level of genetic diversity among the wild johnsongrass versus the domesticated sorghum monocultures. The theory is that the former would promote selection of a variety of genotypes within the pathogen population, while the latter would promote selection of a few individual genotypes. The exception seemed to be the isolates from grain sorghum, which displayed a very high level of richness. Other studies of diversity within grain sorghum populations have also suggested high levels of diversity (30, 135, 184). However, my results may have been an artifact of the fact that these isolates

have been collected across many decades and from different continents. The randomness of sampling and the sample size have a big impact on these measures of diversity. Now that I have developed and characterized molecular fingerprinting tools, these can be applied to additional isolates sampled at various levels in the future. Overall this work has revealed that the pathogen in the region has significant genetic potential to overcome individual resistance genes and may pose a serious threat to widespread sorghum monoculture in the future.

### **Summary**

The predictions for chapter 3 were: (1) isolates from sweet sorghum will resemble those on grain and forage sorghum more than they will resemble isolates from johnsongrass; (2) the population of strains recovered from johnsongrass will be more diverse genetically than the population recovered from cultivated sorghum; and (3) isolates from different hosts will vary in their virulence and aggressiveness to sweet sorghum varieties. Predictions 1 and 2 were upheld, although sampling is likely to be a significant complicating factor in my estimates of diversity. It will be important in future to increase the sampling depth in individual locations to properly address this question. Prediction 3 was not upheld. There appeared to be no difference in the ability of isolates from grain, forage, or sweet sorghum to infect Sugar Drip sweet sorghum in the greenhouse. And, although a majority of isolates from johnsongrass were not pathogenic to sweet sorghum, this was not true for all isolates from johnsongrass.

Table 3.1. RAPD and transposon primers used for fingerprint analyses.

| Primer  | Sequences of Primers     | Reference                  |
|---------|--------------------------|----------------------------|
| OPA 1   | 5'CAGGCCCTTC3'           | Vaillancourt & Hanau, 1992 |
| OPA 2   | 5'TGCCGAGCTG3'           | Vaillancourt & Hanau, 1992 |
| OPA 3   | 5'AGTCAGCCAC3'           | Vaillancourt & Hanau, 1992 |
| UBC 356 | 5'GCGGCCCTCT3'           | Browning et al, 1999       |
| UBC 384 | 5'TGCGCCGCTA3'           | Browning et al, 1999       |
| UBC 391 | 5'GCGAACCTCG3'           | Browning et al, 1999       |
| UBC 357 | 5'AGGCCAAATG3'           | Browning et al, 1999       |
| UBC 345 | 5'GCGTGACCCG3'           | Browning et al, 1999       |
| OPA 11  | 5'CAATCGCCGT3'           | This Publication           |
| OPA 18  | 5'AGGTGACCGT3'           | This Publication           |
| CsTn1F  | 5'AATCCAGGCTGATCTTGGGC3' | This Publication           |
| CsTn1R  | 5'CCTGGCGAGCCTTCTGATAG3' | This Publication           |

Table 3.2. The strains of *Colletotrichum* chosen for the pathogenicity assays.

| Strain        | Host           | State    | County      | Year | Haplotype  |          |      |
|---------------|----------------|----------|-------------|------|------------|----------|------|
|               |                |          |             |      | Transposon | Telomere | RAPD |
| CgS11         | Grain sorghum  | Indiana  | Tippecanoe  | 1975 | 98         | 110      | 300  |
| CsTX430BB     | Grain sorghum  | Texas    | Brazos      | 1975 | 17         | 33       | 33   |
| FS13.001      | Forage sorghum | Alabama  | Brewton     | 2013 | 17         | 33       | 13   |
| FS34.001      | Forage sorghum | Alabama  | Macon       | 2013 | 19         | 33       | 12   |
| FS37.001      | Forage sorghum | Alabama  | Macon       | 2013 | 19         | 33       | 18   |
| FS41.001      | Forage sorghum | Alabama  | Cullman     | 2013 | 17         | 33       | 17   |
| FS5.001       | Forage sorghum | Alabama  | Brewton     | 2013 | 21         | 33       | 9    |
| FS8.001       | Forage sorghum | Alabama  | Brewton     | 2013 | 21         | 33       | 8    |
| GS1001        | Grain sorghum  | Kentucky | Hopkins     | 2013 | 99         | 108      | 298  |
| JG102.001     | johnsongrass   | Georgia  | Tift County | 2014 | 50         | 84       | 54   |
| JG18.001      | johnsongrass   | Kentucky | Fayette     | 2012 | 43         | 61       | 49   |
| JG2.001       | johnsongrass   | Kentucky | Russell     | 2011 | 38         | 53       | 42   |
| JG9.001       | johnsongrass   | Kentucky | Russell     | 2012 | 38         | 57       | 41   |
| JG62.001      | johnsongrass   | Kentucky | Russell     | 2013 | 38         | 73       | 71   |
| JG34.001      | johnsongrass   | Kentucky | Frankfort   | 2013 | 42         | 60       | 109  |
| JG42.001      | johnsongrass   | Kentucky | Fayette     | 2013 | 42         | 60       | 35   |
| JG7.001       | johnsongrass   | Kentucky | Russell     | 2011 | 39         | 56       | 47   |
| JG75.001      | johnsongrass   | Florida  | Gadsden     | 2014 | 47         | 78       | 61   |
| JG86.001      | johnsongrass   | Kentucky | Montgomery  | 2014 | 17         | 33       | 101  |
| JG88.001      | johnsongrass   | Kentucky | Montgomery  | 2014 | 17         | 33       | 102  |
| M30.001       | Maize          | Kentucky | Fayette     | 2012 | 101        | 112      | 32   |
| Sweet sorghum |                | Kentucky | Fayette     | 2012 | 5          | 34       | 301  |

Continue Table 3.2.

| Strain    | Host          | State    | County      | Year | Haplotype  |          |      |
|-----------|---------------|----------|-------------|------|------------|----------|------|
|           |               |          |             |      | Transposon | Telomere | RAPD |
| SS100.001 | Sweet sorghum | Alabama  | Macon       | 2013 | 17         | 33       | 14   |
| SS107.001 | Sweet sorghum | Georgia  | Tift County | 2013 | 24         | 33       | 296  |
| SS124.001 | Sweet sorghum | Georgia  | Tift County | 2013 | 29         | 33       | 208  |
| SS159.001 | Sweet sorghum | Georgia  | Tift County | 2013 | 19         | 33       | 213  |
| SS204.001 | johnsongrass  | Kentucky | Montgomery  | 2014 | 17         | 33       | 274  |
| SS240.001 | johnsongrass  | Georgia  | Tift County | 2014 | 19         | 33       | 225  |
| SS26.001  | Sweet sorghum | Florida  | Live Oak    | 2013 | 1          | 33       | 159  |
| SS29.001  | Sweet sorghum | Florida  | Live Oak    | 2013 | 1          | 33       | 166  |
| SS41.001  | Sweet sorghum | Florida  | Live Oak    | 2013 | 1          | 33       | 150  |
| SS45.001  | Sweet sorghum | Florida  | Live Oak    | 2013 | 1          | 33       | 148  |
| SS63.001  | Sweet sorghum | Florida  | Live Oak    | 2013 | 4          | 33       | 178  |
| SS78.001  | Sweet sorghum | Alabama  | Brewton     | 2013 | 17         | 33       | 16   |

Table 3.3. Number of haplotypes identified among 360 strains isolated from johnsongrass, sweet, forage, and grain sorghum by the number of haplotypes identified upon hybridization with the telomere and transposon probes. \*The forage sorghum strains all had the same, single band that hybridized with the telomere probe, thus this probe did not reveal any polymorphisms among this group of strains.

| Host           | Number of individuals | Number of locations | Probes     | Polymorphic Bands | Individual Haplotypes |
|----------------|-----------------------|---------------------|------------|-------------------|-----------------------|
| Johnsongrass   | 86                    | 7                   | RAPD       | 1388              | 79                    |
|                |                       |                     | Telomere   | 729               | 33                    |
|                |                       |                     | Transposon | 228               | 15                    |
| Sweet sorghum  | 225                   | 6                   | RAPD       | 4035              | 173                   |
|                |                       |                     | Telomere   | 44                | 5                     |
|                |                       |                     | Transposon | 593               | 31                    |
| Forage sorghum | 42                    | 3                   | RAPD       | 391               | 22                    |
|                |                       |                     | Telomere   | 0*                | 1                     |
|                |                       |                     | Transposon | 66                | 3                     |
| Grain sorghum  | 13                    | 7                   | RAPD       | 391               | 11                    |
|                |                       |                     | Telomere   | 99                | 11                    |
|                |                       |                     | Transposon | 266               | 14                    |

Table 3.4. Combined haplotype richness by host identified from isolates collected in the southeastern US.

| Host           | Total number of individuals | Location (number of individuals) | Haplotypes* | Total number of Haplotypes*<br>Haplotype Richness ** |
|----------------|-----------------------------|----------------------------------|-------------|--|
| Johnsongrass   | 89                          | Fayette KY (18)                  | 7           | 35<br>0.39   |
|                |                             | Franklin KY (13)                 | 6           |  |
|                |                             | Russell KY (35)                  | 14          |  |
|                |                             | Montgomery KY (2)                | 1           |  |
|                |                             | Macon AL (7)                     | 1           |  |
|                |                             | Tift GA (6)                      | 2           |  |
|                |                             | Gadson FL (7)                    | 6           |  |
|                |                             | Tippecanoe IN (1)                | 1           |  |
| Sweet sorghum  | 229                         | Brewton AL (24)                  | 1           | 35<br>0.15   |
|                |                             | Macon AL (8)                     | 1           |  |
|                |                             | Tift GA (67)                     | 17          |  |
|                |                             | Live Oak FL (77)                 | 18          |  |
|                |                             | Gainesville FL (1)               | 1           |  |
|                |                             | Montgomery KY (51)               | 1           |  |
|                |                             | Fayette KY (1)                   | 1           |  |
| Forage sorghum | 49                          | Brewton AL (29)                  | 3           | 3<br>0.06  |
|                |                             | Cullman AL (8)                   | 2           |  |
|                |                             | Macon AL (12)                    | 2           |  |
| Grain sorghum  | 17                          | North America (3)                | 2           | 16<br>0.89   |
|                |                             | South America (9)                | 8           |  |
|                |                             | Africa (5)                       | 5           |  |

\* Total number of haplotypes based on the combined haplotypes identified upon hybridization with telomere and transposon probes.

\*\*Haplotype richness is the number of haplotypes divided by the number of individuals.

Figure 3.1. (A) Transposon probe (CsTn1, accession number KY006076) sequence including the forward and reverse primers highlighted in yellow. (B) Similarity of the putative transposase from TX430BB toCsTn1 peptide sequence.

```
>Cstn1
AATCCAGGCTGATCTTGGGCCTCCCTCCAACCCATCAGCAAGTGAGGGAGTTCGCCCAGCGTAT
CCTCTGTATAATGGGTGATCATAAGCCTCTAGGCAAGCGATGGATGGATGGCTTCCTGCGAAG
ATACCCCTCTATCAAGGTCCAGAGAAGTCGTTCTATTGATTCGGGGCGTATTAATGGAGCATC
TACTGAGGTTATTAGGGAATGGTTTAAATACCTCGTGATGCCTCACATTGTTGCCATTA AAC
AGCCAACCGATAACAACATGGATGAGACTGGTATCCTTGAGGGCAAGGGAGATAATGGGCTAG
TGCTGGGCAGAGCTGAGACTAAGTCAGTGAGGAAAAAGCAGCCTGGATCACGAGCCTGGGTA
TCTATCATCGAGTGTATCTCAGCTGAGGGCAAGGCACCTCATCCACTGGTTATATATAAGGGC
AAGACGGTCCAGCAGCAGTGGTTTCTCTAGATCTTGCCCTCATGATGGATGGGAGTTTACA
GCAACAGAGAATGGATGGACTACTGATGATACTGCTGTTGAGTGGTTGACGAGAGTGTTC
CCTCAGTCTCAGCCTTGCCAGCCTTGCCAGCCTTGCCAGCCTGATCAGGCAAGACTACTTATCC
TGGATGGGCATGGGAGCCATAACAACACTGATTTTATGTGGTTATGCTATAACCATAACGTTT
ATCTGTTATTCTGCCTCCACATACCTCCCATGTCCTCCAGCCACTAGATCAGTCAGTCTTTAG
CCCTGTGAAGTCTGCTTATCGCAAGGAGCTTGGATATCTCAGCCTTTGGAATGACTCTACTATT
ATCGGCAAAAAGGAACCTTTATTTCTGCTATCAGAAAGGCTCGCCAG
```

Putative transposase [Colletotrichum sublineola]  
 Sequence ID: KDN72148.1 Length: 538 Number of Matches: 1  
 Related Information  
 Range 1: 70 to 351 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

| Score                 | Expect     | Method  | Identities          | Positives           | Gaps             | Frame     |
|-----------------------|------------|---|---------------------|---------------------|------------------|-----------|
| <b>540 bits(1390)</b> | <b>0.0</b> | <b>Compositional matrix adjust.</b>                           | <b>266/288(92%)</b> | <b>271/288(94%)</b> | <b>6/288(2%)</b> | <b>+2</b> |
| Query                 | 2          | IQADLGLPPTHQQVREFAQRILCIMGDHKPLGKRWMDGFLRRYPSIKVQRSRSIDSGRIN  |                     |                     |                  | 181       |
|                       |            | IQADLGLPPTHQQVREFAQRIL MGDHKPLGKRWMDGFLRR PSIKVQRSRSIDS RIN   |                     |                     |                  |           |
| Sbjct                 | 70         | IQADLGLPPTHQQVREFAQRILRAMGDHKPLGKRWMDGFLRRNPSIKVQRSRSIDSRIN   |                     |                     |                  | 129       |
| Query                 | 182        | GASTEVIREFWKYLVMPHIVAIPANRYNMDETGILEGKGDNGLVLGRAETKSVRKKQPG   |                     |                     |                  | 361       |
|                       |            | GASTEVIREFWKYLVMPHI AIKPANRYNMDETGILEGKGDNGLVLGRAETKSVRKKQPG  |                     |                     |                  |           |
| Sbjct                 | 130        | GASTEVIREFWKYLVMPHIAAIKPANRYNMDETGILEGKGDNGLVLGRAETKSVRKKQPG  |                     |                     |                  | 189       |
| Query                 | 362        | SRAWVSIIECISAEGKALHPLVIYKGTVQQQWFPLDLGPHDGWEFTATENGWTTDDTAV   |                     |                     |                  | 541       |
|                       |            | SRAWVSIIECISAEGKALHPLVIYKGTVQQQWFPLDLGP+DGWEFTATENGWTTDDTAV   |                     |                     |                  |           |
| Sbjct                 | 190        | SRAWVSIIECISAEGKALHPLVIYKGTVQQQWFPLDLGPYDGWEFTATENGWTTDDTAV   |                     |                     |                  | 249       |
| Query                 | 542        | EWLTRVFLpqsqpcqpcqpcqpcDQARLLILDGHGSHTTTDFMWLCYTHNVYLLFLPPHTS |                     |                     |                  | 721       |
|                       |            | EWLTRVFL QPCQPDQARLLI+DGH SHTTTDFMWLCYT+NV+LLFLPPHTS          |                     |                     |                  |           |
| Sbjct                 | 250        | EWLTRVFL-----PQSQPCQPDQARLLIVDGHRSHTTTDFMWLCYTYNVHLLFLPPHTS   |                     |                     |                  | 303       |
| Query                 | 722        | HVLQPLDQSVFSPVKSA YRKELGYLSLWNDSTIIGKRN FISCYQKARQ 865        |                     |                     |                  |           |
|                       |            | +V LQPLDQ V FSPVKSA YRKELGYLSLWNDSTIIGKRN I SCYQKARQ          |                     |                     |                  |           |
| Sbjct                 | 304        | YVLQPLDQLV FSPVKSA YRKELGYLSLWNDSTIIGKRN S I SCYQKARQ 351     |                     |                     |                  |           |



Figure 3.2. Representative Southern blot analysis of genomic DNA digested with HindIII. *Colletotrichum* sp. strain name is indicated on each lane. Blot was hybridized with (A) transposon and (B) telomere probes. Strains marked with the same symbol belong to the same haplotype. The ones without symbols belong to different haplotypes.

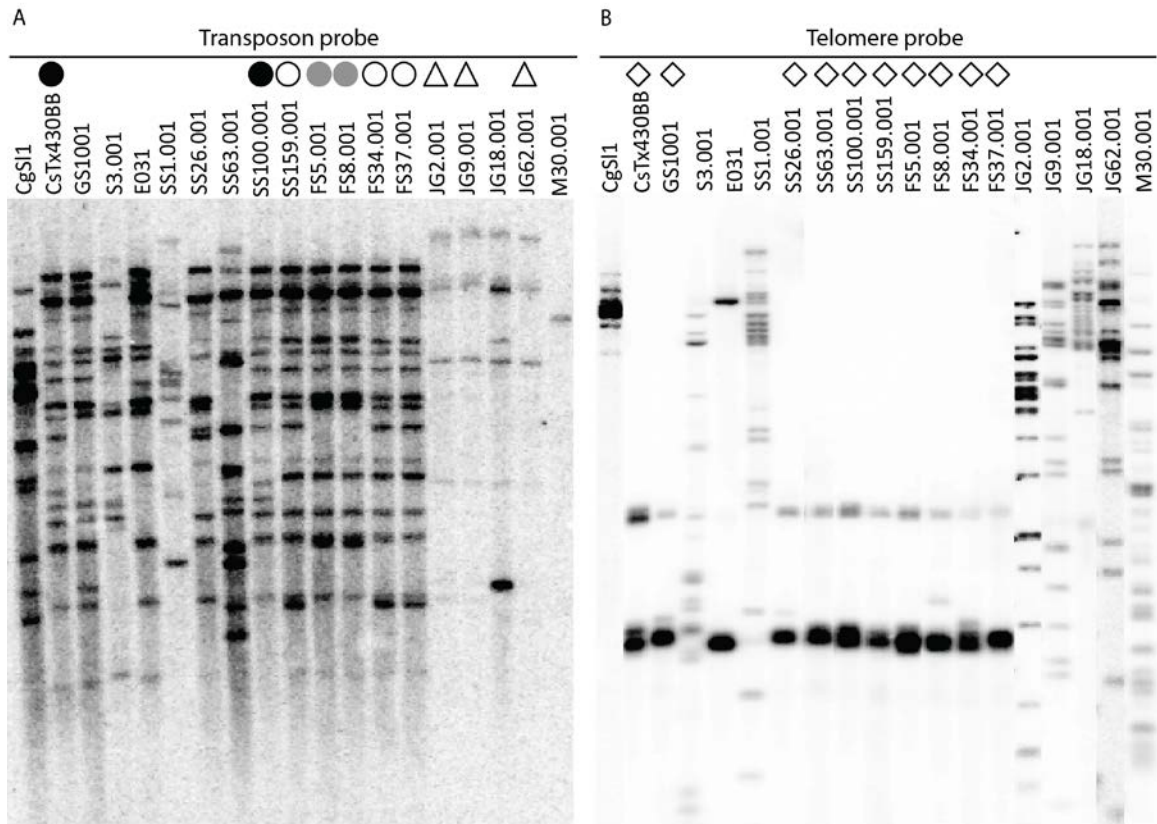


Figure 3.3. Distribution of combined haplotypes. Slices with the same colors represent the same haplotype found in different locations and/or on different host types. Colors match the highlighting in supplemental table 1. Slices in gray tones represent different haplotypes that were found in only that location. The total number of isolates from each location is presented in the center of each pie graph. Empty circles represent locations from which no isolates of that category were obtained.

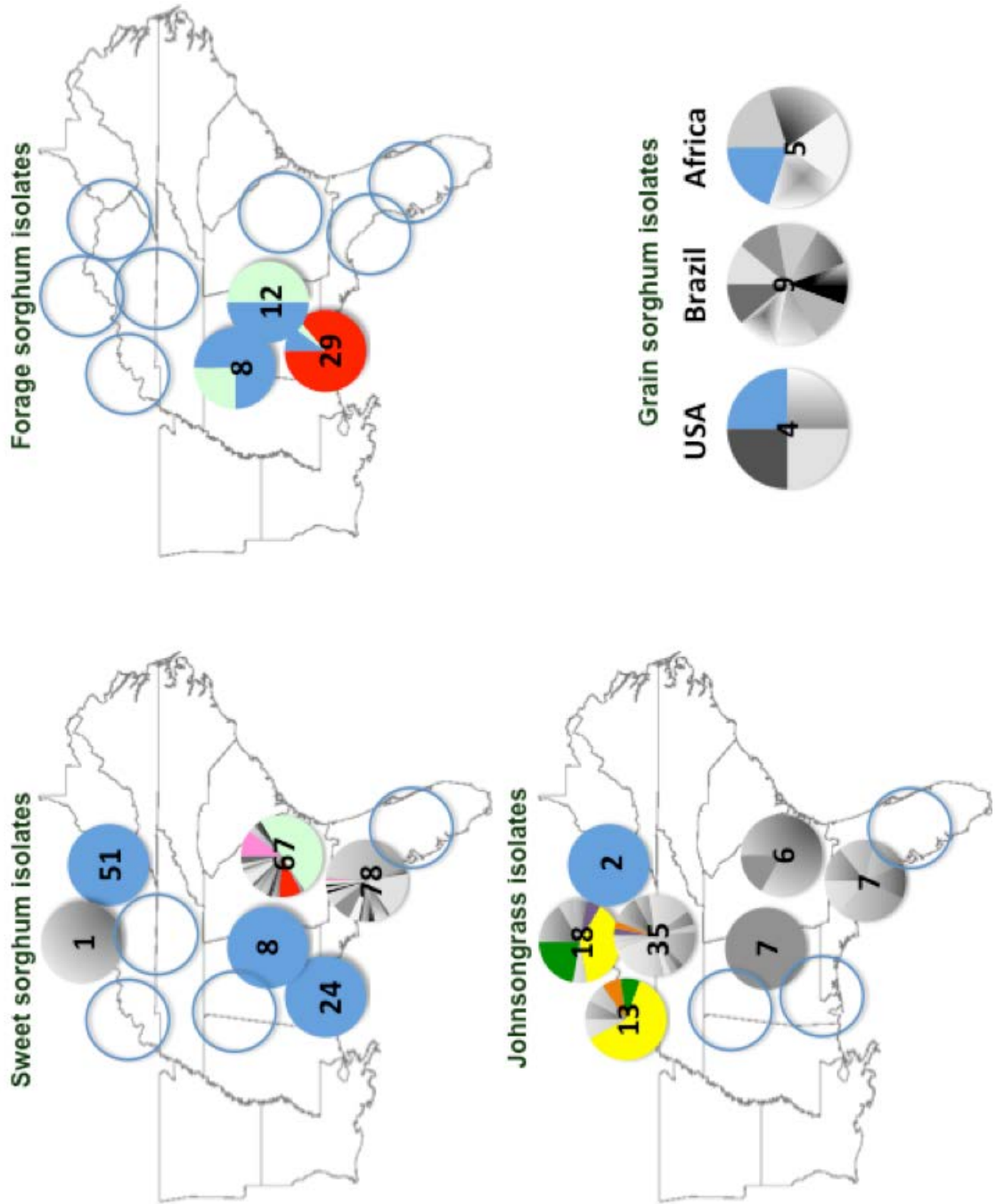


Figure 3.4. Dendrogram illustrating the relatedness of *Sorghum halepense* (JG) (gray boxes), and *S. bicolor* (red boxes) isolates, based on the polymorphic bands revealed by RAPD molecular fingerprinting. The latter group includes isolates from grain (GS1.001, S3.001, S26.001, CgS11, S17.001 RIII42, and E031), sweet (SS), and forage (FS) sorghum. Isolates from sweet sorghum in the *S. halepense* group (\*), and isolates from johnsongrass in the *S. bicolor* group (\*\*). The yellow box (A) includes a representative of a related species *C. graminicola*, which is pathogenic only to maize. Brackets indicate: (A) maize isolate; (B) mostly johnsongrass isolates; (C) johnsongrass isolates only; (D) grain and sweet sorghum isolates from the U.S. and two johnsongrass isolates; (E) mostly sweet sorghum isolates from the U.S., and one grain sorghum isolate; (F) sweet and forage sorghum isolates from the U.S. and (G) grain sorghum isolates from Brazil and Zambia, and sweet sorghum isolates from FL.

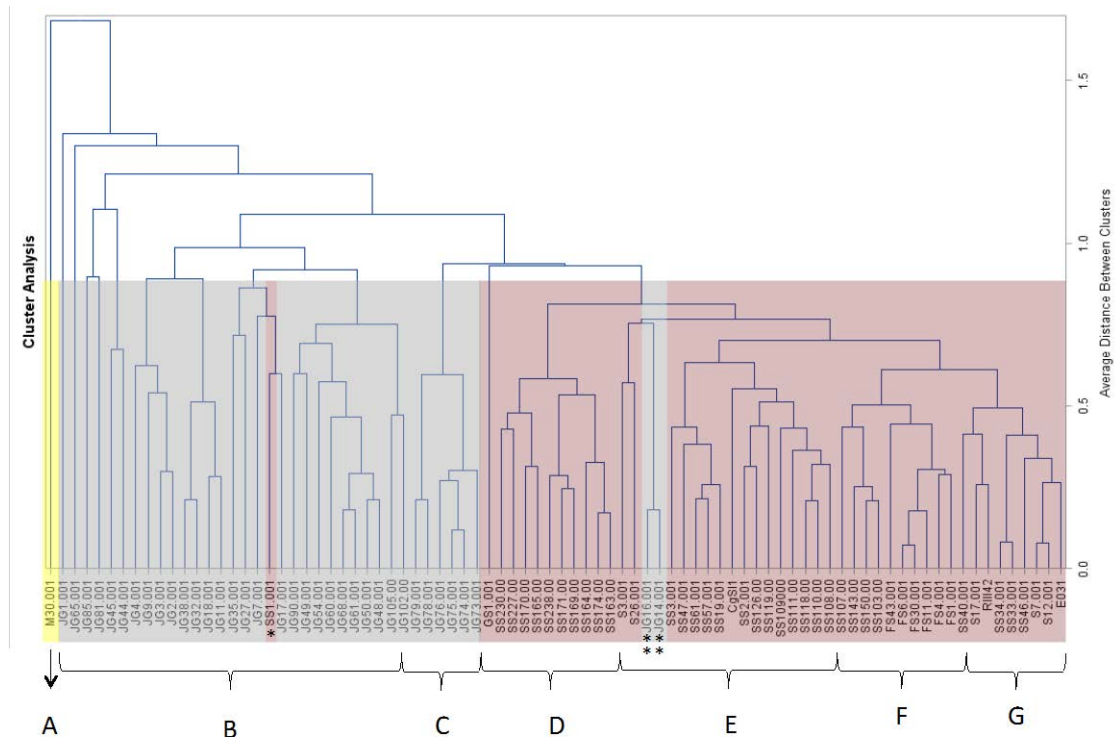


Figure 3.5. Average disease ratings (= the average number of plants rated 0-5) at four weeks post-inoculation in (A) first; (B) second; and (C) third repetition.

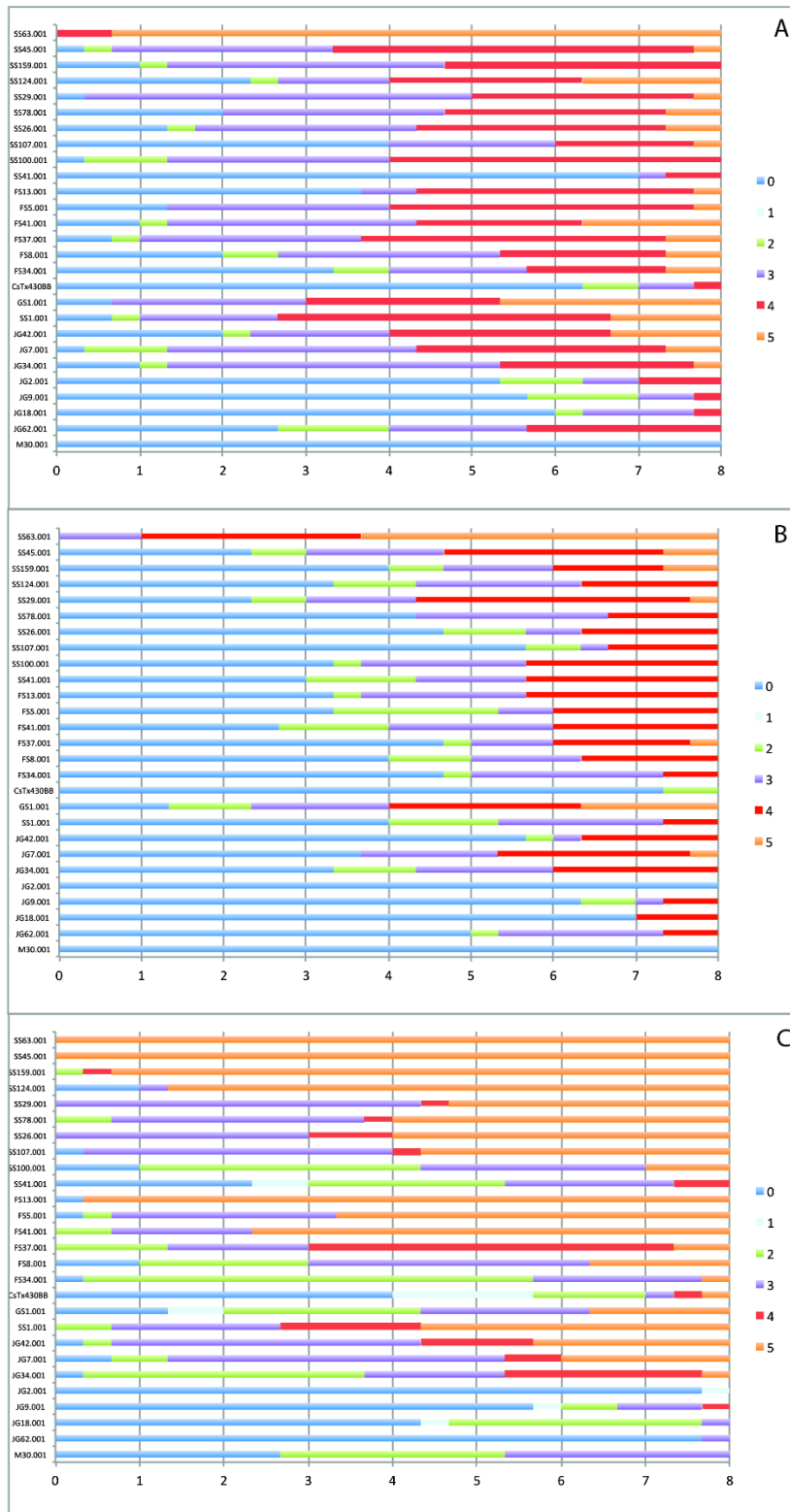


Figure 3.6. Pathogenicity assay of several strains of *Colletotrichum* on Sugar Drip. Incidence values, from the combined data sets of three experiments (summer and fall of 2014; and summer 2016), were used to calculate on the area under the disease progress curve (AUDPC). Black bars are the *S. bicolor* isolates and white bars are the *S. halepense* isolates.

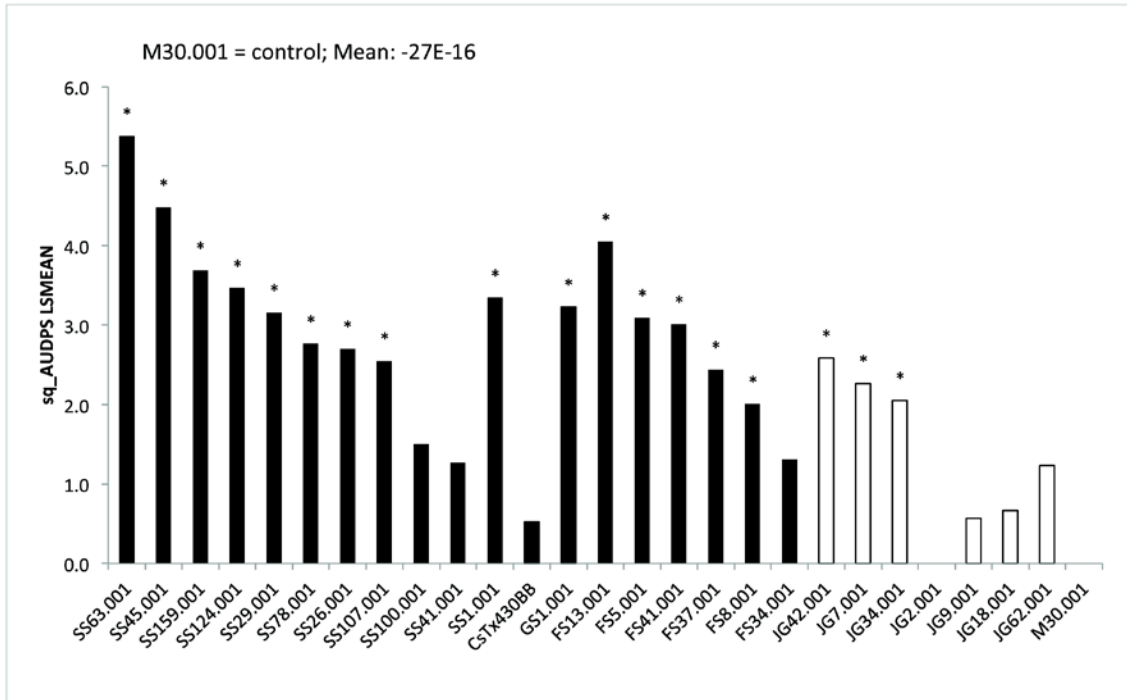


Figure 3.7. Pathogenicity assay of several strains of *Colletotrichum* on Sugar Drip. Incidence values, from the combined data sets of two experiments (July and August of 2016), were used to calculate on the area under the disease progress curve (AUDPC). Black bars are the *S. bicolor* isolates and white bars are the *S. halepense* isolates.

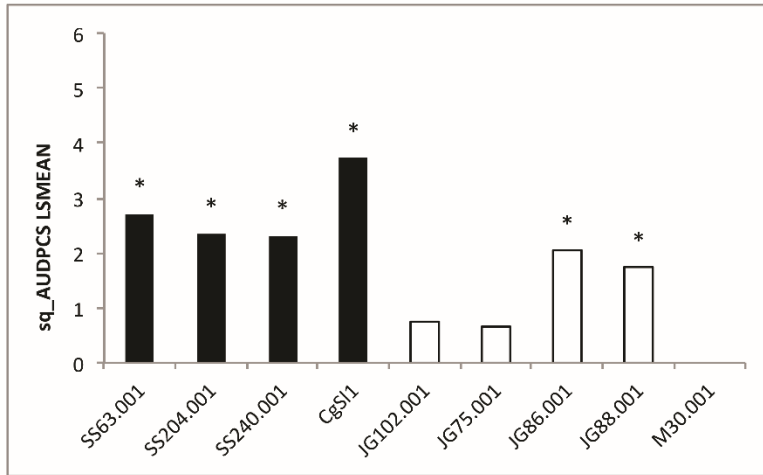


Figure 3.8. Greenhouse experiment comparing 13 fungal strains on Honey, Simon and Bailey. Treatment means were compared with the negative control (M30.001) using the Dunnett's test. An asterisk indicates that the treatment was significantly different from the control at P-value less than 0.05.

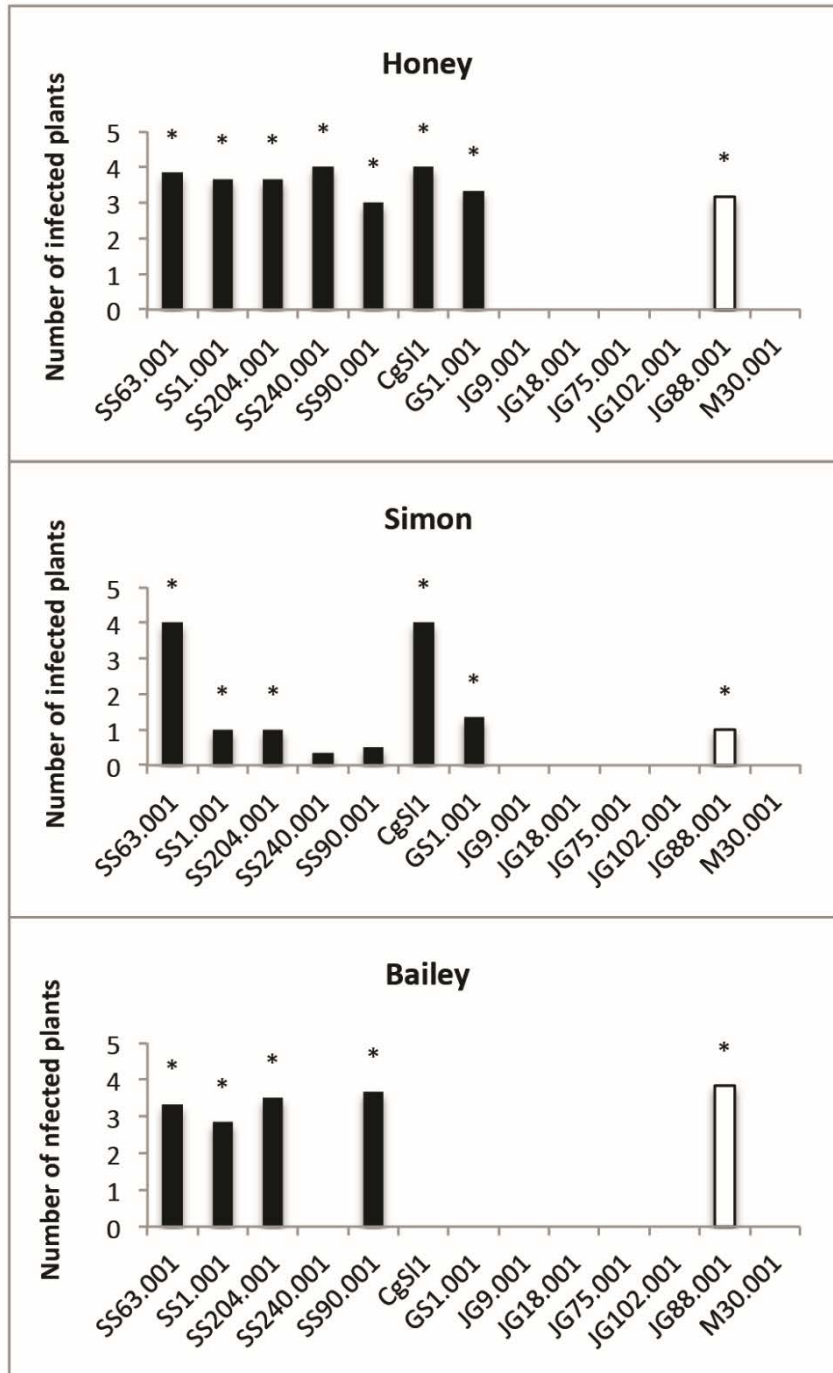


Figure 3.9. Greenhouse experiment comparing 13 fungal strains on Dwarf Yellow Milo, Planter, and Chinese Amber. Treatment means were compared with the negative control (M30.001) using the Dunnett's test. An asterisk indicates that the treatment was significantly different from the control at P-value less than 0.05.

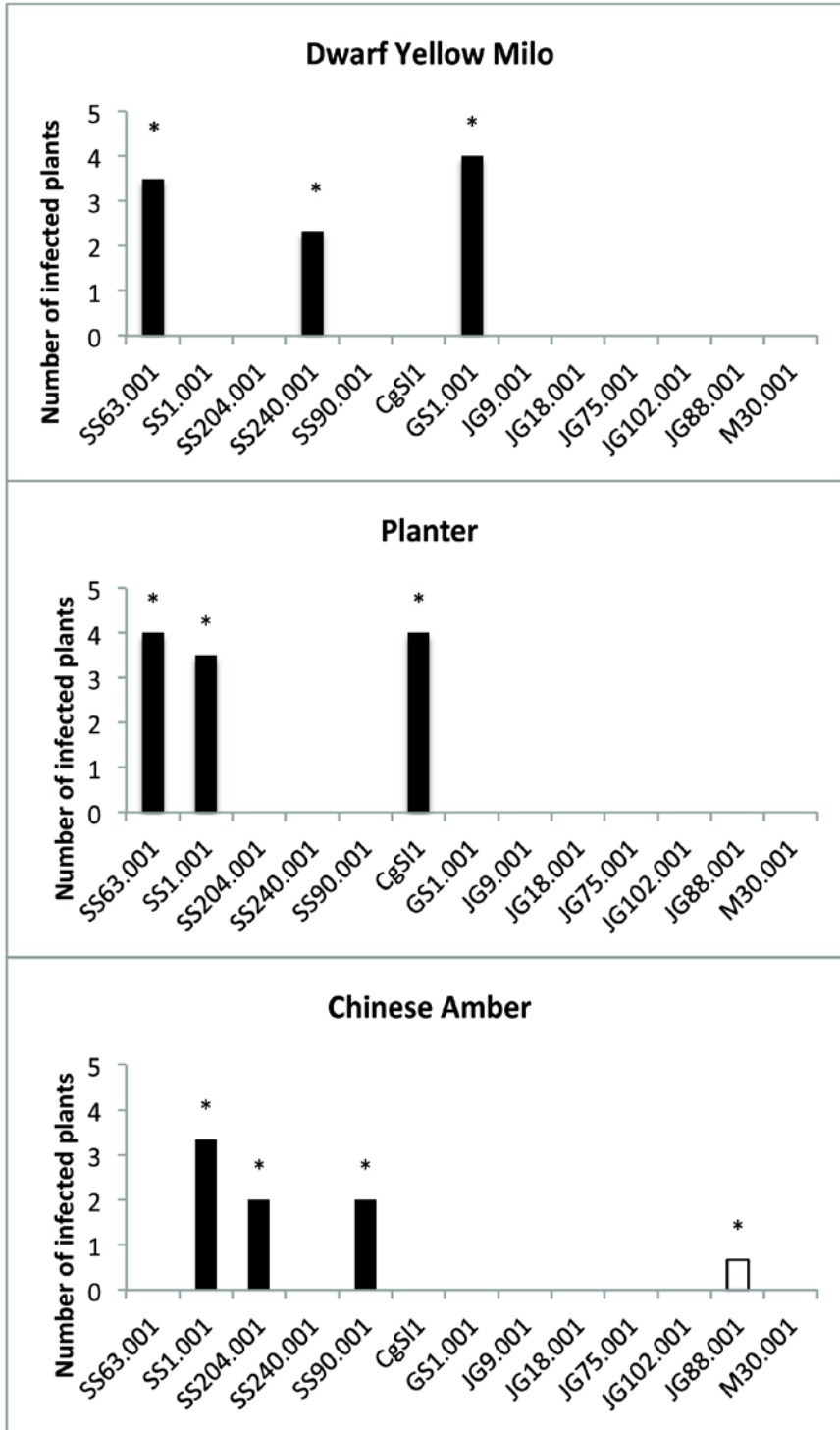




Figure 3.10. Greenhouse experiment comparing 13 fungal strains on Dale, Cowper and Orange. Treatment means were compared with the negative control (M30.001) using the Dunnett's test. An asterisk indicates that the treatment was significantly different from the control at P-value less than 0.05.

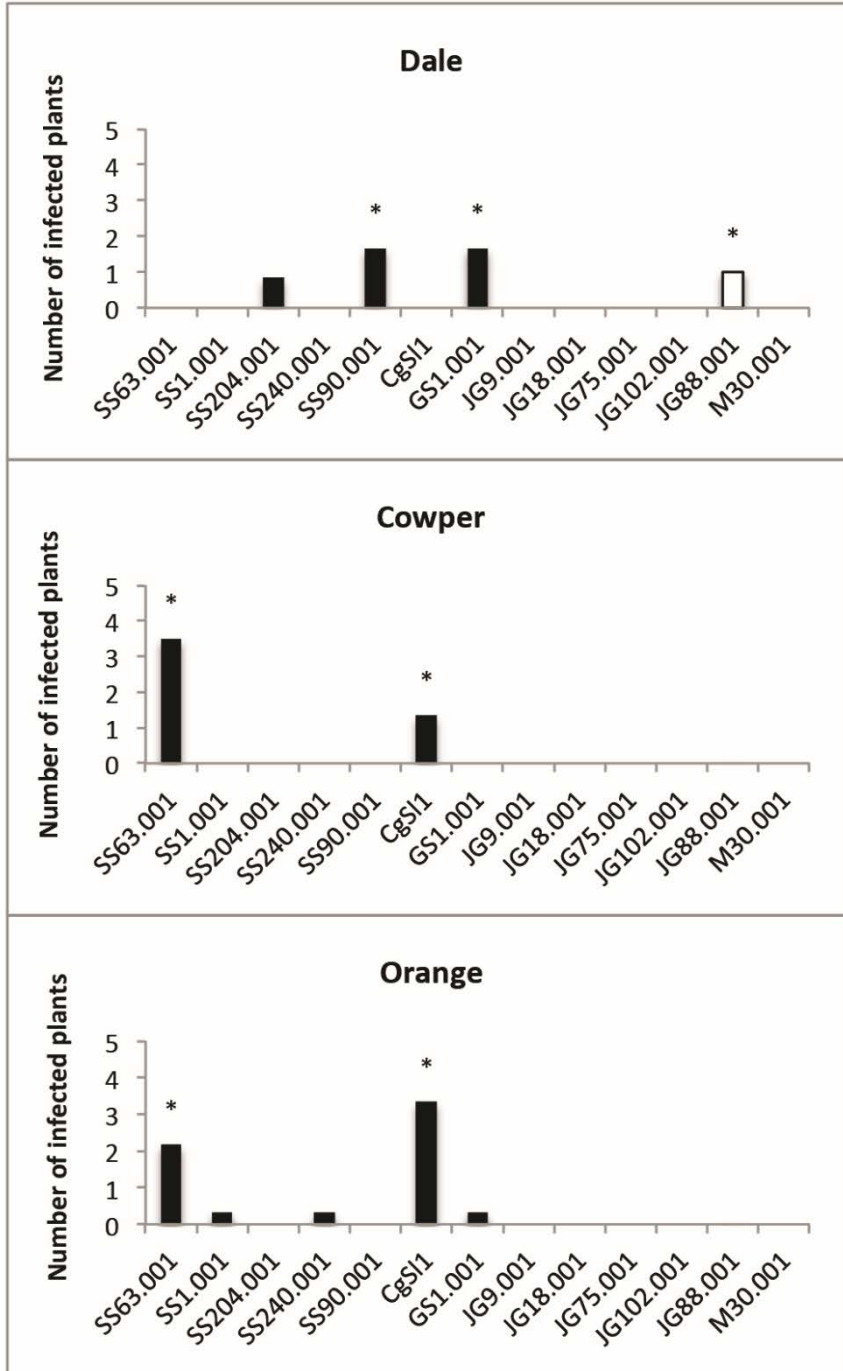


Figure 3.11. Greenhouse experiment comparing 13 fungal strains on N100, Della and Keller. Treatment means were compared with the negative control (M30.001) using the Dunnett's test. An asterisk indicates that the treatment was significantly different from the control at P-value less than 0.05.

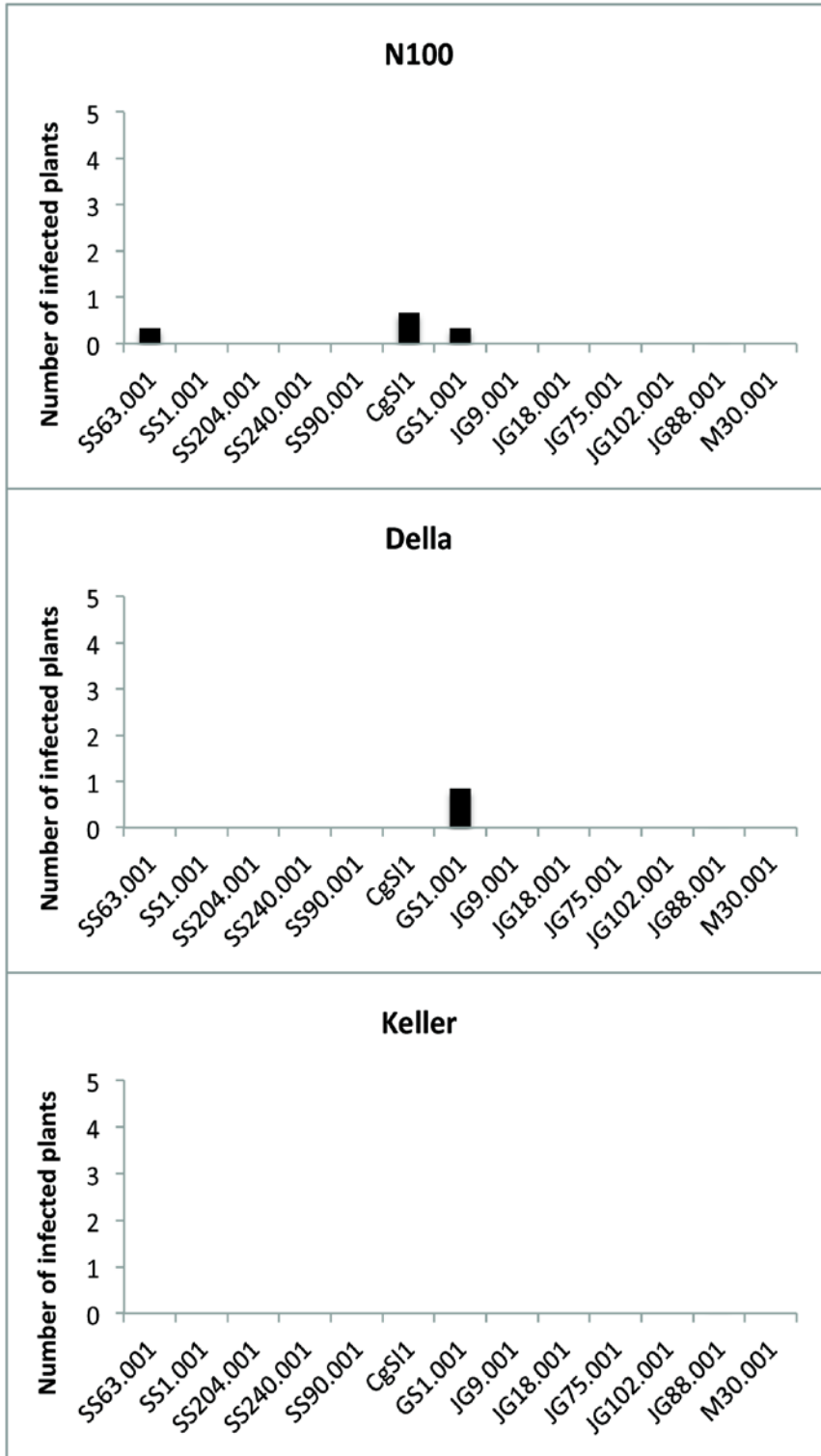


Figure 3.12. Summary of the inoculations performed to evaluate the infection potential of *Colletotrichum* isolates on different varieties of Sweet Sorghum. Table (A) represents the summary of the greenhouse experiment. The green boxes (S) indicate susceptibility and the orange (R) indicate resistance.

| Strains   | S. Drip | Honey | Simon | Bailey | C. amber | Dale | DYM | Cowper | Orange | Planter | N100 | Della | Keller |
|-----------|---------|-------|-------|--------|----------|------|-----|--------|--------|---------|------|-------|--------|
| SS63.001  | S       | S     | S     | S      | R        | R    | S   | S      | S      | S       | R    | R     | R      |
| SS1.001   | S       | S     | S     | S      | S        | R    | R   | R      | R      | S       | R    | R     | R      |
| SS204.001 | S       | S     | S     | S      | S        | R    | R   | R      | R      | R       | R    | R     | R      |
| SS90.001  | -       | S     | R     | S      | S        | S    | R   | R      | R      | R       | R    | R     | R      |
| CgS11     | S       | S     | S     | R      | R        | R    | R   | S      | S      | S       | R    | R     | R      |
| GS1.001   | S       | S     | S     | R      | R        | S    | S   | R      | R      | R       | R    | R     | R      |
| JG88.001  | S       | S     | S     | S      | S        | S    | R   | R      | R      | R       | R    | R     | R      |
| SS240.001 | S       | S     | R     | R      | R        | R    | S   | R      | R      | R       | R    | R     | R      |
| JG9.001   | R       | R     | R     | R      | R        | R    | R   | R      | R      | R       | R    | R     | R      |
| JG18.001  | R       | R     | R     | R      | R        | R    | R   | R      | R      | R       | R    | R     | R      |
| JG75.001  | R       | R     | R     | R      | R        | R    | R   | R      | R      | R       | R    | R     | R      |
| JG102.001 | R       | R     | R     | R      | R        | R    | R   | R      | R      | R       | R    | R     | R      |
| M30.001   | R       | R     | R     | R      | R        | R    | R   | R      | R      | R       | R    | R     | R      |



**Chapter 4 - A comparative genomic analysis of putative pathogenicity genes in the host-specific sibling species *Colletotrichum graminicola* and *Colletotrichum sublineola***

**Abstract**

The fungal sibling species *Colletotrichum graminicola* and *C. sublineola* cause anthracnose leaf blight and stalk rot of maize and of sorghum, respectively. In spite of their close evolutionary relationship, the two species appear to be completely host-specific. I confirmed the presence of inducible non-host resistance to *C. sublineola* by maize, and to *C. graminicola* by sorghum, in epidermal cells. This resistance was manifested as a rapid deployment of visible resistance responses and cell death. Host specificity in plant-pathogen interactions can often be attributed to the presence or absence of pathogen virulence factors, including small secreted protein (SSP) effectors and specialized secondary metabolites (SSM). I identified numerous non-conserved SSM-associated predicted proteins that could play a role in this non-host recognition. Probes against some of the genes encoding these proteins, and against several putative non-conserved SSP genes, were used to conduct RFLP analysis of a representative group of *Colletotrichum* strains from *Sorghum bicolor* and *S. halepense*. The strains could be assigned to one of three distinct groups based on allelic similarity, and these groups were correlated with the fingerprinting and pathogenicity data from chapter 3. The three groups could be differentiated from one another by the presence or absence of specific alleles of some of these pathogenicity genes (aka apparent allelic fixation).

## Introduction

Members of the fungal genus *Colletotrichum* cause anthracnose diseases on nearly every plant species grown for food or fiber worldwide (38, 51). *Colletotrichum graminicola* (Ces.) Wils., and *C. sublineola* Henn., cause economically important anthracnose leaf blight and stalk rot diseases of maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* [L.] Moench), respectively (88, 92, 165, 181). These two fungal sibling species are morphologically very similar, but reproductively isolated (181). Results of molecular phylogenetic analyses suggest that they diverged from a common ancestor relatively recently, perhaps at the same time as the split between maize and sorghum (thought to be approximately 12 million years ago) (39, 40, 61, 157, 165, 167, 181). There are no reports in the literature of *C. graminicola* infecting sorghum or of *C. sublineola* infecting maize in the field, and most studies agree that the two species are host-specific (45, 92, 105, 202). We have found that *C. sublineola* can infect maize stalk epidermal cells, and maize leaf sheath cells that are dead or dying (175, 187). This ability of *C. sublineola* to conditionally infect some maize tissues might explain two earlier papers that reported that maize was susceptible to isolates of *Colletotrichum* from sorghum (31, 198). It also suggests that host range is determined by active recognition of and response to the non-pathogen by most healthy tissues of the non-host, rather than structural barriers or the absence of some vital nutrient or other factor.

The determination of host range in plant pathogens is often attributed to the presence or absence of pathogen virulence factors, particularly specialized secondary metabolites (SSMs), and small secreted proteins (SSPs) (aka. effectors) (7, 36, 48, 59, 90, 103, 180).

The presence of particular SSMs has been associated with the determination of host range in some phytopathogenic fungi including *Alternaria* spp. (90) and *Cochliobolus* spp. (36). The major classes of fungal SSMs include polyketides, peptides, terpenes, and indole alkaloids (10, 11, 108). Each of these classes is associated with a specific family of proteins. These SSM-associated proteins are: polyketide synthases (PKS); nonribosomal peptide synthetases (NRPS); terpene synthases (TS); and dimethylallyl transferases (DMAT), respectively. Genes encoding these enzymes and other proteins involved in the production of the SSMs are often found physically associated in transcriptionally co-regulated gene clusters (113, 115).

Fungal effectors have been defined as SSPs that alter the structure or modulate the function of host cells to facilitate infection (62, 186). Some effectors are translocated and operate in the host cytoplasm (53, 60, 99, 100). Others function in the plant cell apoplast (95). Some effectors act as host specific toxins and induce apoptosis only in certain plant genotypes, conferring host specificity in several important necrotrophic pathogens (185, 189). Examples of known effector categories include serine proteases, necrosis and ethylene-inducing protein 1-like proteins (NEP1-like proteins), and small cysteine-rich proteins (48, 122, 141).

Some plants have evolved an ability to recognize and respond to certain effectors by activating defense pathways via specific resistance (R) proteins, a phenomenon known as effector-triggered immunity (ETI). In these cases, the effectors act as avirulence (avr) factors. Multiple rounds of mutation and selection of R and avr genes during a co-evolutionary “arms-race” leads to the presence of multiple pathogenic races expressing different combinations of avr genes within the pathogen population (93). Recent evidence

suggests that inducible non-host resistance in many agriculturally-important pathosystems, particularly involving closely related hosts, is due to ETI. In these cases all members of the non-host plant species contain the same R gene(s), whereas all members of the nonpathogenic microbial species contain the corresponding avr gene(s) (33, 96, 114, 125, 153, 154, 168, 177, 178, 183).

A number of recent comparative genomics studies have confirmed that genes encoding SSM-associated proteins and SSPs show evidence of rapid evolution in related pathogens with different host ranges (7, 17, 21, 36, 50, 58, 74, 107, 127, 137, 138, 149, 152, 163, 203).

My goal in this chapter was to see if the differences in host specificity and molecular fingerprints among strains from *S. bicolor* versus *S. halepense* that I reported in chapter 3 were associated with differences in potential pathogenicity genes. I had three predictions: (1) that the sibling species *C. sublineola* and *C. graminicola* would differ in the presence of one or more genes predicted to encode SSPs and SSM-associated proteins (aka pathogenicity genes); (2) that pathogenicity genes found in *C. sublineola* but not in *C. graminicola* would be conserved among all members of the population infecting *Sorghum bicolor* in Kentucky and the southeastern U.S. (suggesting the possibility that they were involved in triggering ETI in maize); and (3) that populations affecting *S. bicolor* would be distinguishable from populations affecting *S. halepense* by allelic differences in individual pathogenicity genes, ie. allelic fixation (suggesting that the two populations are genetically isolated).



## **Material and Methods**

**Plant and fungal growth and inoculation:** Fungal strains were preserved on silica gel at  $-80^{\circ}\text{C}$  (179). Strains were cultured on potato dextrose agar (PDA, BD Difco, Franklin Lakes, NJ) under continuous fluorescent lights at  $23^{\circ}\text{C}$ . Spores were harvested from 2-week-old culture plates by gently scraping them from the surface, and washed three times before use.

Seeds of sweet sorghum (Sugar Drip) or maize (Mo17) were sown in a mixture of two parts sterile topsoil and three parts of Pro-Mix BX (Premiere Horticulture, Ltd, Riviere du Loup, PQ, Canada). Seedlings were maintained in the greenhouse with 14 hours of light, watered every other day to saturation using an automated overhead irrigation system, and fertilized beginning one week after emergence two or three times per month as needed with a solution of 150 ppm of Peters 20-10-20 (Scotts-Sierra Horticultural Product Co., Marysville, OH).

Maize and sorghum seedlings at the V6 stage were inoculated by applying a suspension of  $5 \times 10^6$  spores per ml with a compressed-air sprayer (Preval Model 267 Paint Spray Gun). After inoculation, the plants were incubated for 18 h in the dark at  $25^{\circ}\text{C}$  in a dew chamber at 100% relative humidity before being returned to the greenhouse bench. Maize leaf sheaths were inoculated with a suspension of  $5 \times 10^5$  spores per ml as described previously in this dissertation.

**Sequencing, assembly, and annotation of fungal genomes:** The genomes of *C. graminicola* strain M5.001 and *C. sublineola* strain CgS11 were sequenced to 29X and 43X, respectively, in the Advanced Genetic Technologies Center (AGTC) at the University of Kentucky. Genome assembly was done by using Newbler version 2.9. Annotation was done by using MAKER. The genome assemblies and predicted proteomes for *C.*

*graminicola* strain M1.001 and for *C. sublineola* strain TX430BB were downloaded from the NCBI BioProjects database (accession numbers PRJNA37879 and PRJNA246670, respectively). Genome assemblies and predicted proteomes for *C. sublineola* strain S3.001 and for *C. falcatum*, *C. somersetensis*, *C. caudatum*, *C. eremochloae*, and *C. zoysiae* were downloaded from the Joint Genomes Institute Genome Portal (<http://genome.jgi.doe.gov/>). The comparative genome analysis and general protein characterization (other than specifically mentioned in this dissertation chapter) was done by AGTC staff, Dr. Neil Moore, and Dr. Ester Buiate (2016).

**Identification of orthologous and unique genes:** Fungal protein sequences were downloaded from the Broad Institute (*C. graminicola*, *C. higginsianum*, *Fusarium graminearum*, *F. oxysporum*, *Verticillium dahliae*, *Aspergillus flavus*) and the Joint Genome Institute Genome Portal (*Trichoderma reesei*, *C. falcatum*, *C. somersetensis*, *C. caudatum*, *C. eremochloae*, *C. zoysiae*, *C. sublineola* strain S3.001). Protein sequences from *Epichloë festucae* were the FGENESH gene predictions previously used in the Clavicipitaceae analysis (151). Putative orthologs were identified by using two methods. The first method was application of Ortho-MCL and COCO-CL (COrrrelation COefficient-based CLustering) to the annotations (94, 111), following a procedure previously used for ortholog identification within the Clavicipitaceae (151). The species included for comparison in the Ortho-MCL/COCO-CL analysis were: *C. graminicola*; *C. higginsianum*; *C. sublineola* CgS11; *M. oryzae*; *E. festucae*; *F. graminearum*; *F. oxysporum*; *T. reesei*; *V. dahlia*; and *A. flavus*. The second method used for ortholog identification was Reciprocal Best Hit (RBH) with an expect-value cutoff of 1e-5 (119,

190). This method was used to compare proteins from *C. graminicola*, *C. sublineola*, and *C. higginsianum*.

**Identification of SSM-associated proteins in *Colletotrichum sublineola*:** I identified the five classes of candidate SSM-associated genes (PKS, NRPS, PKS-NRPS hybrid, DMAT, and TS) from *C. sublineola* by applying a process that included Pfam and Ortho-MCL/COCO-CL analysis; followed by manual annotation and domain validation in the Conserved Domain Database (CDD) (<http://www.ncbi.nlm.nih.gov/cdd/>); blastp comparisons with the NCBI nr database; and InterproScan analysis. This protocol has been described in more detail previously (131).

I manually annotated *C. sublineola* SSM gene clusters by evaluating Ortho-MCL/COCO-CL results for the genes that were located upstream and downstream of the SSM-associated backbone genes. Genes that had no or few orthologs were considered to belong to the clusters, while genes that were conserved in most or all of the ten species included in the analysis defined the outside boundaries of the clusters.

**Phylogenetic analysis of *Colletotrichum* SSM-associated proteins:** I performed a phylogenetic analysis of SM genes by using the tools on the phylogeny.fr website (<http://www.phylogeny.fr/index.cgi>). The A and KS N-terminal and C-terminal domains of the NRPS, PKS, and NRPS-PKS hybrids were identified by using the NCBI CCD. Amino acid sequences were aligned by using MUSCLE version 3.7, and phylogenies were inferred by maximum-likelihood using PhyML version 3.0. Statistical branch support was provided by an approximation to the standard likelihood ratio test, aLRT (52).

**Preparation of probes and Southern blot:** Genomic DNA extraction was performed following the protocol of (174). Genomic DNA from the *C. sublineola* strain CgS11 was used as a template for PCR. Primers used for amplification of pathogenicity gene probes are listed in Table 4.1. PCR conditions consisted of 3 min of denaturation at 94°C; followed by 35 cycles of denaturation for 30 sec at 94°C, 60 sec of annealing at 59°C, and 40 seconds of extension at 72°C; and a final extension of 10 min at 72°C. The identities of representative amplicons of the expected size were confirmed by sequencing.

A total of 88 isolates were selected to represent the diversity among the 400 individuals in my collection, based on the molecular fingerprinting analyses described in chapter 3 of this dissertation (Supplemental Table 1, Supplemental Table 2). The Southern blot was performed as described previously. Each of the strains was hybridized with each of the probes (Table 4.1).

**Analysis of allelic diversity among populations:** Polymorphic RFLP bands were scored as present (1) or absent (0) (Supplemental Table 2). Jaccard's coefficients (91) were applied to the data by using the Statistical Analysis Software (SAS) package, in order to group the isolates in a dendrogram based on the overall similarity of the band patterns.

## **Results**

### **Characterization of strain CgS11, and confirmation of host specificity**

Partial sequences of four genes have been used previously for multigene phylogenetic analysis of *Colletotrichum* (131). These included portions of the actin (*Act*) gene; the chitin synthase 1 (*Chs-1*) gene; the histone3 (*His3*) gene; and the beta-tubulin (*Tub2*) gene. These sequences from CgS11 shared 100% identity with those of strain S.3001, the designated

epitype specimen for *C. sublineola* (131) (Figure 4.1). The 5.8S nuclear ribosomal gene and the two flanking internal transcribed spacer (ITS) sequences from CgS11 also shared 99.6% identity with the ITS sequence of S3.001. This indicates that CgS11 belongs to the *C. sublineola* species as it is presently defined (Figure 4.1).

*Colletotrichum graminicola* strain M1.001 was isolated from maize in Missouri in the late 1970s (70). *Colletotrichum sublineola* strain CgS11 was isolated in the early 1980s from grain sorghum in Indiana (92). I confirmed that these isolates were host-specific, and I also determined that the non-host reaction was associated with rapid cell death, typical of ETI (33, 96, 114, 125, 153, 154, 168, 177, 178, 183). Strain M1.001 caused typical, sporulating anthracnose lesions on maize leaves (cv. Mo17) within three days post inoculation (dpi), but on leaves of sorghum (cv. Sugar Drip) it produced only small reddish flecks, which failed to expand or sporulate even up to 7 dpi (Figure 4.2 A, D). Strain CgS11 caused large, sporulating anthracnose lesions on sorghum, but not on maize leaves (Figure 4.2 B, C). Strain M1.001 readily infected and colonized multiple cells of detached leaf sheaths of maize by 48 hr after inoculation (hpi) and strain CgS11 did the same in sorghum sheaths by 72 hpi (Figure 4.3 A, B). In contrast, *C. graminicola* failed to infect leaf sheath cells of sorghum, and *C. sublineola* failed to infect maize leaf sheath cells, even up to 6 dpi (Figure 4.3 C, D). Sorghum responded within 48 hpi to *C. graminicola* appressoria by an accumulation of numerous vesicles containing red pigments, and maize responded to *C. sublineola* appressoria by the formation of iridescent papillae (Figure 4.3 C, D). Previous studies have determined that the red pigments consist of various anthocyanin phytoalexins (162). The maize papillae are composed primarily of callose (117). Visible primary hyphae were always very small, and were produced in fewer than 1% of infection attempts in both

non-host combinations. Unpenetrated cells beneath *C. sublineola* appressoria in maize leaf sheaths typically retained their ability to plasmolyze even up to 48 hpi, but cells containing rare penetration hyphae appeared granulated, and did not plasmolyze normally (Figure 4.4 A, B). Sorghum cells beneath *C. graminicola* appressoria usually plasmolyzed at 24 hpi, but by 48 hpi most of the cells had lost the ability to plasmolyze, whether they contained infection hyphae or not (Figure 4.4 C, D, Figure 4.5). Most of the cells in the mock-inoculated maize and sorghum controls still plasmolyzed normally up to 72 hpi (Figure 4.6). *Colletotrichum sublineola* and *C. graminicola* were able to colonize non-host leaf sheaths readily if the cells were killed first by a localized application of liquid nitrogen (Figure 4.7). These observations suggest that host specificity is based on active recognition of the non-pathogen by living non-host plant cells, followed by rapid deployment of defense responses targeting the infection sites, and ultimately plant cell death prior to, or coincident with, penetration.

### **Identification of SSM-associated genes in *C. sublineola* strain CgS11**

The program Ortho-MCL and the refiner COCO-CL were used to identify genes in *C. sublineola* that were orthologous to the previously identified SSM-associated genes of *C. graminicola* and *C. higginsianum* (131). Using this approach, combined with manual annotation, I identified 31 PKS genes, eight NRPS genes, six PKS-NRPS hybrid genes, 14 TS genes, and eight DMAT genes in *C. sublineola* (Table 4.2). Pfam analysis of the *C. sublineola* protein predictions identified 172 putative SSM domains. All of the SSM-associated genes that were identified by Ortho-MCL and COCO-CL (above) were included among the SSM genes identified after manual annotation of the Pfam domains. However, the Pfam analysis identified additional genes in each class (three TSs and one DMAT)

encoded by *C. sublineola* that were not found in either *C. graminicola* or *C. higginsianum* (Table 4.2).

### **Phylogenetic analysis of the SSM-associated proteins**

A phylogenetic analysis was performed to address the relationships among the putative SSM-associated proteins in *C. graminicola* and *C. sublineola*. The more distantly-related species *C. higginsianum*, a pathogen of Brassicaceae, was also included for comparison. SSM-associated genes in *C. graminicola* and *C. higginsianum* were previously published (131). After manual annotation and identification of overlapping gene models, I reduced the 58 PKS genes that were previously identified in *C. higginsianum* (131) to 36 complete genes for analysis (Table 4.2). The amino acid sequences of the adenylation domain (A domain) of NRPS proteins and PKS-NRPS hybrids (16, 164); the keto-synthase (KS) N-terminal and C-terminal domains of PKS proteins and PKS-NRPS hybrids (101); and the entire DMAT and TS protein sequences, were used for the phylogenetic analyses. The alignments are provided in Appendix 3 of this dissertation.

Results of the analysis revealed a high degree of diversity, with relatively few SSM-associated protein ortholog families that were conserved across all three *Colletotrichum* species (Figures 4.8-4.12). As expected, *C. graminicola* and *C. sublineola* shared more ortholog families than either shared with *C. higginsianum*, consistent with a more recent common ancestor. The presence of some ortholog families only in *C. higginsianum* and *C. graminicola*, or only in *C. higginsianum* and *C. sublineola*, suggested that some members of these families may have been lost since the divergence of *C. higginsianum* from the other two species. The PKS proteins were the largest and most diverse group of SSM-associated proteins, with 79 proteins or protein ortholog families across the three species.

The NRPS proteins comprised the smallest group, with only 15 different proteins or ortholog families. *Colletotrichum graminicola* and *C. sublineola* shared about half of their PKS proteins, and also about half of their PKS-NRPS hybrid and TS proteins. The DMAT and NRPS proteins were more highly conserved, with about two thirds represented in both species. Searches of the NCBI nr database, and of the predicted proteomes of five close relatives in the JGI database, revealed that there were no SSM-associated protein genes in either *C. sublineola* or in *C. graminicola* that were unique to either species (Supplemental Table 3).

**Conservation of gene clusters:** I identified gene clusters in *C. sublineola* by manual analysis of the genes located on either side of the “backbone” SSM-associated genes (i.e. the genes encoding PKS, NRPS, TS, DMAT, and PKS-NRPS hybrids) that had been identified by using Ortho-MCL/COCO-CL and Pfam. A total of 67 putative SSM-associated gene clusters in the *C. sublineola* genome (Supplemental Table 2), were compared with the 42 clusters that were previously identified from *C. graminicola* (131). There were 25 PKS gene clusters that appeared to be shared (with more than 50% of the genes in common) between *C. sublineola* and *C. graminicola*. One of these is the melanin cluster (Figure 4.13) (131), and another is likely to be responsible for the production of monorden because it is identical in gene structure and content with the RADS cluster of *Pochonia chlamydospora* (Figure 4.14) (176). *Colletotrichum sublineola* and *C. graminicola* also shared five DMATs clusters, six NRPSs gene clusters, four hybrids and fifteen TSs gene clusters (Supplemental table 2). One of these conserved TS clusters is probably involved in the production of carotenoids (131).



I found five and four PKS clusters that were not shared in *C. sublineola* and *C. graminicola*, respectively. Examples of non-conserved PKS gene clusters are shown in (Figures 4.15-4.16). *C. sublineola* also has four DMATs, two TSs, two NRPSs, and two hybrid gene clusters that are unique (Supplemental Table 2). An example of a unique *C. sublineola* NRPS cluster is shown in Figure 4.17.

### **Comparison of the *C. graminicola* and *C. sublineola* SSP genes**

*Colletotrichum graminicola* M1.001 encodes 143 predicted SSPs that are not found in *C. sublineola* strain CgS11, while *C. sublineola* has 301 that are not shared with *C. graminicola* (Buiate, 2016). After comparisons with the NCBI nr database and available genome data from a group of five close relatives of *C. graminicola* and *C. sublineola* (<http://genome.jgi.doe.gov/>), there appeared to be 21 SSPs that were specific only to *C. sublineola*, and there were no lineage specific SSM-associated genes. One gene, CSUB\_000060, appears to be shared only by *C. graminicola* and *C. sublineola*, and is not present in any other species currently in the databases.

### **Genomic analysis of SSP and SSM diversity among different isolates**

Two other genome assemblies are available for *C. sublineola*. The TX430BB strain was isolated from grain sorghum in Texas in the late 1980s, and was sequenced by Baroncelli et al., (2016) (7). The S3.001 strain is the epitype for the species (40, 41), and its genome assembly can be accessed from JGI (<http://genome.jgi.doe.gov/>). This strain was isolated in the late 1980s in Burkina Faso (181). *C. sublineola* isolate CgS11 shared 99% of its predicted gene models with TX430BB and S3.001. Only 23 CgS11 SSP genes were not found in the TX430BB assembly and only seven were not found in S3.001. Only 39 gene sequences were not found in either of the other two other strains, including 2 SSPs. All of

the SSM-associated genes in CgS11 appeared to be conserved in both other strains of *C. sublineola*.

**RFLP analysis: putative effector proteins and secondary metabolites**

Two secondary metabolite-associated genes from CgS11, CSUB\_020320 (PKS14) and CSUB\_060040 (PKS40), were selected for analysis. PKS14 is conserved with *C. graminicola* and *C. higginsianum*, while PKS40 is found only in *C. sublineola* (Supplemental Table 3). PCR primers were designed to amplify the sequences encoding the KS domains for each gene (Table 4.1). Twelve putative SSP genes, seven of which appeared to be unique to *C. sublineola*, and not found in *C. graminicola* and *C. higginsianum* (Supplemental Table 4), were also chosen for use as probes. Two SSP genes, (CSE21 and CSE24) that were shared by *C. sublineola* and *C. graminicola* but not *C. higginsianum*, and three that were conserved in all three species (CSE33, CSE53, and CSE56) were also chosen for comparison (Supplemental Table 4).

All of the probes identified restriction fragment length polymorphisms among the isolates that were analyzed. Some isolates did not hybridize at all to some of the probes. Representative results are shown in (Figure 4.18). A dendrogram was built to illustrate the relatedness of 88 representative fungal strains (Supplemental Table 4) isolated from johnsongrass (JG), grain sorghum (GS1.001, S3.001, E031, CgS11, TX430BB), sweet sorghum (SS), and forage sorghum (FS), based on RFLP allelic variation for the 14 putative effector and SMM-associated probes. This dendrogram separated most of the isolates from cultivated sorghum from most of the isolates from johnsongrass (Figure 4.19). However, there were some exceptions. For example, strain SS1.001 from sweet sorghum grouped with johnsongrass isolates, while JG88.001, JG14.001, and JG35.001 clustered with the

cultivated sorghum isolates. Similar results were found in the fingerprinting analysis that was reported in chapter 3 of this dissertation. Among the isolates from johnsongrass (and SS1.001), there were two distinct clusters, marked on the dendrogram by orange and blue boxes. Isolates within the blue box that were tested were pathogenic to *Sorghum bicolor*, while isolates in the orange box were not (chapter 3 of this dissertation). The isolates in the blue box could be differentiated from the others by several of the probes. Thus, none of the isolates in the blue box hybridized to CSSM3. All of them had unique alleles of CSE4, CSE17, CSE21, CSE53, and CSE56. The johnsongrass isolates in the orange box could be differentiated from the other two groups by the absence of CSE6. Furthermore, JG14.001, JG88.001, and JG35.001 shared alleles of CSE23 and CSE33 that were otherwise found only in isolates from cultivated sorghum, confirming their close relationship as revealed in the dendrogram.

## **Conclusions**

This work was part of a larger project in which our team compared gene models from two contemporaneous, co-occurring strains of the sibling species *C. graminicola* and *C. sublineola*, and identified those that do not appear to be conserved as potential candidates for involvement in host specificity. This approach was based on previous studies that have shown that gene gain and loss is associated with host range in many plant pathogens, including *Colletotrichum* (7, 74).

The analysis confirmed that the genomes of the *C. graminicola* and *C. sublineola* strains were very similar to one another in both gene content and gene order, consistent with a relatively recent common ancestor. I confirmed that each strain was able to successfully

colonize its own living host (maize and sorghum, respectively), while the closely related non-host underwent an apparent hypersensitive response upon challenge.

We found that 14% of the *C. graminicola* gene models, and 22% of the *C. sublineola* gene models, were not conserved in the other species. Certain categories of genes were especially likely to be non-conserved (NC) including genes that were predicted to encode SSPs and SSM-associated proteins that may play important roles in early events related to host recognition and the induction of compatibility. Some of the NC SSP gene sequences were also not conserved among different strains within each species, which suggested the possibility of selection within the population and a potential avr function. Races of both *C. sublineola* and *C. graminicola* have been reported to occur (3, 14, 30, 44, 128, 143, 184, 199). There were relatively few strain-specific SSP gene sequences, however, and no strain specific SSM sequences. This differs from some other fungal species, e.g. *Magnaporthe oryzae*, where the deletion of effector genes seems to be common, and to play an important role in the rapid evolution of new races (205, 207). However, comparisons with genome assemblies of the five closely related species within the graminicolous clade, accessed from JGI (<http://genome.jgi.doe.gov/>), suggests a more important role for deletion of SSP and SSM genes in speciation and host species adaptation, a finding that has also been reported by others based on comparative analyses of a wider range of *Colletotrichum* genera (7, 74). Within the population of *Colletotrichum* strains from *Sorghum* spp., RFLP analysis revealed the presence of three distinct groups, based on allelic variations of several of the putative pathogenicity loci that were identified in this chapter. These groups were highly correlated with the fingerprinting data from chapter 3. Thus, one group consisted primarily of isolates from cultivated sorghum, while the other consisted primarily of isolates from

johnsongrass. Exceptions to the rule in the RFLP analysis, were also exceptions in the fingerprinting analysis (e.g. JG88.001 and SS1.001), additional evidence that these represent occasional cross-infections of *S. bicolor* by members of the *S. halepense* population, and *vice versa*.

The isolates from johnsongrass were further divided into at least two different groups (the orange and blue groups) based on the RFLP data. These groups were consistent with pathogenicity data from chapter 3. Thus, the orange group contained isolates that appear to be host-specific to johnsongrass, while the members of the blue group (including SS1.001) are able to infect both *S. bicolor* and *S. halepense*. All the members of the orange group appear to lack one SSP gene (CSE6) that is present in all of the isolates that are able to infect *S. bicolor*. The orange group also differs from nearly all of the rest of the strains in having unique alleles of CSE56. Only JG35.001 outside of the orange group also shares one of these alleles.

All members of the blue group share the same allele of CSE6, which differs from the allele that is shared by all of the members of the green group of isolates that come mostly from *S. bicolor*. Other fixed allelic differences between the blue group and the other strains can be seen for CSE4, CSE17, CSE21, CSE53, and CSE56. Members of the blue group also lack the CSSM3 gene that is found in all of the other strains affecting *Sorghum* spp.

The allelic differences revealed by the RFLP analysis support the presence of three non-recombining sub-groups within the population of *Colletotrichum* strains affecting *Sorghum* spp. in the southeastern U.S.

## Summary

I had three predictions for this chapter: (1) that the sibling species *C. sublineola* and *C. graminicola* would differ in the presence of one or more genes predicted to encode SSPs and SSM-associated proteins (aka. pathogenicity genes); (2) that pathogenicity genes found in *C. sublineola* but not in *C. graminicola* would be conserved among all members of the population infecting *Sorghum bicolor* in Kentucky and the southeastern U.S. (suggesting the possibility that they could be involved in triggering ETI in maize); and (3) that populations affecting *S. bicolor* would be distinguishable from populations affecting *S. halepense* by allelic differences in individual pathogenicity genes, ie. allelic fixation (suggesting that the populations are genetically isolated).

Prediction 1 was upheld. *Colletotrichum graminicola* and *C. sublineola* differed in the presence or absence of a large number of putative SSP and SSM genes and gene clusters. Prediction 2 was mostly supported. Analysis of RFLPs using probes against pathogenicity genes that were shared between *C. sublineola* and *C. graminicola* (CSE33, CSE53, CSE56, CSSM2, CSE21 and CSE24) revealed that all of them were present in all isolates. Among the genes that were not conserved with *C. graminicola* (CSE1, CSE4, CSE6, CSE12, CSE23, CSE29, and CSSM3), most were also shared among all the strains. However, one of the genes (CSE6) was absent from the orange group of strains from johnsongrass, and another (CSSM3) was not found among the members of the blue group. Prediction three was upheld, with the caveat that allelic variation differentiates two groups within the johnsongrass isolates, rather than one, and that one of those groups also includes a strain from sweet sorghum (SS1.001). These groups are consistent with pathogenicity assays in which one (the orange group) appears to be host-specific for johnsongrass while the other

(the blue group) can affect either johnsongrass or sweet sorghum. The evidence points to these three groups being genetically isolated from one another.

Table 4.1. Primers used as probes

| Gene                     | Primer name | Sequences of Primers                                  | Notes               |
|--------------------------|-------------|---|---------------------|
| CSUB_000030              | CSE1        | 5'GGACAACGACAGAGCATCCC3'<br>5'TGTCTGCAGTGGAGGAGGAG3'  | UNIQUE Csub         |
| CSUB_000060              | CSE4        | 5'TGTGTAGCTGAACGTCGCAT3'<br>5'GTTTGCTGTCGGGACACACT3'  | UNIQUE Csub         |
| CSUB_000110              | CSE6        | 5'GCTATGGGAGCCCTAGCAGA3'<br>5'CTAGCATTTCACACAGCGGC3'  | UNIQUE Csub         |
| CSUB_000120              | CSE12       | 5'ATTATTGCTACCCTCGCGGC3'<br>5'GAGAGTGTCTCAGGGTGGG3'   | UNIQUE Csub         |
| CSUB_000150              | CSE17       | 5'AGCTTCTGGCTTTAGTCGCC3'<br>5'CTATCAGTCGTGCCCGTA3'    | UNIQUE Csub         |
| CSUB_054660              | CSE21       | 5'GAAGGCCGTCACTATCCTCA3'<br>5'CAGGCGAAGGATCGGAAG3'    | SHARED<br>CsubCgram |
| CSUB_014250              | CSE23       | 5'CACCTAGGCCAGACCAAATG3'<br>5'TCACTACTTCCTCCGTCTTGG3' | UNIQUE Csub         |
| CSUB_101250              | CSE24       | 5'GTCCCACCAATTTGGACTTG3'<br>5'CTCGTCGTTCTAGGCTCACC3'  | SHARED<br>CsubCgram |
| CSUB_133010              | CSE29       | 5'TGCGTCCTCAATGTGTTTTG3'<br>5'CGAGACTTGGAGCGGAGATA3'  | UNIQUE Csub         |
| CSUB_020960              | CSE33       | 5'TGCAGGTCGTAACAAAGTGG3'<br>5'CTCATTATGCACGCCATCAC3'  | CsubCgramChig       |
| CSUB_054650              | CSE53       | 5'CTTTCCGCCCACATCCT3'<br>5'ACAAAATTTCCGGGTCGAT3'      | CsubCgramChig       |
| CSUB_075920              | CSE56       | 5'TTGGCTTCTTTGGGAATACG3'<br>5'GGACAATCCGGTCAGACCTA3'  | CsubCgramChig       |
| CSUB_020320<br>aka PKS14 | CSSM2       | 5'GGCCTACCACGAGACTACCA3'<br>5'TTGGCTGTGTGATACCTTGC 3' | CsubCgramChig       |
| CSUB_060040<br>aka PKS40 | CSSM3       | 5'GTCACCTTGATGCCCGATTT3'<br>5'GGTCTCGCTAGGACATTGGA3'  | UNIQUE Csub         |



Table 4.2. Ortho-MCL prediction of shared SSM genes for the three species of *Colletotrichum*. PKS, polyketide synthases; NRPSs, non-ribosomal peptide synthetases; PKS-NRPS hybrids that contain at least one PKS and at least one NRPS domain; DMAT, dimethylallyl transferases; and TS, terpene synthases. The numbers in parentheses represent the total number of genes in each category based on Pfam predictions for *C. sublineola*. \*Manual annotation was performed on data retrieved from O’Connell et al, 2012.

| Type     | <i>C. sublineola</i> | <i>C. graminicola</i> * | <i>C. higginsianum</i> * |
|----------|----------------------|-------------------------|--------------------------|
| PKS      | 31 (31)              | 36                      | 36                       |
| NRPS     | 8 (8)                | 7                       | 12                       |
| PKS-NRPS | 6 (6)                | 4                       | 3                        |
| DMAT     | 8 (9)                | 7                       | 10                       |
| TS       | 14 (17)              | 14                      | 17                       |

Figure 4.1. Alignments of sequences of CgSl1 with species type S3.001. A: actin, B: chitin synthase, C: histone H3, D: beta-tubulin, E: ITS. Alignments were performed with MUSCLE version 3.7.

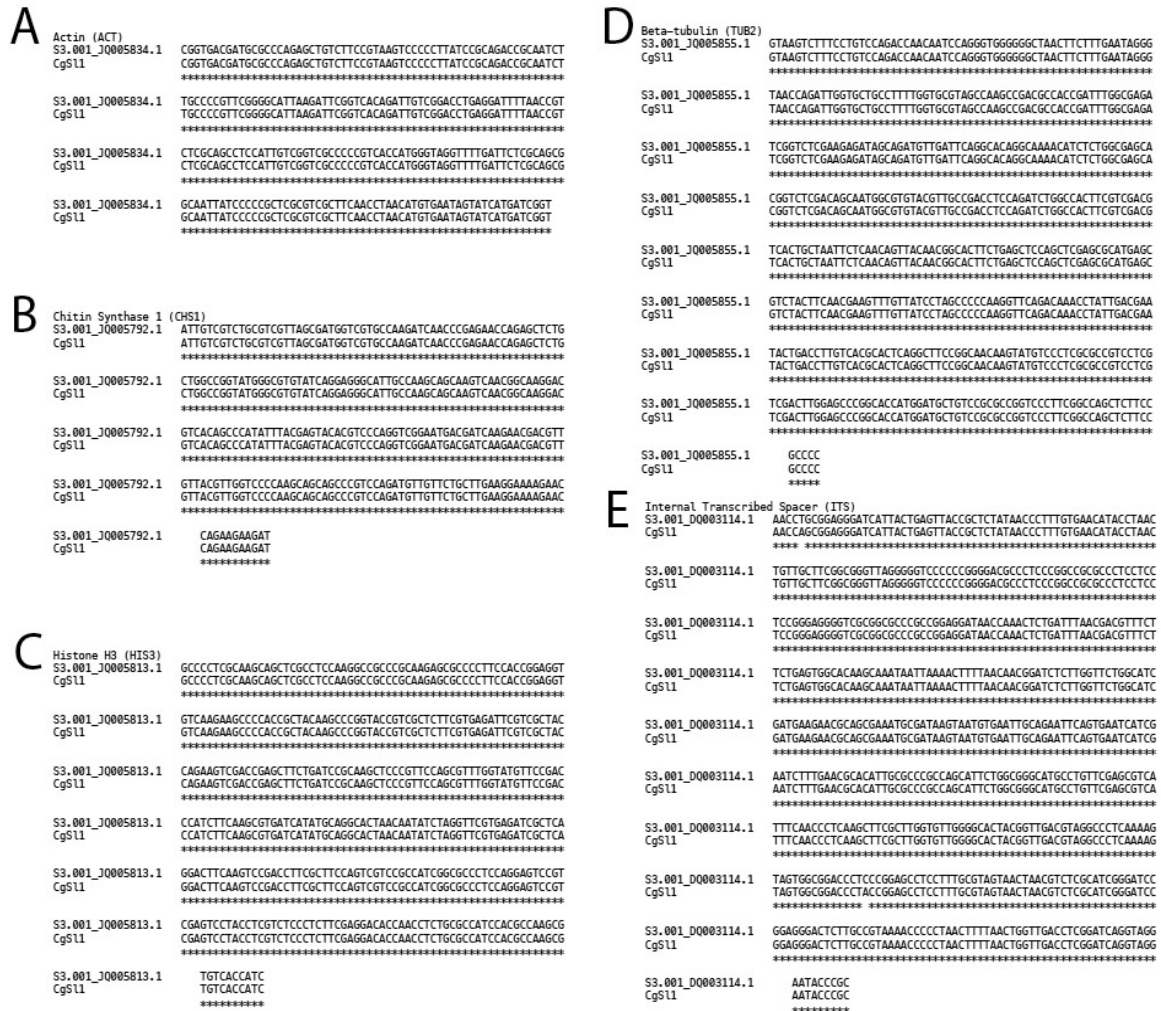


Figure 4.2. A, maize inoculated with *C. graminicola*, B, sorghum inoculated with *C. sublineola*, C, maize inoculated with *C. sublineola*, and D, sorghum inoculated with *C. graminicola*, E, maize mock inoculated with water, and F, sorghum mock inoculated with water. All at 7 dpi.

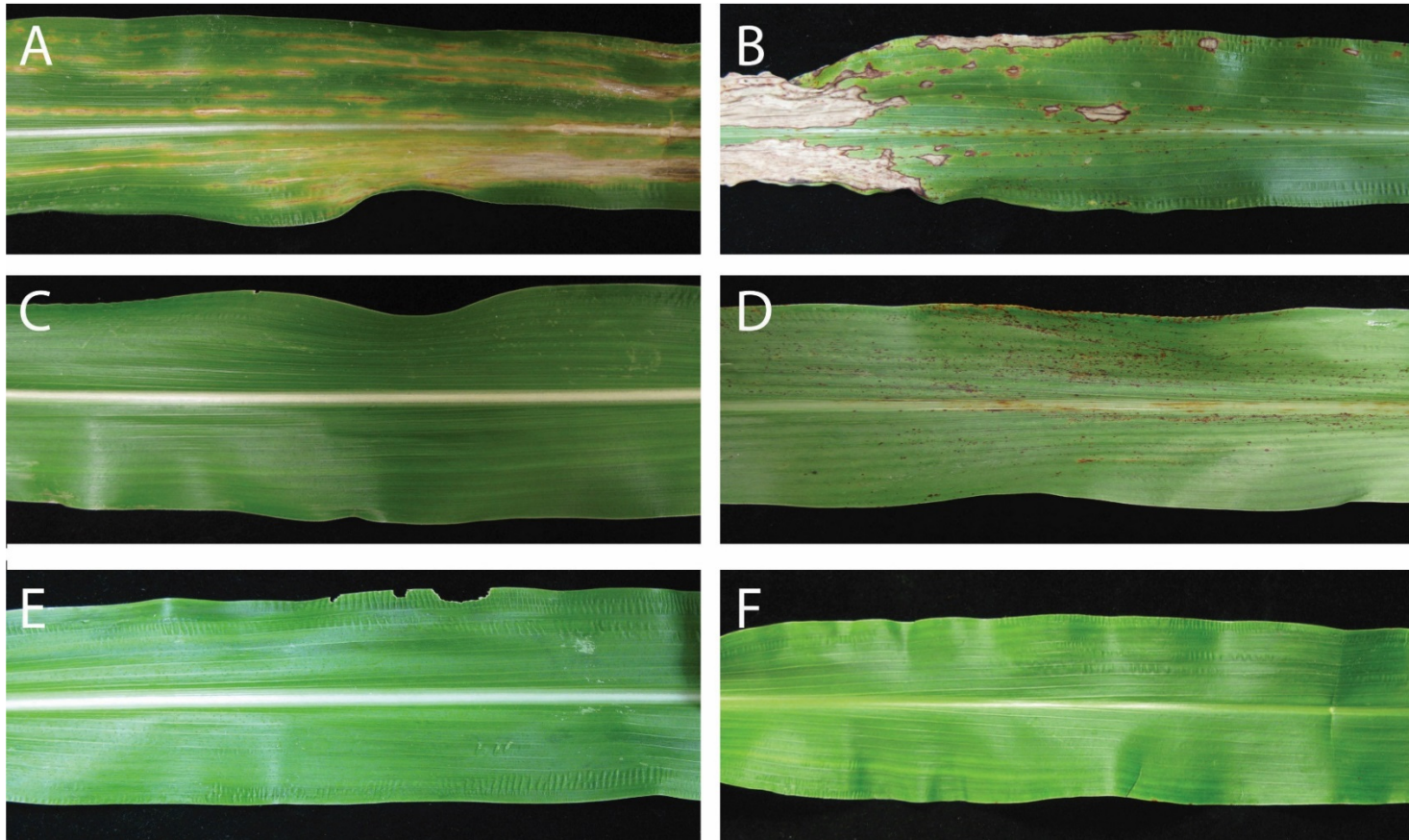


Figure 4.3. A, *C. graminicola* hyphae in maize leaves, 48 dpi. B. *C. sublineola* hyphae in sorghum leaves, 72 dpi. C. *C. graminicola* on sorghum, 48 dpi, white arrow indicates red vesicles surrounding the appressorium. D. *C. sublineola* on maize, 48 dpi, white arrow indicates an iridescent papillum beneath a melanized appressorium. Scale bars equal to 50  $\mu$ m.

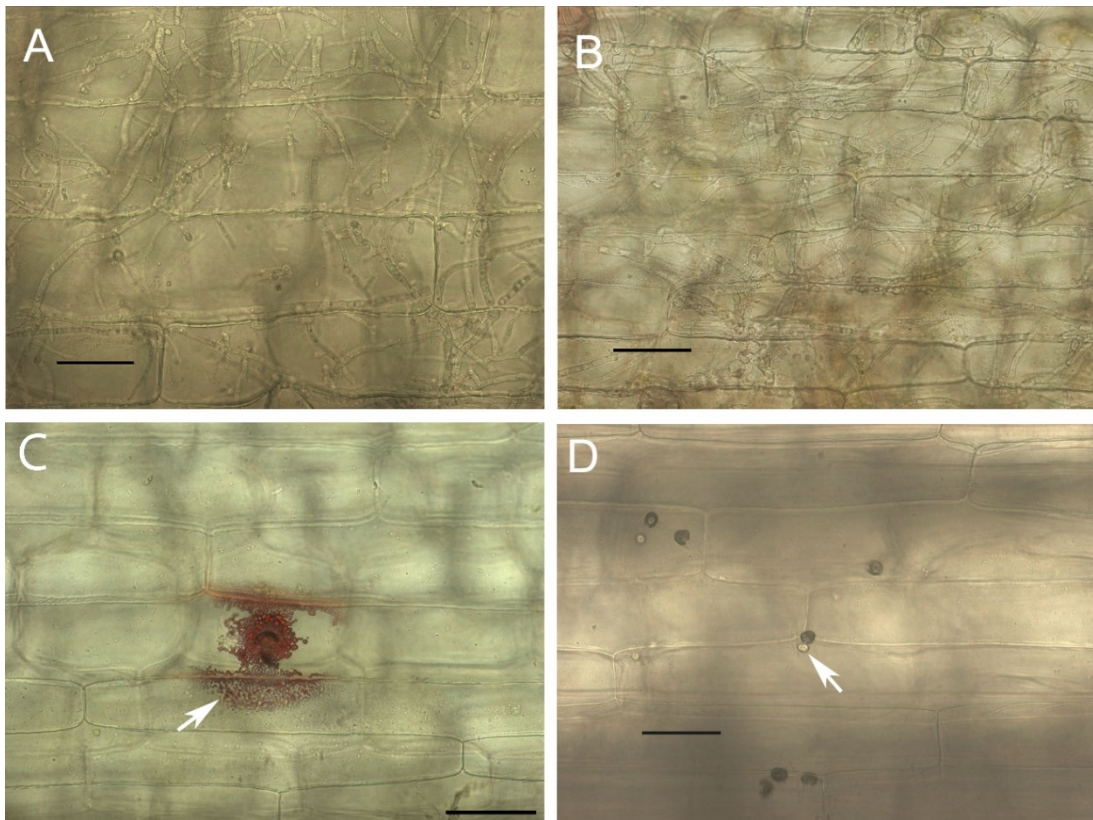


Figure 4.4. A. CgS11 on maize sheath, 48hpi. Cell beneath appressorium (white arrow) plasmolyzes normally. B. CgS11 on maize sheath, small penetration hypha (white arrow) 48hpi. Adjacent cell (black arrow) plasmolyzes normally. Cell containing penetration hypha appears granulated, plasma membrane visible but appears abnormal. C. M1.001 on sorghum sheath, 24 hpi., cell beneath appressorium (white arrow) plasmolyzes normally. D. M1.001 on sorghum sheath, 48 hpi. Treated with sucrose but no plasmolysis evident in any of the cells in the vicinity of the appressoria (white arrows). Scale bars equal to 50  $\mu\text{m}$ .

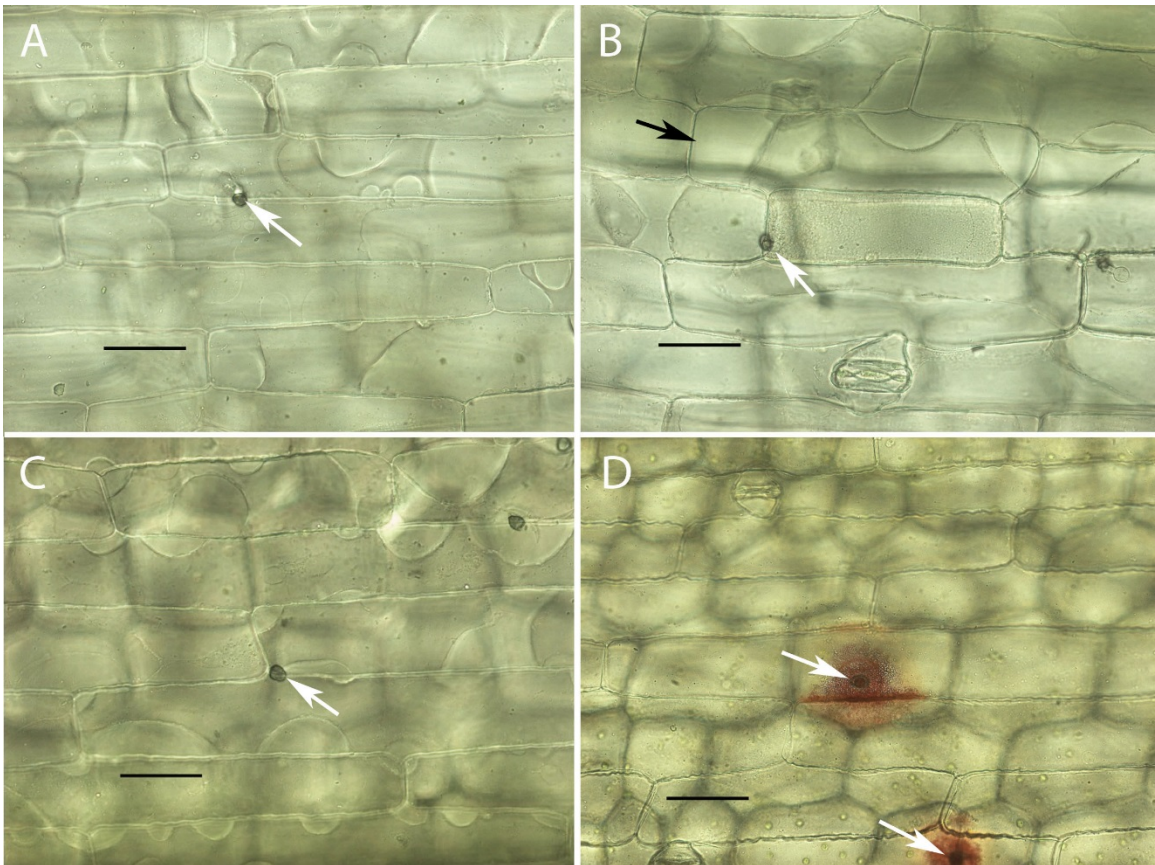


Figure 4.5. M1.001 on Sugar Drip sorghum, 48 hpi, cells beneath appressoria (white arrows) plasmolyzed (result not typical). Scale bars equal to 50  $\mu$ m.

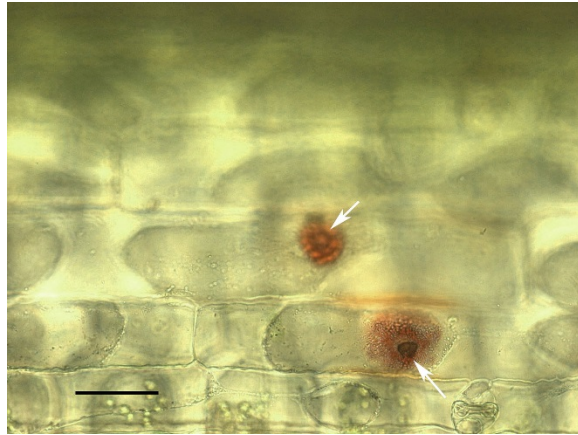


Figure 4.6. Plasmolysis controls. A: Maize leaf sheath, 72 hours after mock inoculation, most cells still plasmolyze. B: Sugar Drip leaf sheath, 72 hours after mock inoculation, most cells still plasmolyze. Scale bars equal to 50  $\mu\text{m}$ .

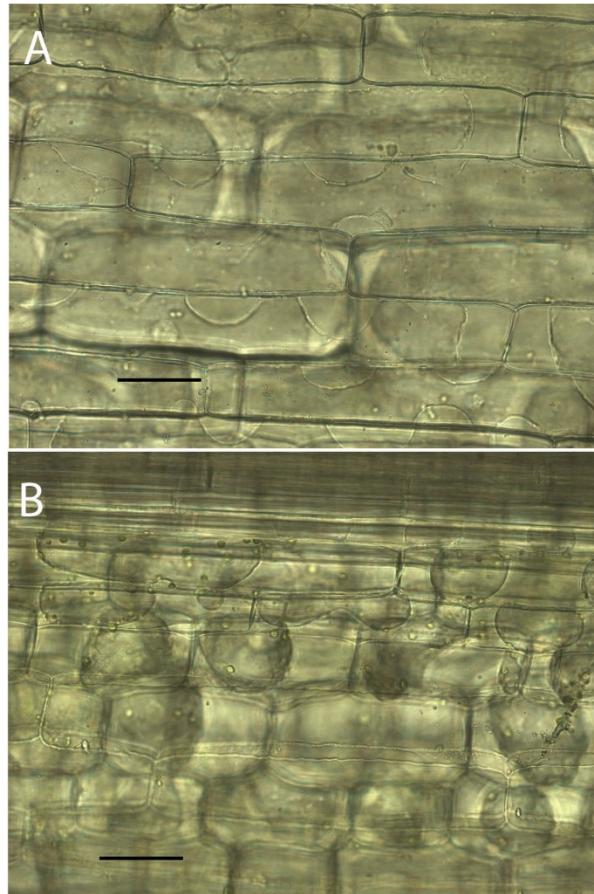
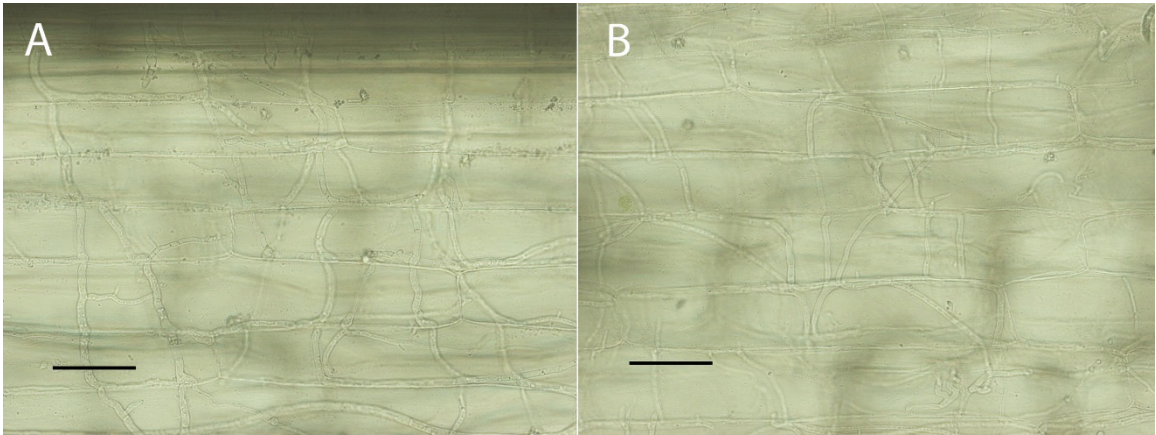


Figure 4.7. A. CgS11 growing in cells of maize sheaths killed by liquid nitrogen, 48 hpi. B. M1.001 growing in cells of sorghum sheaths killed by liquid nitrogen, 48 hpi. Scale bars equal to 50  $\mu\text{m}$ .





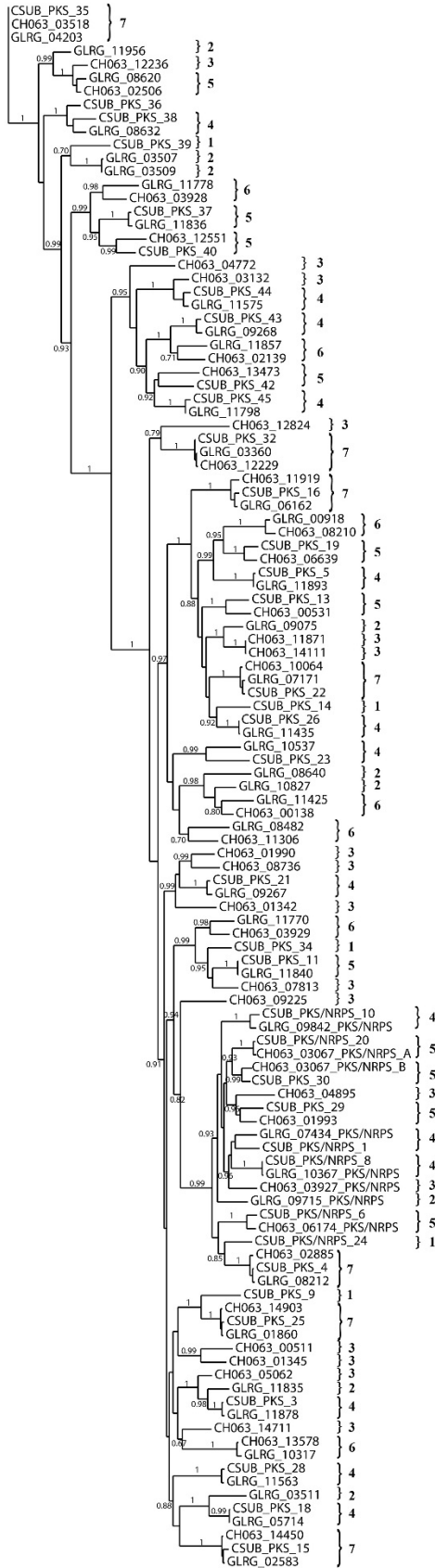


Figure 4.8. Phylogenetic tree of the ketoacyl CoA synthetase domain sequences of putative PKSs and PKS-NRPS hybrids. Sequences were aligned by using MUSCLE version 3.7, and phylogenies were inferred by maximum-likelihood using PhyML version 3-0 Statistical. The numbers on the branch nodes indicate support values above 50%, calculated by an approximation to the standard Likelihood Ratio Test, aLRT. Brackets refer to proteins found only in (1) *C. sublineola*, (2) *C. graminicola*, (3) *C. higginsianum*; (4) *C. sublineola* and *C. graminicola*, (5) *C. sublineola* and *C. higginsianum*, (6) *C. graminicola* and *C. higginsianum*, (7) *C. sublineola*, *C. graminicola* and *C. higginsianum*.

Figure 4.9. A. Phylogenetic tree of the terpene synthase sequences. Sequences were aligned by using MUSCLE version 3.7, and phylogenies were inferred by maximum-likelihood using PhyML version 3-0 Statistical. The numbers on the branch nodes indicate support values above 50%, calculated by an approximation to the standard Likelihood Ratio Test, aLRT. Brackets refer to proteins found only in (1) *C. sublineola*, (2) *C. graminicola*, (3) *C. higginsianum*; (4) *C. sublineola* and *C. graminicola*, (5) *C. sublineola* and *C. higginsianum*, (6) *C. graminicola* and *C. higginsianum*, (7) *C. sublineola*, *C. graminicola* and *C. higginsianum*.

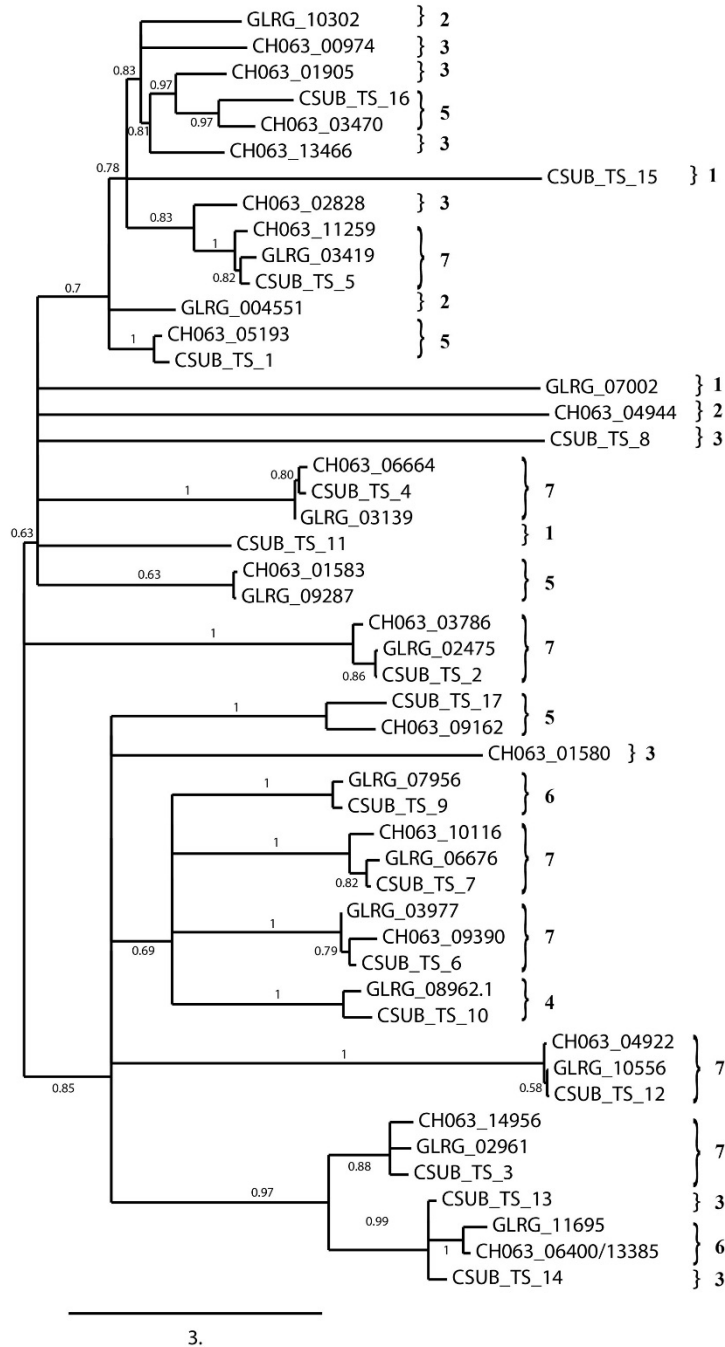
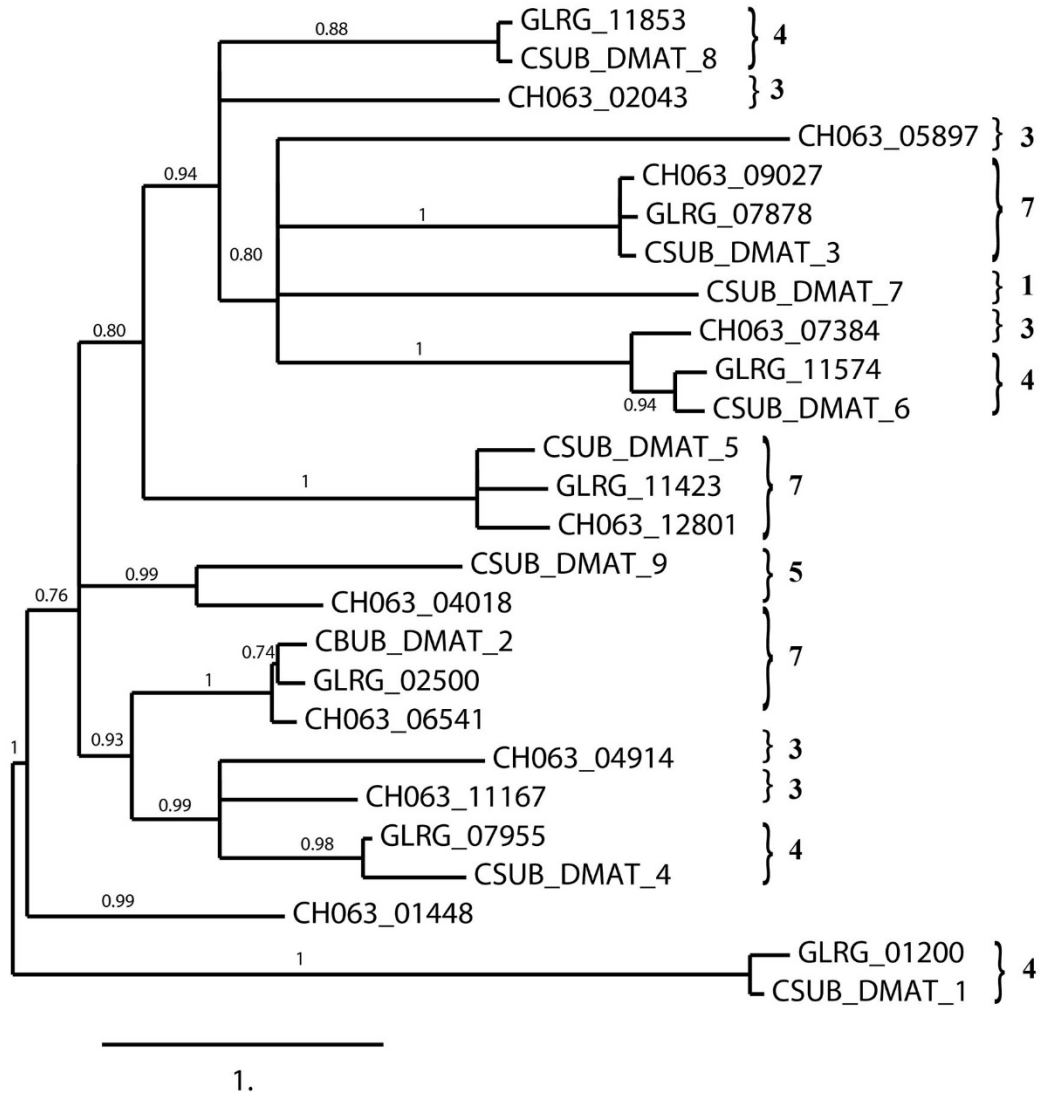


Figure 4.10. Phylogenetic tree of the dimethylallyl transferase sequences. Sequences were aligned by using MUSCLE version 3.7, and phylogenies were inferred by maximum-likelihood using PhyML version 3-0 Statistical. The numbers on the branch nodes indicate support values above 50%, calculated by an approximation to the standard Likelihood Ratio Test, aLRT. Brackets refer to proteins found only in (1) *C. sublineola*, (2) *C. graminicola*, (3) *C. higginsianum*; (4) *C. sublineola* and *C. graminicola*, (5) *C. sublineola* and *C. higginsianum*, (6) *C. graminicola* and *C. higginsianum*, (7) *C. sublineola*, *C. graminicola* and *C. higginsianum*.



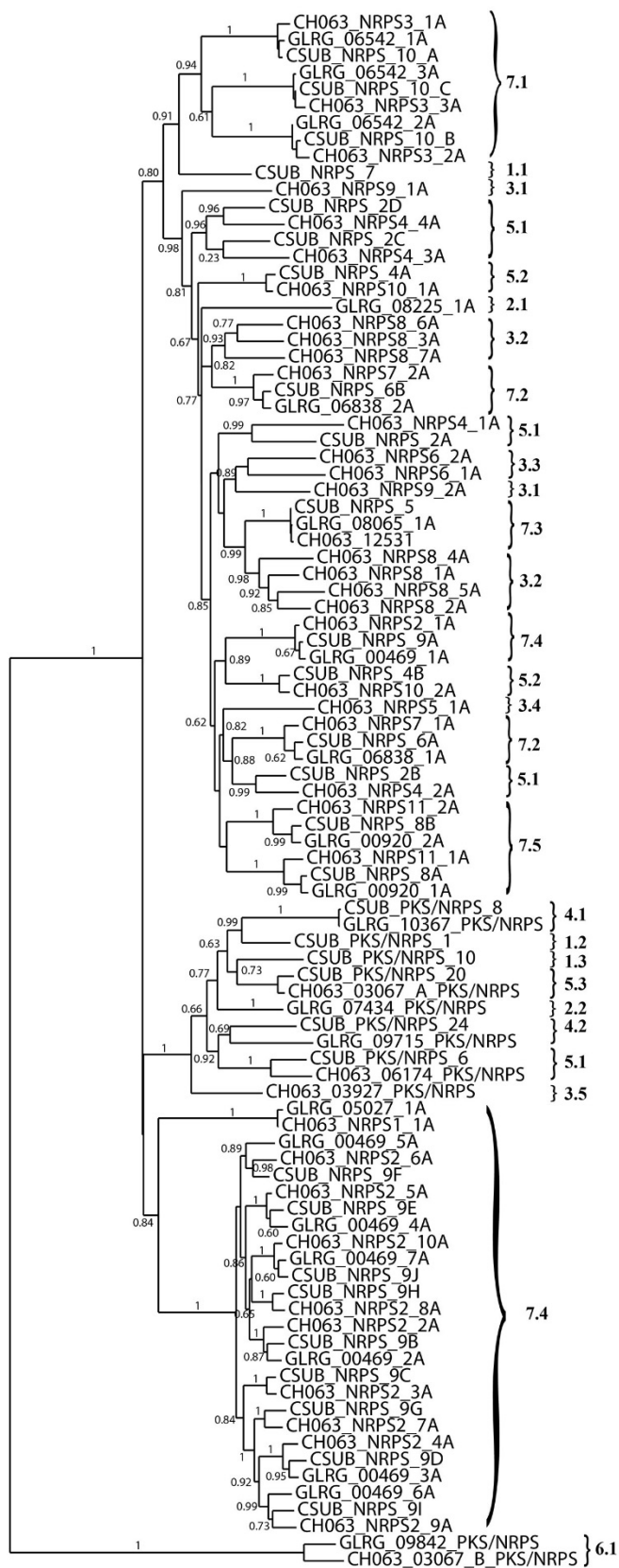
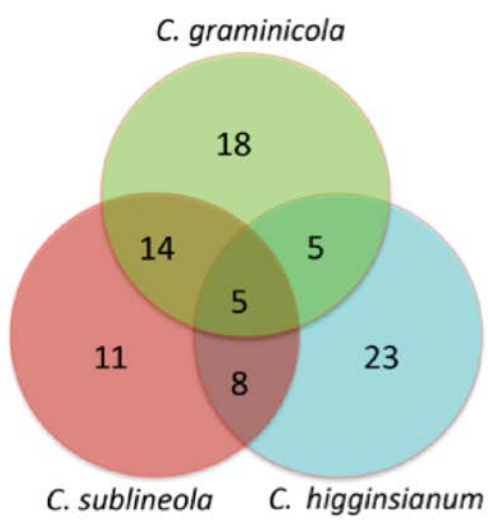
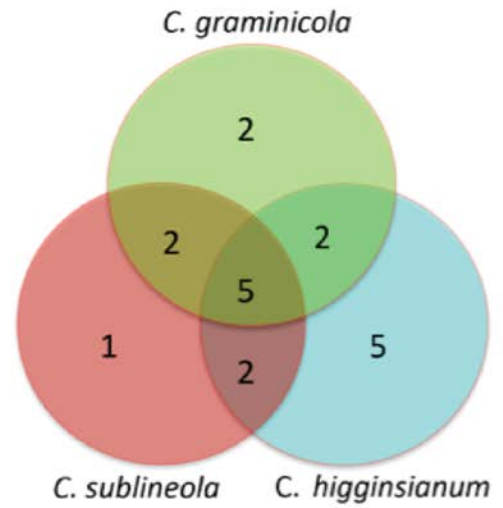


Figure 4.11. Phylogenetic tree of the AMP binding domains of putative NRPS and PKS-NRPS hybrids. Sequences were aligned by using MUSCLE version 3.7, and phylogenies were inferred by maximum-likelihood using PhyML version 3-0 Statistical. The numbers on the branch nodes indicate support values above 50%, calculated by an approximation to the standard Likelihood Ratio Test, aLRT. Brackets refer to proteins found only in (1) *C. sublineola*, (2) *C. graminicola*, (3) *C. higginsianum*; (4) *C. sublineola* and *C. graminicola*, (5) *C. sublineola* and *C. higginsianum*, (6) *C. graminicola* and *C. higginsianum*, (7) *C. sublineola*, *C. graminicola* and *C. higginsianum*. The number after the decimal point refers to different domains of the same gene.

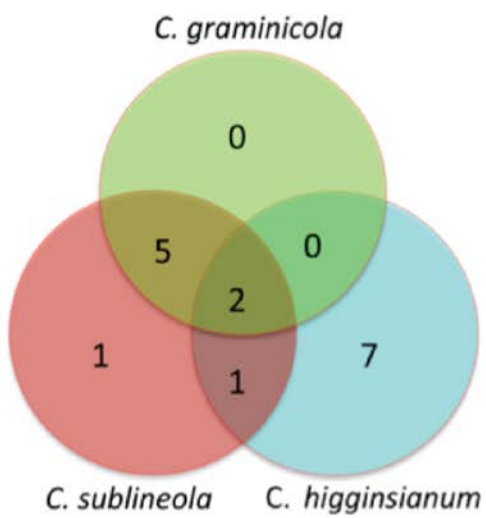
Figure 4.12. Venn diagram summarizing the numbers of shared and unique sequences among the three species.



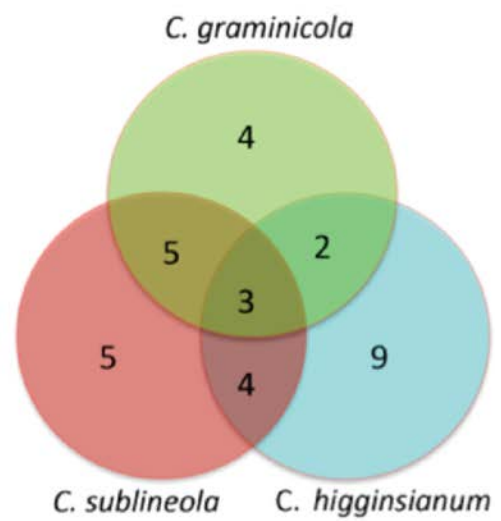
PKS/Hybrid



NRPS/Hybrid



DMAT



TS

Figure 4.13. The organization of the conserved melanin gene clusters from *Colletotrichum sublineola*, *C. graminicola* and *C. orbiculare* are shown, with related genes depicted in the same color. The gray genes encode hypothetical proteins. Microsynteny is indicated by the gray bars.

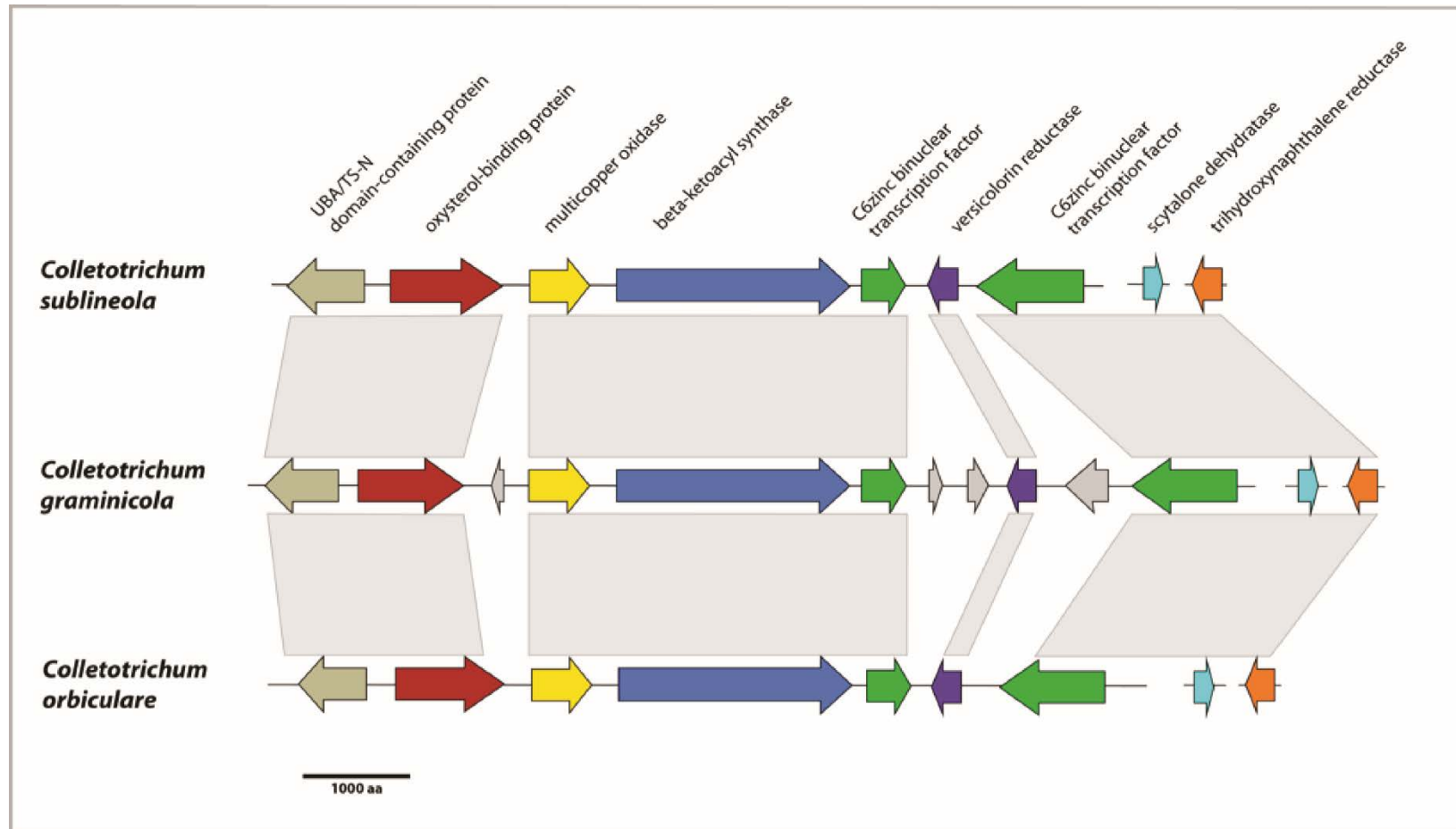


Figure 4.14. The organization of radicicol (RADS) gene clusters from *Pochonia chlamydospora*, *Colletotrichum graminicola* and *C. sublineola* are shown, with related genes depicted in the same color. Microsynteny is indicated by the gray bars.

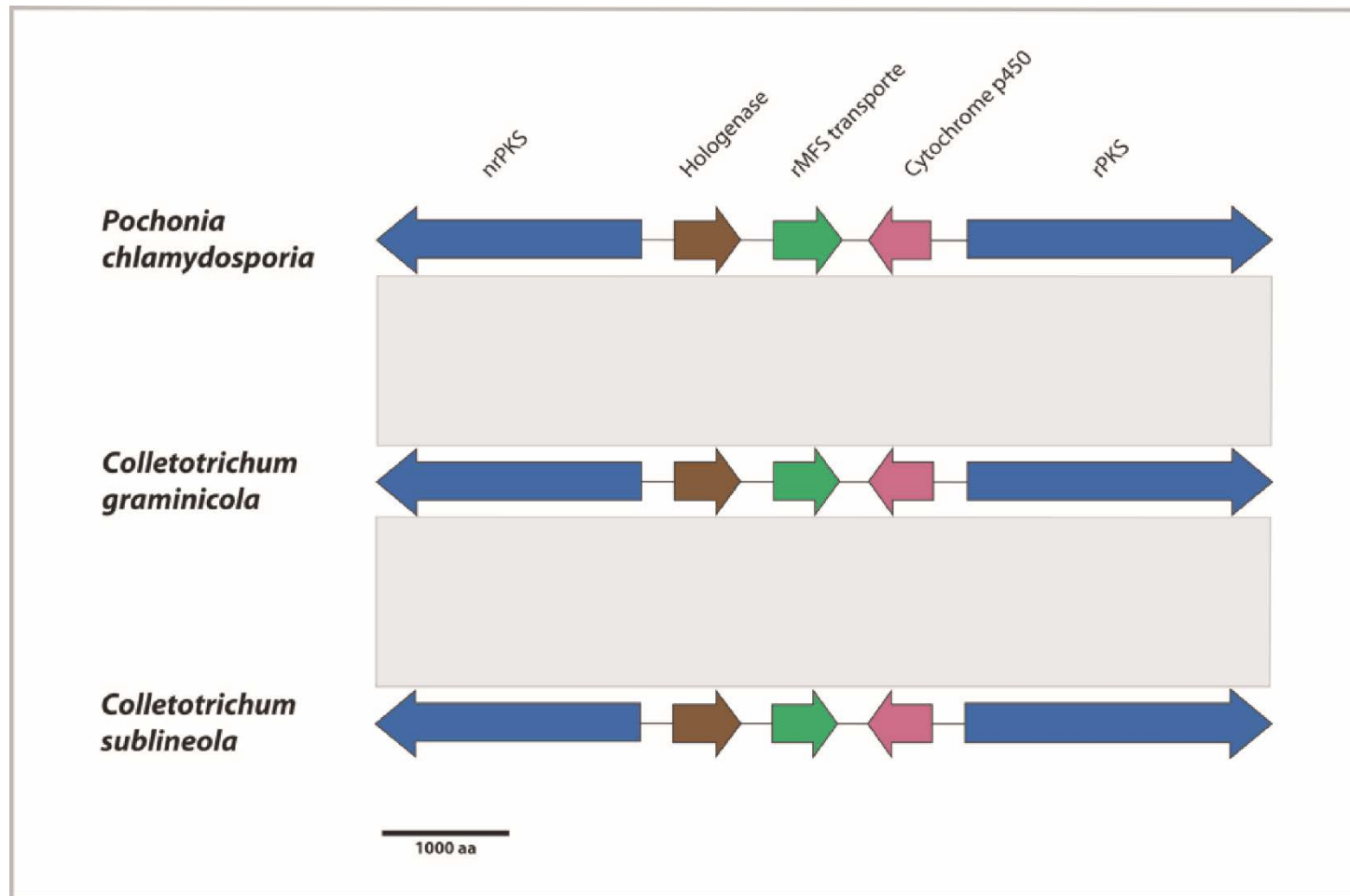


Figure 4.15. Diagrams of representative SM gene cluster (PKS 42) that appears to be unique to *C. sublineola*. Microsynteny is indicated by the gray bars.

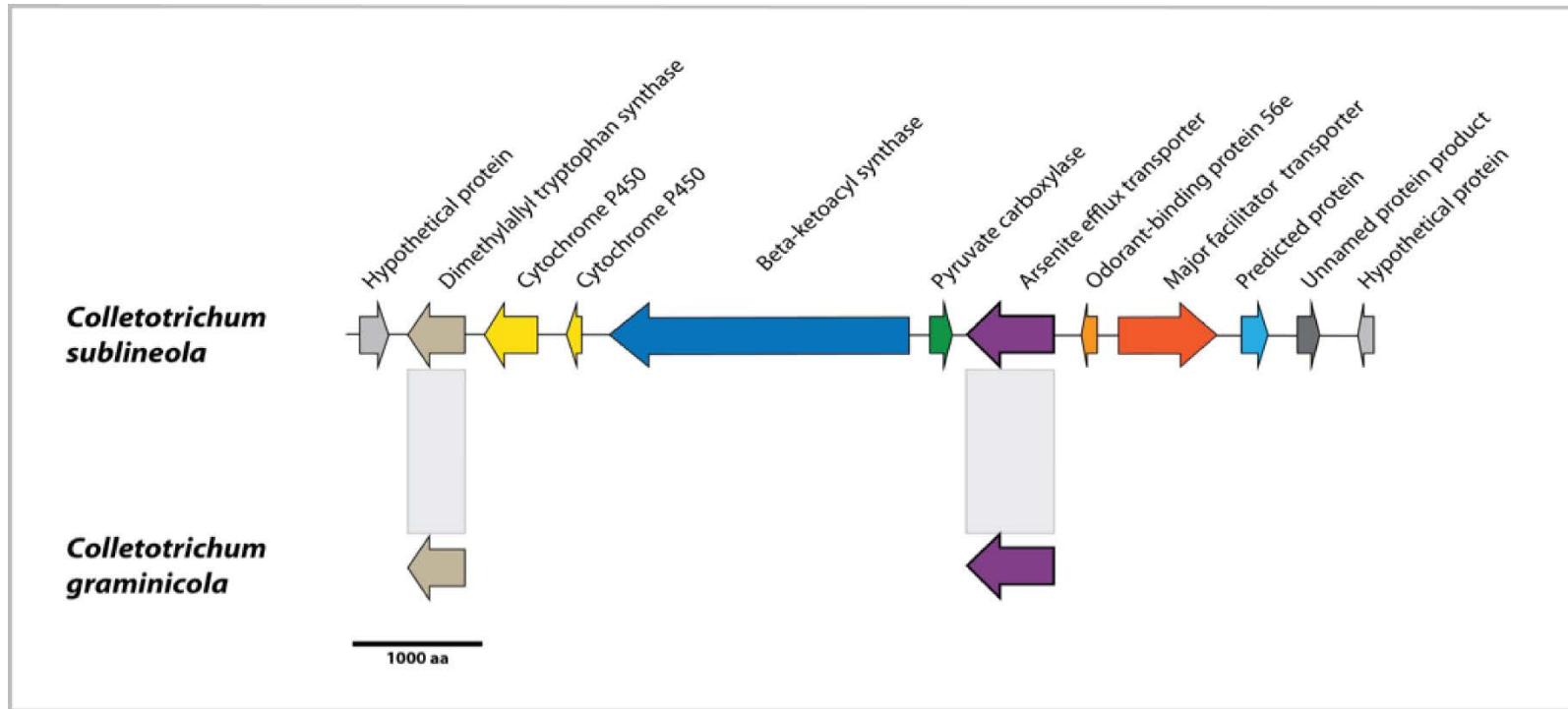




Figure 4.16. Diagrams of representative PKS gene cluster that appears to be present in *C. graminicola* but not in *C. sublineola*.

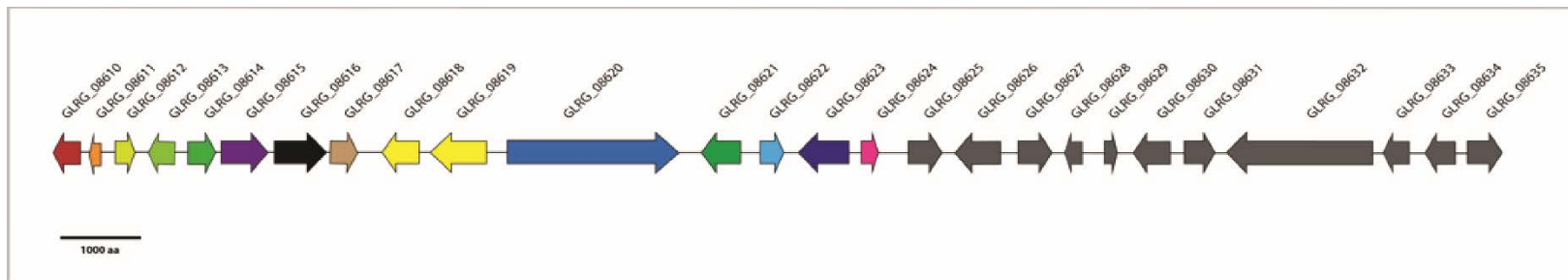


Figure 4.17. An example of a NRPS (NRPS 7) gene cluster unique *Colletotrichum sublineola* in comparison with *C. graminicola*, with related genes depicted in the same color. The gray genes encode hypothetical proteins. Microsynteny is indicated by gray area.

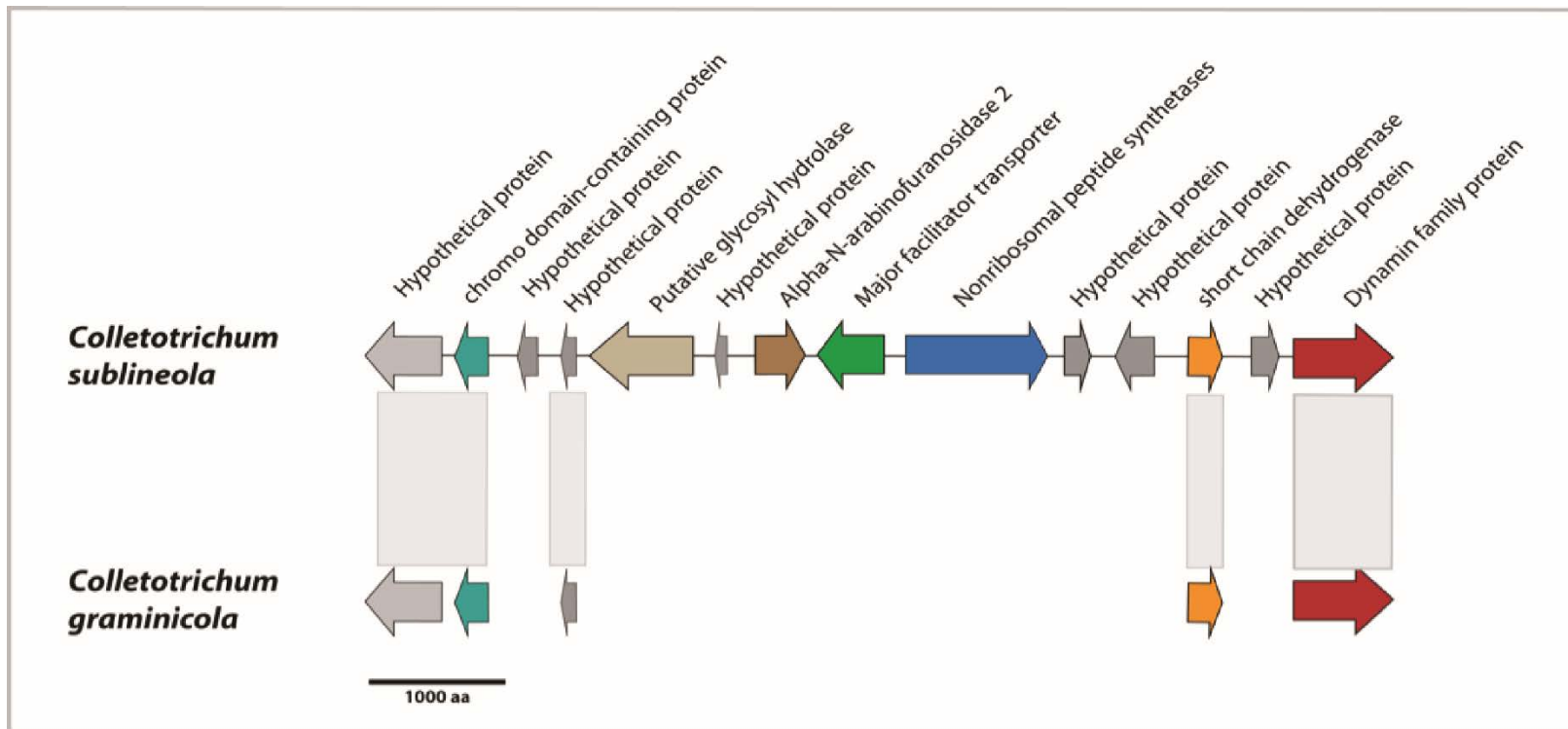


Figure 4.18. Southern blot analysis of genomic DNA digested with HindIII. *Colletotrichum sp.* strain name is indicated on each lane. Blot was hybridized with (A) CSE6, (B), CSE21, (C) CSSM3, and (D) actin probe control.

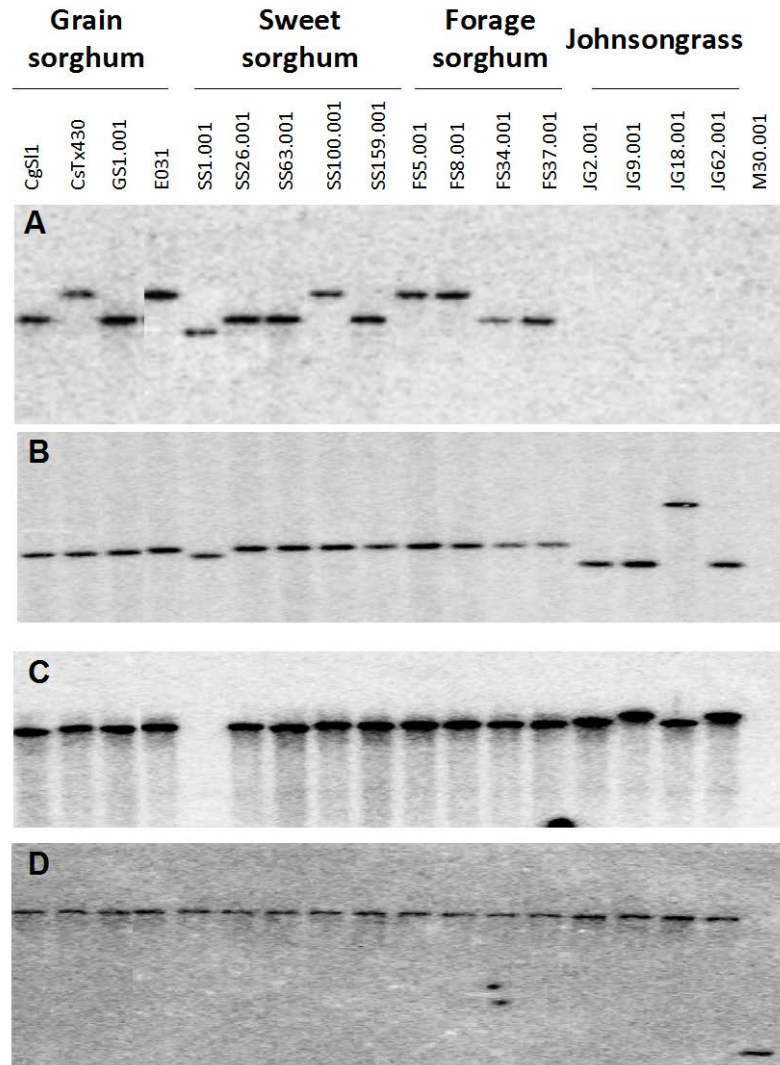
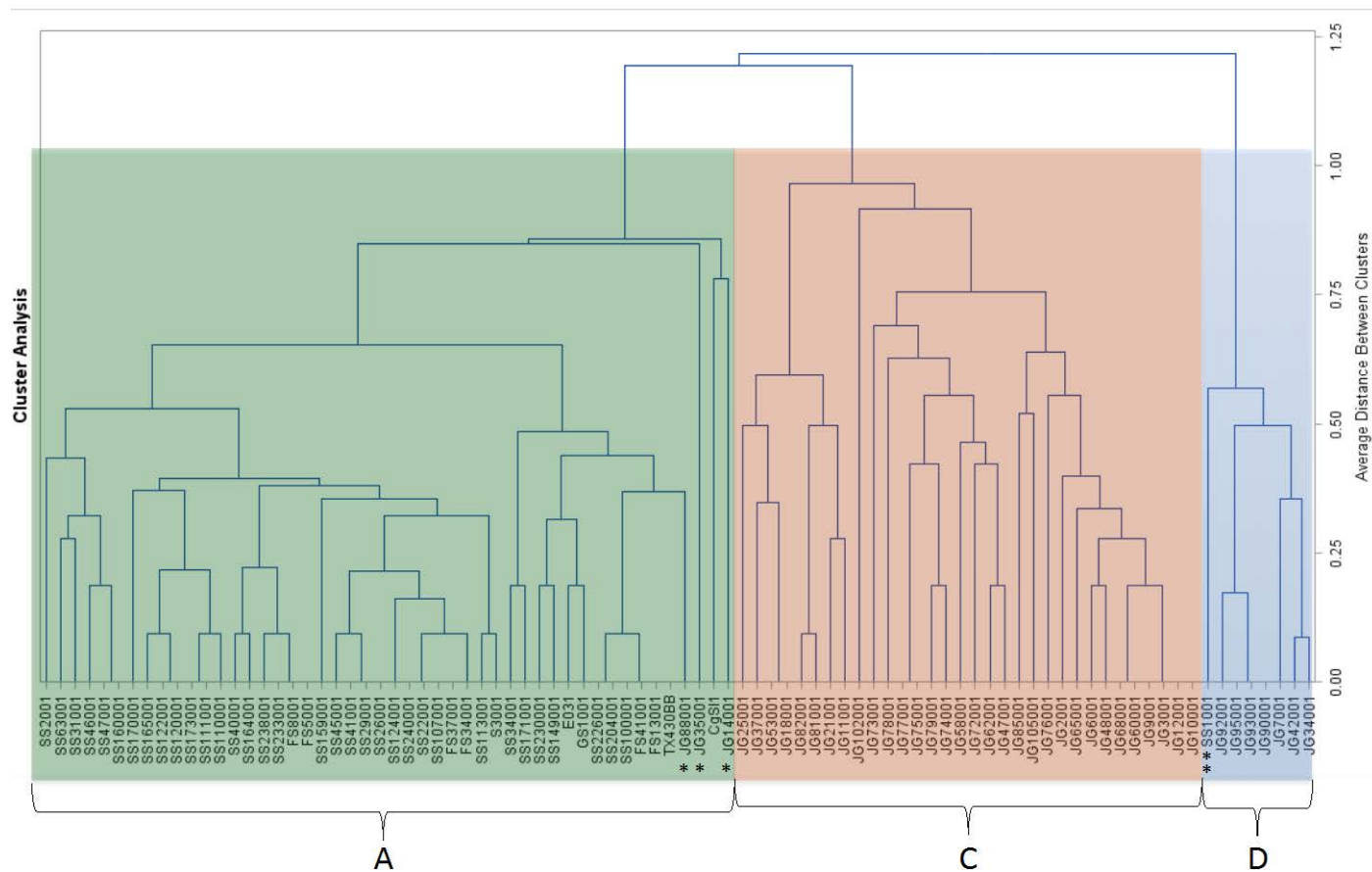


Figure 4.19: Dendrogram illustrating the relatedness of isolates from *Sorghum halepense* (JG) (blue box and orange box), and *S. bicolor* (green box), which includes isolates from grain (GS1.001, S3.001, CgS11, and E031), sweet (SS) and forage (FS) sorghum, based on single locus probes using RFLP. There were some exceptions, marked with (\*) isolates from *S. halepense* in the *S. bicolor*



## **Chapter 5 - Identification of two new species of *Colletotrichum* infecting *Sorghum bicolor* and *S. halepense* in the Southeastern United States**

### **Abstract**

*Colletotrichum sublineola* reportedly causes anthracnose disease on grain, forage and sweet sorghum (*Sorghum bicolor*), and also on the weed johnsongrass (*S. halepense*). My goal in this chapter was to analyze populations of *Colletotrichum* from cultivated and wild sorghum by evaluating individual genetic markers that are relevant to phylogenetic species determinations within this group of *Colletotrichum* fungi. Phylogenetic analysis was performed using 32 representative *Colletotrichum* strains that had been isolated from johnsongrass, and from forage, grain, and sweet sorghum. Phylogenetic trees were inferred based on portions of the DNA lyase gene (*Apn2*); the manganese superoxide dismutase gene (*Sod2*); and a region between the *Apn2* and the *Mat1* genes (*Mat1/Apn2*). The trees distinguished *C. sublineola* from two novel species. One of these new species was associated only with *S. halepense* throughout the southeastern U.S. Accordingly, I named this species *Colletotrichum halepense* (Xavier & Vaillancourt). There was evidence for cross-infection of *S. bicolor* and *S. halepense* by *C. sublineola* and by the second new species, which I named *C. caselae* (Xavier & Vaillancourt). These findings have significant implications for the development and deployment of resistant sweet sorghum varieties in areas where johnsongrass is common. This information will help to evaluate the potential for *Colletotrichum* spp. to cause epidemics in sweet sorghum if acreages continue to expand in the SE in the future.

**Taxonomic novelties: Description of new species:** *C. halepense* (Xavier & Vaillancourt) and *C. caselae* (Xavier & Vaillancourt).

## **Introduction**

Anthrachnose, caused by the fungus *Colletotrichum sublineola*, is the most important disease of sorghum (*Sorghum bicolor*) worldwide (3, 29, 71, 92, 193). The primary control for anthracnose in grain sorghum is the use of resistant cultivars, although no cultivar is immune to the disease (28). Furthermore, resistance frequently fails, which is thought to be related to the high degree of genetic variability in the population of *C. sublineola* affecting grain sorghum (25, 27).

Sweet sorghum has been grown in the Southeastern U.S. for more than 150 years, but it has been on a limited scale, primarily for forage and for the production of syrup. Recently, interest in sweet sorghum as a potential source of biofuel has increased in the U.S., especially in the Gulf States, where infrastructure used for the sugar cane industry could be converted to the production of ethanol from sorghum juice and biomass (102). Anthracnose is becoming common on the hybrids that are increasingly grown across the region (80). However, relatively little is known the populations of *Colletotrichum* that affect sweet sorghum in the region.

*C. sublineola* is also reported to be a pathogen of the weed johnsongrass (*Sorghum halepense*), which is related to *Sorghum bicolor* (194), and which is ubiquitous across the southeastern U.S., often found within and alongside fields of cultivated sorghum. Johnsongrass is known to be genetically highly diverse (120), and this diversity could result in balancing selection that maintains multiple pathotypes within the pathogen population.

Thus, johnsongrass could serve as a refuge and an incubator for genetic diversity, which could impact the durability of resistance genes deployed in nearby cultivated sorghum crops. Rosewich (1996) found that isolates from johnsongrass belonged to a population that was distinct from isolates from the grain sorghum varieties grown in nearby fields in Texas, suggesting that cross-infection did not occur in that location (144). My own analyses of genetic diversity using fingerprinting markers confirmed that most isolates from johnsongrass were genetically distinct from most isolates on cultivated sorghum in the southeastern U.S. I also found that most johnsongrass isolates were not aggressive to *S. bicolor* (chapter 3 of this dissertation). However, I did find evidence suggesting that a minority of isolates were capable of cross-infection of both hosts (chapter 3 of this dissertation). Analysis of allelic diversity at several putative pathogenicity loci showed that the populations of *Colletotrichum* from *S. bicolor* and from *S. halepense* could be separated into three distinct groups (chapter 4 of this dissertation). These groups were differentiated from one another by the presence or absence of specific alleles of several of these pathogenicity genes (aka apparent allelic fixation) (chapter 4 of this dissertation).

My goal in this final chapter of my dissertation was to test the hypothesis that the three groups identified in chapter 4 represent different species. Sequences from the *Sod2* gene, the *Apn2* gene, and from the intergenic region between *Apn2* and *Mat1*, have been used previously to identify new species within the *C. graminicola* species complex that affects graminaceous hosts (40). To test my hypothesis, I analyzed these sequences, as well as measuring various morphological traits in culture, for several representatives of each of the three groups of *Colletotrichum* isolates.

## Material and Methods

**Fungal Strains:** A total of 32 isolates from sweet, forage, and grain sorghum (*S. bicolor*), and from johnsongrass (*S. halapense*), from a variety of locations were used for this study (Table 5.1). The strains were chosen to represent the genetic diversity that was revealed by the fingerprinting, pathogenicity, and RFLP analyses (chapters 3 and 4 of this dissertation). Isolates were purified by single-sporing, and preserved on silica gel at -80°C (179). Fungal strains were routinely cultured on PDA at 23°C under continuous fluorescent light.

**Multigene Phylogenetic Analysis:** A multigene phylogenetic analysis of the 32 representative strains of *Colletotrichum* was performed. The primer pair ITS4 and ITS5 was used to amplify the internal transcribed spacer (ITS) sequence of the rDNA repeats (200). The primer pair SOD507F and SOD507F/R was used to amplify a 533bp fragment of the manganese superoxide dismutase gene *Sod2* (39). In order to amplify the DNA lyase gene (*Apn2*) the primers Apn1W1F and Apn1W1R were used, generating a 799bp fragment (40). Primers MAT1/APN2F and MAT1/APN2R were used in an attempt to amplify the region between the *Apn2* gene and the *Mat1* gene (*Mat1/Apn2*) (40). However, these primers failed to amplify a product from any of the *Colletotrichum* strains used in my study. The *Mat1/Apn2* sequence from *C. graminicola* (accession number FJ377991) was then used to search for similar sequences in the genome of the CgS11 strain of *C. sublineola* by blastn. A matching sequence from *C. sublineola* was retrieved, and used to design a new *Mat1/Apn2* primer pair (MAT1/APN2F and MAT1/APN2R), which amplified a product of 860bp from all 32 strains. Primer sequences are shown in Table 5.2.

PCR reactions were performed in a final volume of 25µL containing: 25 ng of genomic DNA, 10X PCR buffer, 50 mM MgCl<sub>2</sub>, 200 µM dNTPs, one U of *Taq* DNA polymerase,



and 5  $\mu$ M of each primer. The PCR cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing for 30 sec at (i) 53°C for *Sod2*, (ii) 51°C for ITS, (iii) 55°C for *Mat1/Apn2*, and (iv) 55°C for *Apn2*; extension at 72°C for 1 min; with a final extension at 72°C for 10 min.

The amplified fragments were visualized on 0.7% agarose gels, and then excised and purified by using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). The purified PCR amplicons were submitted to the University of Kentucky Advanced Genetic Technologies Center (UK-AGTC) for sequencing. Forward and reverse primers were used to sequence the amplicons with the BigDye Terminator cycle-sequencing chemistry (Applied Biosystems, Foster City, CA) on an ABI 3730 DNA Capillary Sequencer following the manufacturer's protocol, except in a reaction volume of 10  $\mu$ L.

I also included sequences from the *C. sublineola* epitype strain CBS131301, aka S3.001, as a control. *Colletotrichum eremochloae*, the closest known relative of *C. sublineola* (41), was included as an outgroup in all the trees. The ITS, *Sod2*, *Apn2*, and *Mat1/Apn2* sequences of these strains were identified by blastn searches of the genome databases that are available from the Joint Genome Institute Genome Portal (<http://genome.jgi.doe.gov>). The sequences were aligned by using MUSCLE version 3.7. Alignments were checked in Geneious Pro (version 5.4.7) and Mesquite (version 3.1), followed by trimming and manual editing and optimization of the alignments as required. Phylogenetic analysis was performed by using phylogeny.fr (<http://www.phylogeny.fr/>) with default parameters. Phylogenies were inferred by maximum-likelihood using PhyML version 3.0. Statistical branch support was provided by an approximation to the standard Likelihood Ratio Test-aLRT (52).

**Morphological analysis:** Fungal cultures were grown on PDA at 23°C under continuous fluorescent light for 14 days. Fungal spores were harvested from the PDA cultures and washed three times with sterile water. A conidial suspension was filtered through a layer of cheesecloth and resuspended in water. The spores were mounted on microscope slides in distilled H<sub>2</sub>O. For the production of appressoria, spores were germinated in drops of deionized water on plastic cover slips in a moist chamber overnight. Hyphopodial appressoria were produced by overlaying small blocks of carrot agar containing mycelia with glass cover slips. The mycelia and cover slips were then placed in Petri dishes containing filter paper moistened with distilled sterile H<sub>2</sub>O. The Petri dishes were sealed with Parafilm and incubated at 23°C under continuous fluorescent light for 4-7 days. The cover slips were removed from the agar and then placed onto drops of water on fresh microscope slides. Lengths and widths of 50 randomly chosen conidia, appressoria, or hyphopodia per strain were measured on a Zeiss Axioscop at 400x magnification with the measurements module of the Openlab computer program.

## **Results**

### **Multigene sequencing**

Four sequences (ITS, *Sod2*, *Mat1/Apn2* and *Apn2*) were amplified from 32 isolates, sequenced, and aligned (Alignments are provided in Appendix 4 of this dissertation). Two clades were well-separated by the ITS sequences (Figure 5.1). One of the clades contained the epitype for *C. sublineola*, S3.001, while the other included five isolates that had previously been grouped together based on fingerprints, belonging to the “blue group” identified by RFLP analysis of putative pathogenicity genes (chapters 3 and 4 of this dissertation). Strain CgS11 belonged to the same clade as S3.001, but it was located on a

long branch due to the presence of a SNP. The closely related species *C. eremochloae* was not separated from *C. sublineola* in the ITS tree.

A phylogenetic tree constructed by using an alignment of the concatenated *Sod2*, *Apn2*, and *Mat1/Apn2* sequences resolved three major clades (Figure 5.2). The alignment of the concatenated gene sequences included 2018bp of which 1917bp were phylogenetically informative. One clade contained the epitype S3.001, and so I concluded that members of this clade belong to the species *C. sublineola*. S3.001 differs all from the rest of the members of that group by one SNP. The strain CgS11 differed from the rest by the presence of a 15bp indel, representing one copy of a sequence that is repeated twice. All the other strains in the clade had only one copy of this sequence. Two other clades were consistent with the two other groups (the “orange” and the “blue” groups) previously identified based on RFLP analysis (see chapter 4 of this dissertation).

Separate trees constructed by using each of three gene sequences (*Sod2*, *Apn2*, and *Mat1/Apn2*) all grouped the isolates into the same three major clades with a high degree of confidence (Figure 5.3-5.5). However, within the largest clade I observed reticulation, with the positions of CgS11, GS1.001, SS63.001, and S3.001 varying relative to one another and to the other members of the clade in each tree. This indicates the possibility of recombination among the genes, and it suggests that this large clade (highlighted in green) represents a single species, according to the multilocus genealogical concordance method described by (169). Because this clade includes the epitype S3.001, I conclude that it represents the previously described species *C. sublineola*. The clade included isolates from both *S. bicolor* and *S. halepense*, confirming that *C. sublineola* has the ability to infect both. The other two clades did not exhibit reticulation and were consistent in all three trees. One

of these clades (highlighted in orange) included only strains isolated from johnsongrass, while the other, highlighted in blue, included strains from both hosts. The strains in this blue clade were also separated from the rest by the ITS sequence (Figure 5.1). The three clades were consistent with the pathogenicity data, and with the three groups identified by the results of RFLP analysis using single-gene probes corresponding to putative pathogenicity genes (chapters 3 and 4 of this dissertation).

### **Morphology**

Measurements were made of spores, hyphopodia, and appressoria for representative isolates from each of the phylogenetic groups (Figures 5.6-5.8). The average lengths and widths of conidia, appressoria, and hyphopodia and the ranges, are shown in table 5.3. The ranges overlapped broadly among the three groups, and the averages were similar (Table 5.3).

Colony morphology (upper surface) varied among the different isolates but most produced salmon spores masses in the center of the colony, surrounded by white-to-gray mycelial growth (Figures 5.6-5.8). The lower surfaces also varied among the isolates, with color ranging from orange to black. Isolates produced sclerotia on the bottom of the plates. Isolates in the orange clade produced more aerial mycelia than the other groups (Figures 5.6-5.8).

Spores were similar in shape for the three groups, ranging from mostly falcate to fusiform. Appressoria were also similar, ranging from nearly circular to oval. The hyphopodia were more irregular and lobed. Overall, strains within the group varied quite a lot in appearance and there was no consistent character that could be reliably used to differentiate the clades based on morphology alone.

## Taxonomy

I propose that these 32 isolates belong to three different *Colletotrichum* species, based on fingerprinting, RFLP, pathogenicity, and DNA sequence data. I propose that two of these (the orange and blue clades) are new species, which I have named *Colletotrichum halepense*, associated only with the weed *S. halepense*, and *C. caselae* (in honor of Dr. Carlos Casela, retired from EMBRAPA Milho e Sorgo), pathogenic to both *S. halepense* and *S. bicolor*. The third group (green clade) conforms to the canonical *C. sublineola* species, based on gene sequence similarity with the epitype for the species S3.001. It should be noted that the *Sod2* and *Mat1/Apn2* sequences of S3.001 that are deposited in Genbank (42) do not match the genome sequence from the same strain at JGI. I confirmed the JGI sequences (CBS131301 v1.0/581817 and CBS131301 v1.0/ 572845, respectively) by personally sequencing *Sod2* and *Mat1/Apn2* in this isolate, which we have in our laboratory strain collection. Alignments of the S3001 sequences from JGI, from GenBank, and from the strain in our lab are shown in figure 5.9A-B. It appears that there are some sequencing errors in the Genbank accessions.

I confirmed that *Colletotrichum sublineola* is pathogenic to both *S. bicolor* and *S. halepense*. (39). I also confirmed that *C. caselae* (represented by SS1.001) is also pathogenic to both *Sorghum* spp. In contrast to the other two species, *C. halepense* appears to be confined to a single host, *S. halepense*.

*Colletotrichum halepense* K. V. Xavier & L. J. Vaillancourt, sp. nov. Figures 5.6 (A- P).

The colonies vary in appearance on potato dextrose agar medium, from pale yellowish to dark gray with black sclerotia visible from the lower surface. Colonies produce abundant aerial mycelium. The salmon spore masses are restricted to the center of the colonies

(Figure 5.6A-C). The conidial shape for *C. halepense* is falcate or fusiform, apices acute, 24.15-42.11 (av. 28.75  $\mu\text{m}$ ) by 4-6  $\mu\text{m}$  (av. 5.44  $\mu\text{m}$ ). Hyphopodial appressoria round, globose to lobate, apices cylindrical to lobate, 8.54-26.47 (av. 13.90  $\mu\text{m}$ ) by 7.6-1- 24.92  $\mu\text{m}$  (av. 10.83  $\mu\text{m}$ ). Appressoria are round to irregular, 4.61-12.54  $\mu\text{m}$  (av. 8.56  $\mu\text{m}$ ) by 5.08-11.12 (av. 6.66  $\mu\text{m}$ ).

Morphologically similar to other species of *Colletotrichum* isolated from gramineaceous hosts, but differs based on molecular phylogenetic analyses of *Sod2*, *Mat1/Apn2* and *Apn2* genes, and unique association with the host *Sorghum halepense*. Type strain JG18.001 from Russell County KY.

*Colletotrichum caselae* K. V. Xavier & L. J. Vaillancourt, sp. nov. Figures 5.7 (A- P).

The colonies vary from orange to dark gray in color on potato dextrose agar medium from the lower surface. Salmon spore masses were produced in the centers of the colony, surrounded by gray mycelia. Alternatively, producing mycelia all over the plate, with no conidial masses visible.

The conidial shape for *C. caselae* is falcate or fusiform, apices acute, 23.08-37.5 (av. 28.72  $\mu\text{m}$ ) by 4.5-7.02  $\mu\text{m}$  (av. 5.69  $\mu\text{m}$ ). Hyphopodial appressoria are round, globose to lobate, apices cylindrical to lobate, or flattened, 9.47-19.65 (av. 14.01  $\mu\text{m}$ ) by 6.25-13.12  $\mu\text{m}$  (av. 9.73  $\mu\text{m}$ ). Appressoria are round to irregular, 5.4-10.11 (av. 7.87  $\mu\text{m}$ ) by 4.2-8.62  $\mu\text{m}$  (av. 6.04  $\mu\text{m}$ ).

Morphologically similar to other species of *Colletotrichum* isolated from gramineaceous hosts but differs based on molecular phylogenetic analyses of ITS, *Sod2*, *Mat1/Apn2* and *Apn2* genes. *Colletotrichum caselae* is associated with the hosts *Sorghum halepense* and *Sorghum bicolor*. Type strain SS1.001 from Fayette County, Kentucky.

## Discussion

In this chapter, I tested the prediction that three genetically distinct groups that I had observed within the populations of *Colletotrichum* affecting cultivated sorghum and johnsongrass actually represent different species. Sequences from the *Sod2* gene, the *Apn2* gene, and from the intergenic region between *Apn2* and *Mat1*, have been used previously to identify new species within the *C. graminicola* species complex that affects graminaceous hosts (40). To test my prediction, I analyzed these sequences together with the internal transcribed spacer (ITS) of the ribosomal DNA, as well as measuring various morphological traits in culture, for several representative *Colletotrichum* isolates.

Morphological characters are usually not sufficient to distinguish among closely related *Colletotrichum* species. For example, based on morphology *C. sublineola*, infecting *S. bicolor*, was erroneously called *C. graminicola* up until relatively recently. Molecular phylogenetic analyses finally established that these were two sibling species with specific host ranges (39, 40, 61, 157, 165, 167, 181). Colony appearance on PDA and the shapes and sizes of conidia, appressoria and hyphopodia among the three genetically distinct groups I identified were all very similar. There was no consistent character that distinguished the three groups morphologically.

However, results from the multigene phylogenetic analyses strongly supported the hypothesis that *Colletotrichum* isolates that infect *Sorghum* spp. in the southeastern U.S. belong to three different, closely related species. One is the previously described *C. sublineola*, and the other two are undescribed. There was evidence for reticulation among individual gene trees within *C. sublineola*, suggesting the possibility of recombination. A telemorph has been described for *C. sublineola* (181), although it has not been observed in

nature. The *C. sublineola* clade included strains from both *S. bicolor* and *S. halepense*. One of the two new species appears to be limited to johnsongrass, while the other, like *C. sublineola*, apparently has the ability to infect either *S. halepense* or *S. bicolor* (see chapter 3 of this dissertation). I have named these new species *C. halapense*, and *C. caselae*, respectively. The ability of *C. sublineola* and *C. caselae* to infect both *Sorghum* species has significant implications for sorghum breeding and anthracnose disease management in regions where johnsongrass is common. The presence of three presumably genetically isolated species also has implications for gene flow, including of pathogenicity genes.



Table 5.1. Strains of *Colletotrichum sublineola*, *C. halepense* and *C. caselea* studied in this chapter, with collection details.

| Species              | Strain    | Host           | Country       | State        | County       | Year |
|----------------------|-----------|----------------|---------------|--------------|--------------|------|
| <i>C. sublineola</i> | CgS11     | Grain sorghum  | United States | Indiana      | Tippecanoe   | 1975 |
|                      | E031      | Grain sorghum  | Brazil        | Minas Gerais | Indianapolis | 2009 |
|                      | FS13.001  | Forage sorghum | United States | Alabama      | Brewton      | 2013 |
|                      | FS34.001  | Forage sorghum | United States | Alabama      | Macon        | 2013 |
|                      | FS37.001  | Forage sorghum | United States | Alabama      | Macon        | 2013 |
|                      | FS5.001   | Forage sorghum | United States | Alabama      | Brewton      | 2013 |
|                      | FS8.001   | Forage sorghum | United States | Alabama      | Brewton      | 2013 |
|                      | GS1.001   | Grain sorghum  | United States | Kentucky     | Hopkins      | 2013 |
|                      | JG88.001  | johnsongrass   | United States | Kentucky     | Montgomery   | 2014 |
|                      | SS100.001 | Sweet sorghum  | United States | Alabama      | Macon        | 2013 |
|                      | SS107.001 | Sweet sorghum  | United States | Georgia      | Tift County  | 2013 |
|                      | SS124.001 | Sweet sorghum  | United States | Georgia      | Tift County  | 2013 |
|                      | SS159.001 | Sweet sorghum  | United States | Georgia      | Tift County  | 2013 |
|                      | SS171.001 | Sweet sorghum  | United States | Florida      | Live Oak     | 2014 |
|                      | SS204.001 | Sweet sorghum  | United States | Kentucky     | Montgomery   | 2014 |
|                      | SS26.001  | Sweet sorghum  | United States | Florida      | Live Oak     | 2013 |
|                      | SS29.001  | Sweet sorghum  | United States | Florida      | Live Oak     | 2013 |
|                      | SS41.001  | Sweet sorghum  | United States | Florida      | Live Oak     | 2013 |
|                      | SS45.001  | Sweet sorghum  | United States | Florida      | Live Oak     | 2013 |
|                      | SS63.001  | Sweet sorghum  | United States | Florida      | Live Oak     | 2013 |

Table 5.1. (continued)

| Species              | Strain    | Host          | Country       | State    | County      | Year |
|----------------------|-----------|---------------|---------------|----------|-------------|------|
| <i>C. sublineola</i> | SS63.001  | Sweet sorghum | United States | Florida  | Live Oak    | 2013 |
|                      | SS78.001  | Sweet sorghum | United States | Alabama  | Brewton     | 2013 |
|                      | SS240.001 | Sweet sorghum | United States | Georgia  | Tift County | 2014 |
| <i>C. caselae</i>    | JG34.001  | johnsongrass  | United States | Kentucky | Frankfort   | 2013 |
|                      | JG42.001  | johnsongrass  | United States | Kentucky | Fayette     | 2013 |
|                      | JG7.001   | johnsongrass  | United States | Kentucky | Russell     | 2011 |
|                      | SS1.001   | Sweet sorghum | United States | Kentucky | Fayette     | 2012 |
| <i>C. halepense</i>  | JG102.001 | Sweet sorghum | United States | Georgia  | Tift County | 2014 |
|                      | JG18.001  | johnsongrass  | United States | Kentucky | Fayette     | 2012 |
|                      | JG2.001   | johnsongrass  | United States | Kentucky | Russell     | 2011 |
|                      | JG62.001  | johnsongrass  | United States | Kentucky | Russell     | 2013 |
|                      | JG75.001  | johnsongrass  | United States | Florida  | Gadsden     | 2014 |
|                      | JG9.001   | johnsongrass  | United States | Kentucky | Russell     | 2012 |

Table 5.2. Primers used for PCR amplification and DNA sequencing of specific genes

| Gene    | Primer     | Sequences of Primers           | Reference           |
|---------|------------|--------------------------------|---------------------|
| ITS     | ITS5       | 5' GGAAGTAAAAGTCGTAACAAGG 3'   | White et al., 1990  |
|         | ITS4       | 5' TCCTCCGCTTATTGATATGC 3'     | White et al., 1990  |
| SOD2    | SOD507F    | 5' ATGGCAGCCTTTCCGTTGAGATAC 3' | Crouch et al, 2006  |
|         | SOD507F/R  | 5' AGTTGACATGAAGCCACCTACAGC 3' | Crouch et al, 2006  |
| APN2    | Apn1W1F    | 5' ATGGAGCACAAAAACGAACA 3'     | Crouch et al, 2009b |
|         | Apn1W1R    | 5' GCGGAGCAGAGGATGTAGTC 3'     | Crouch et al, 2009b |
| MAT/APN | MAT1/APN2F | 5' CCCGAGTTCGACCGGAGACG 3'     | accession #         |
|         | MAT1/APN2F | 5' TTCCGAGAGGCCGGGGACAA 3'     | accession #         |

Table 5.3. Characteristics of the spores, appressoria, and hyphopodia of the three phylogenetic groups.

| Species  |         | Spore       |           | Hyphopodium |            | Appressorium |            |
|--|---------|-------------|-----------|-------------|------------|--------------|------------|
|  |         | Length      | Width     | Length      | Width      | Length       | Width      |
| <i>Green Clade</i><br>( <i>C. sublineola</i> ) | Range   | 19.82-36.78 | 4.11-7.68 | 7.03-20.85  | 6.57-16.76 | 6.44-11.64   | 4.85-9.02  |
|  | Average | 28.10       | 5.54      | 12.34       | 10.00      | 8.57         | 6.70       |
| <i>Orange Clade</i>                            | Range   | 24.15-42.11 | 4-6.65    | 8.54-26.47  | 7.6-24.92  | 4.61-12.54   | 5.08-11.12 |
|  | Average | 28.75       | 5.44      | 13.90       | 10.83      | 8.56         | 6.66       |
| <i>Blue Clade</i>                              | Range   | 23.08-37.5  | 4.5-7.02  | 9.47-19.65  | 6.25-13.12 | 5.4-10.11    | 4.2-8.64   |
|  | Average | 28.72       | 5.69      | 14.01       | 9.73       | 7.87         | 6.04       |

Figure 5.1. Phylogenies of *C. sublineola* isolates inferred from ITS gene. Isolates are labeled as johnsongrass (JG), grain (GS1001, E031, CgSI1), sweet (SS) and forage (FS) sorghum isolates. Clades are highlighted in green and blue. Outgroup isolate, *C. eremochloae* is highlighted in yellow. Isolates highlighted in orange belong to the “orange” group of isolates identified by RFLP analysis in chapter 4 of this dissertation.

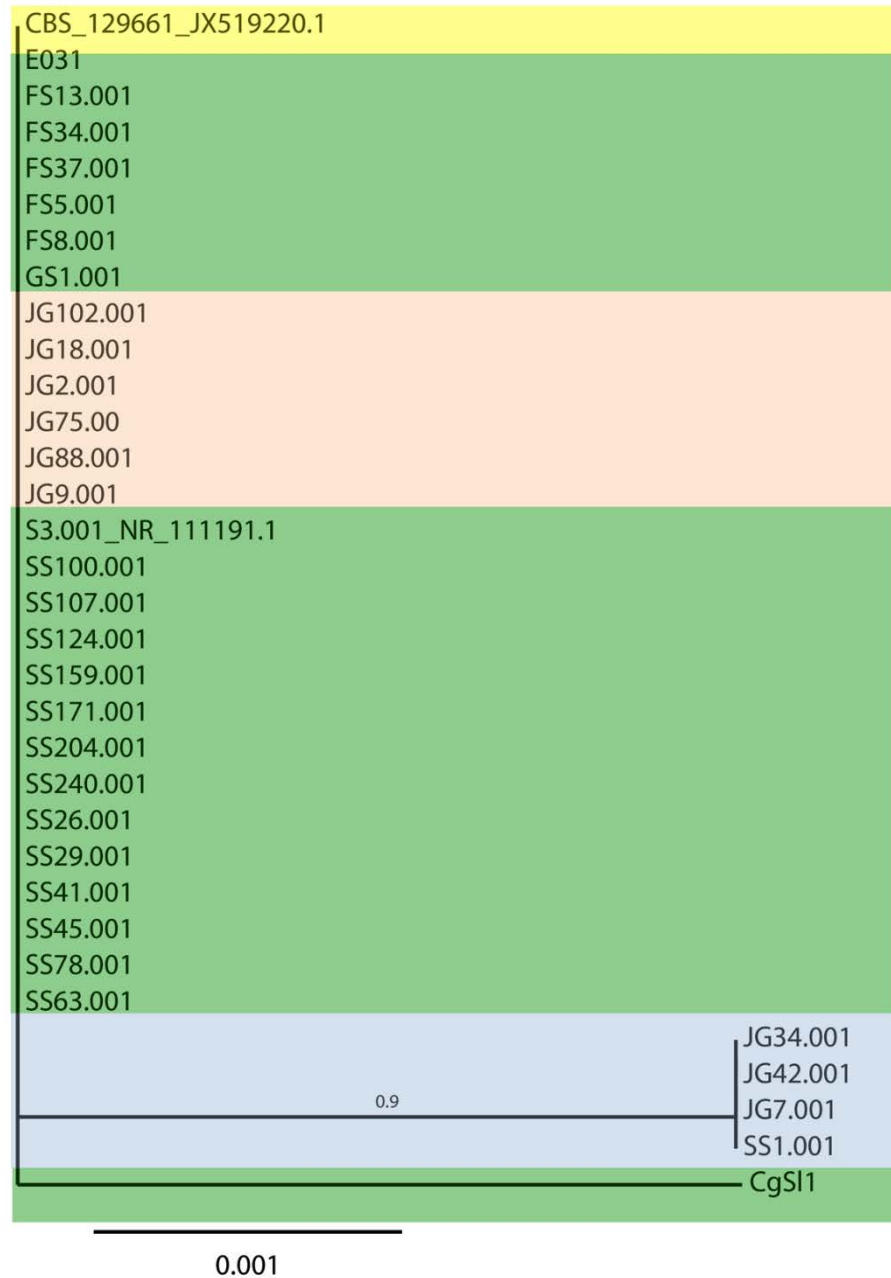


Figure 5.2. Phylogenies of *C. sublineola* isolates inferred from three concatenated gene sequences (*Sod2*, *Apn2*, and *Mat1/Apn2*). Isolates are labeled as johnsongrass (JG), grain (GS1001, E031, CgS11), sweet (SS) and forage (FS) sorghum isolates. Clades are highlighted in green, orange, and blue. Outgroup isolate, *C. eremochloae* is highlighted in yellow.

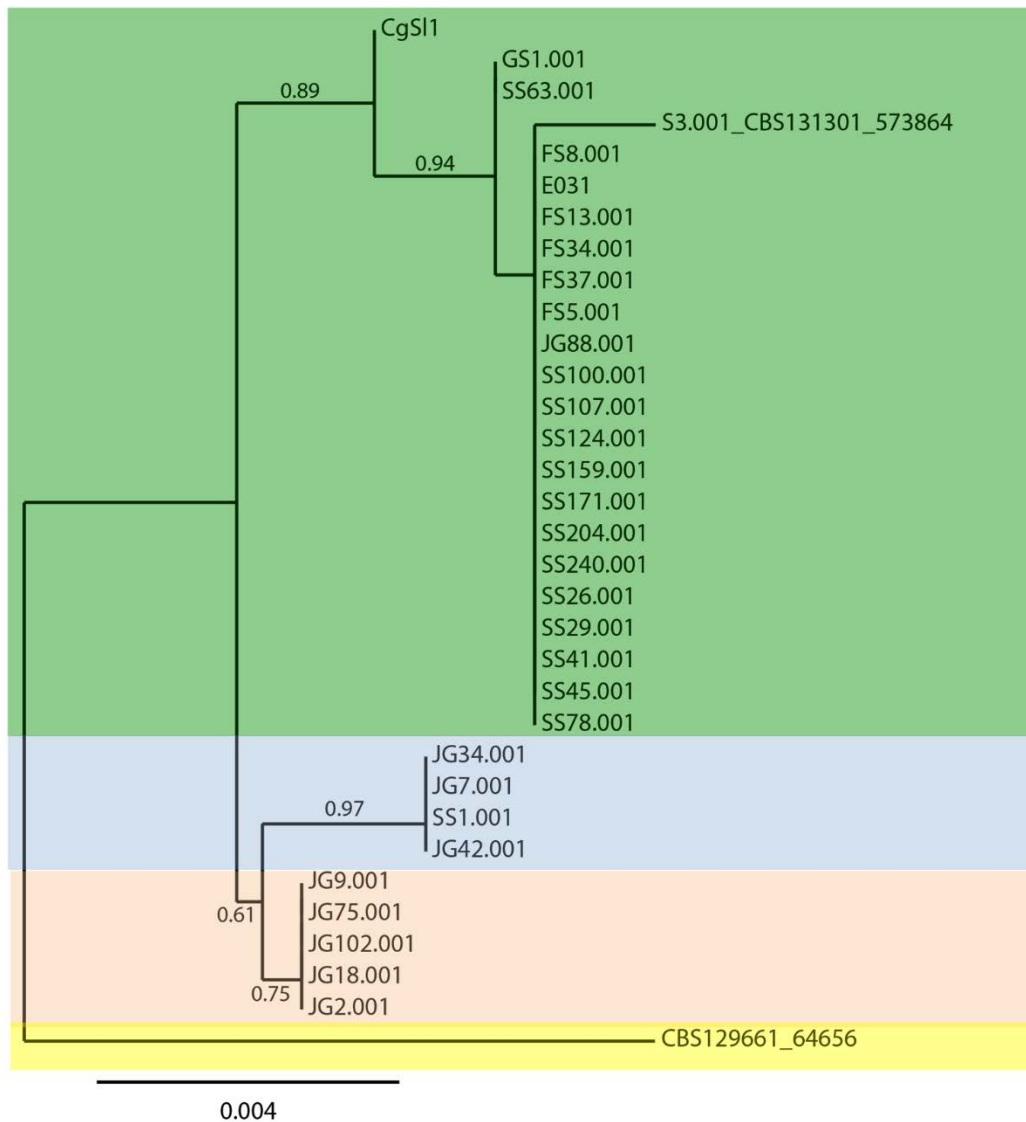


Figure 5.3. Phylogenies of *C. sublineola* isolates inferred from the *Apn2* gene. Isolates are labeled as johnsongrass (JG), grain (GS1001, E031, CgSI1), sweet (SS) and forage (FS) sorghum isolates. Clades are highlighted in green, orange, and blue. Outgroup isolate, *C. eremochloae* is highlighted in yellow.

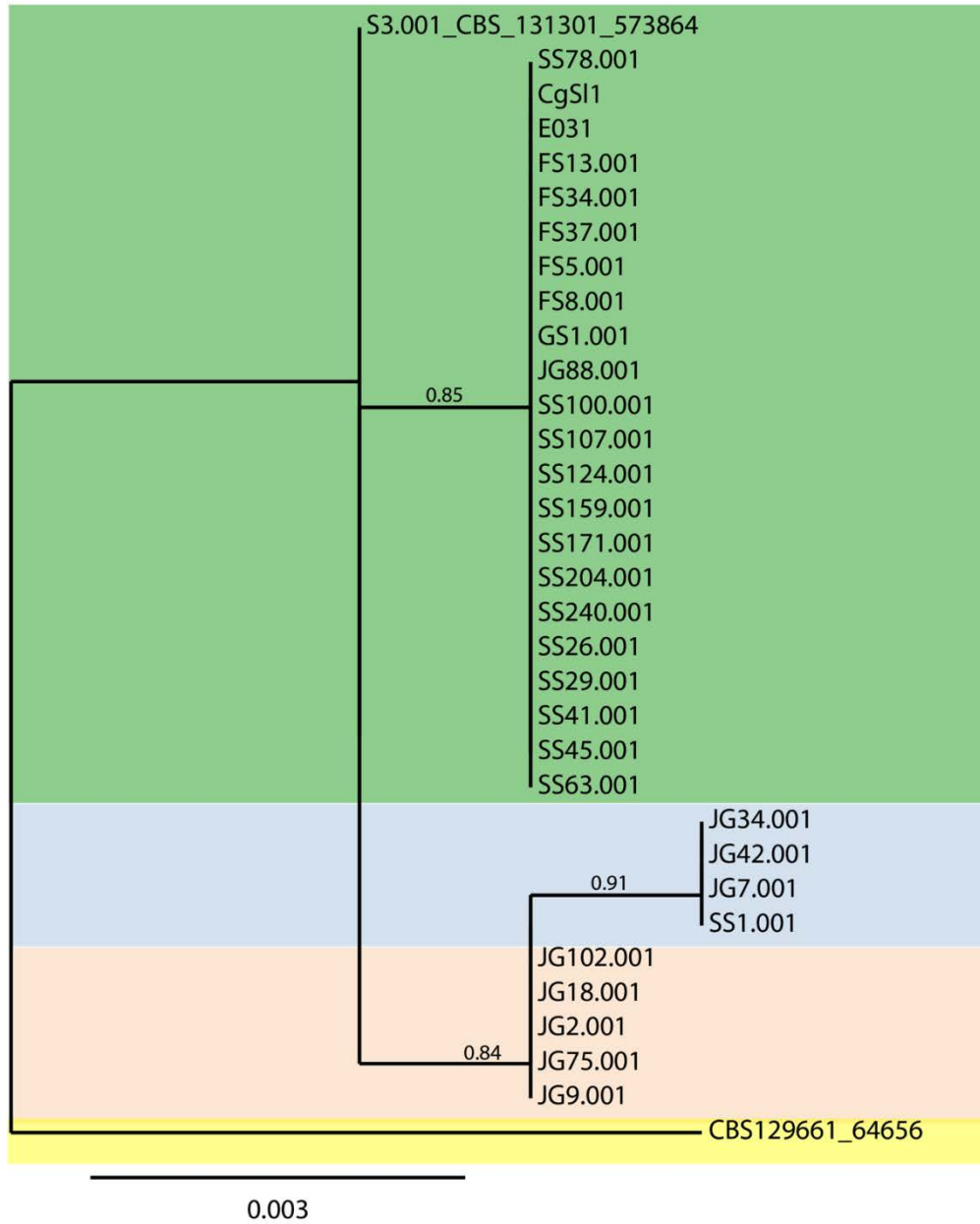


Figure 5.4. Phylogenies of *C. sublineola* isolates inferred from the *Mat1*/*Apn2* sequence. Isolates are labeled as johnsongrass (JG), grain (GS1001, E031, CgS11), sweet (SS) and forage (FS) sorghum isolates. Clades are highlighted in green, orange, and blue. Outgroup isolate, *C. eremochloae* is highlighted in yellow.

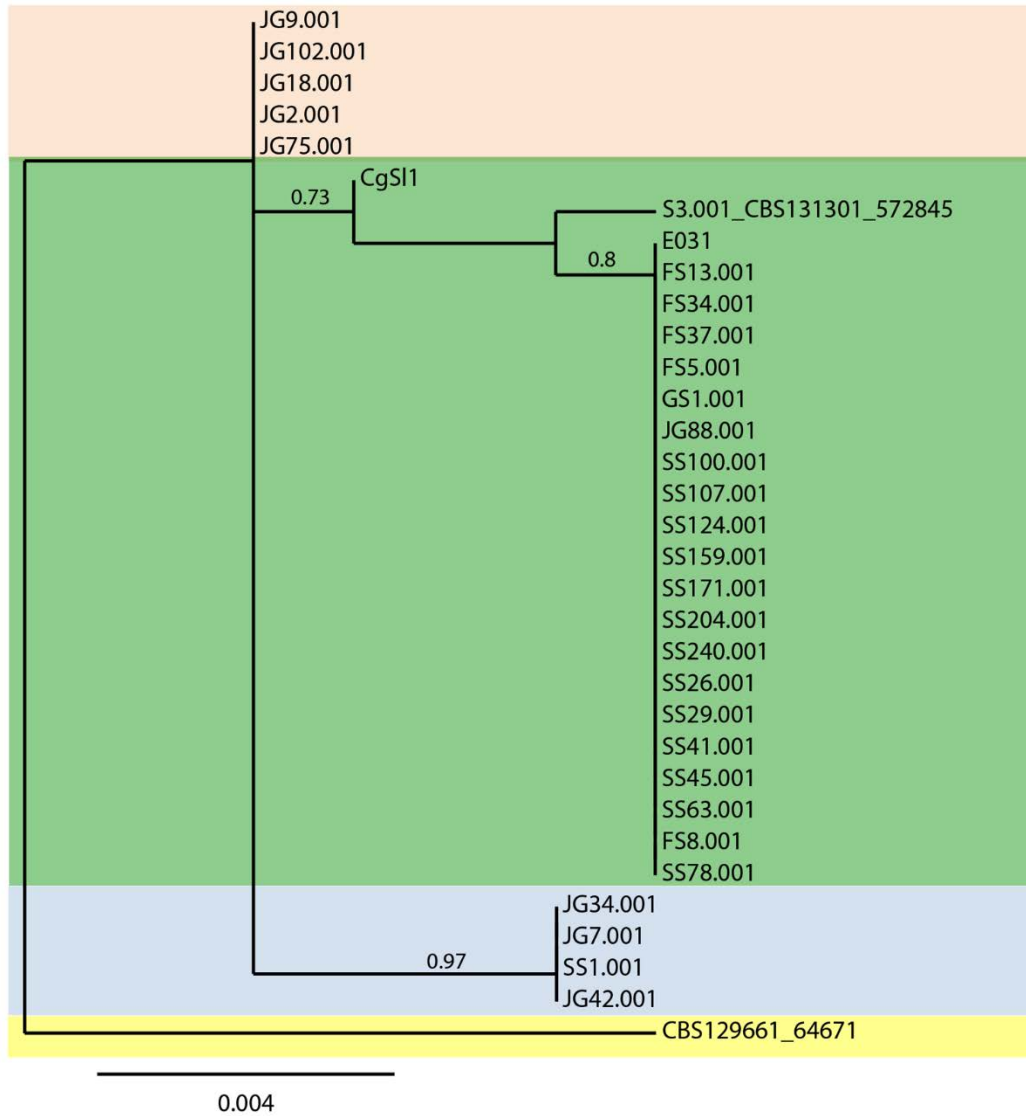




Figure 5.5. Phylogenies of *C. sublineola* isolates inferred from the *Sod2* gene. Isolates are labeled as johnsongrass (JG), grain (GS1001, E031, CgS11), sweet (SS) and forage (FS) sorghum isolates. Clades are highlighted in green, orange, and blue. Outgroup isolate, *C. eremochloae* is highlighted in yellow.

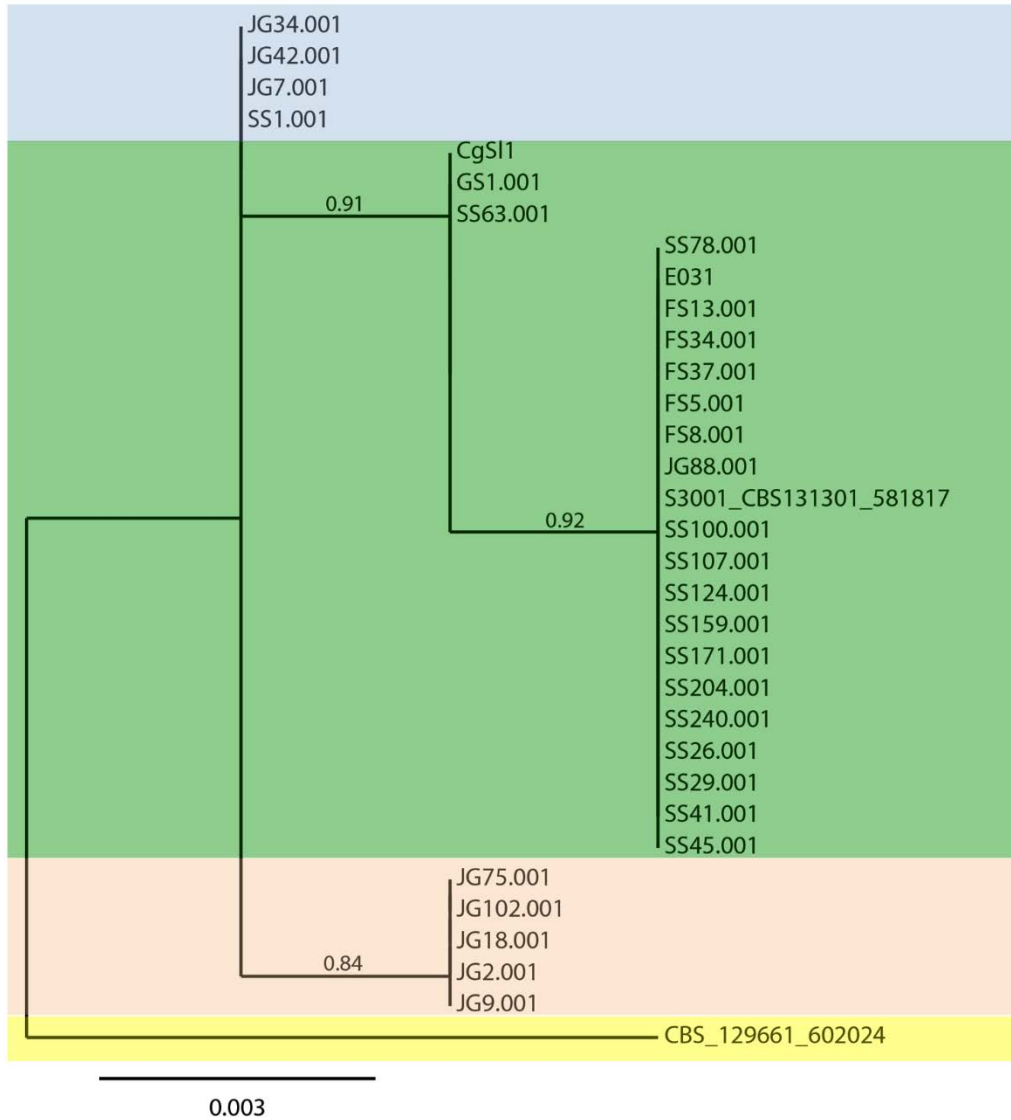


Figure 5. 6. Morphological characters, Orange Clade (*Colletotrichum halepense*) (A- D) Cultures on PDA, 15 d growth, from above and below; (E-H) Hyphopodia; (I- L) Appressoria; and (M- P) Conidia. Scale bars = 20  $\mu$ m. Isolates (A, E, I, M) JG2.001, (B, F, J, N) JG9.001, (C, G, K, O) JG75.001, (D, H, L, P).

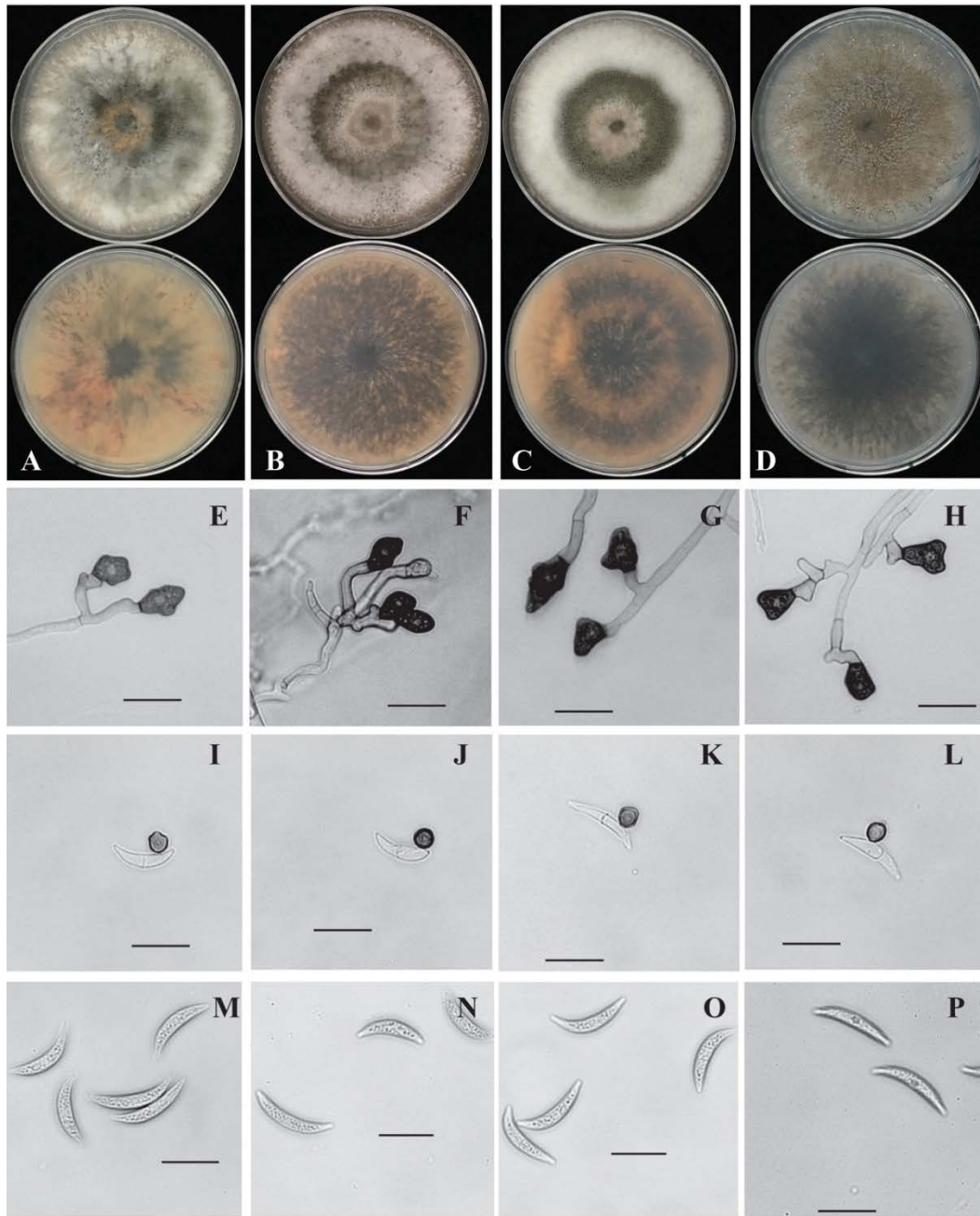


Figure 5.7. Morphological features, Blue Clade, (*Colletotrichum caselae*). (A- D) Cultures on PDA, 15 d growth, from above and below; (E-H) Hyphopodia; (I- L) Appressoria; and (M- P) Conidia. Scale bars = 20  $\mu$ m. Isolates (A, E, I, M) JG34.00, (B, F, J, N) SS1.001, (C, G, K, O) JG7.001, (D, H, L, P) JG42.001.

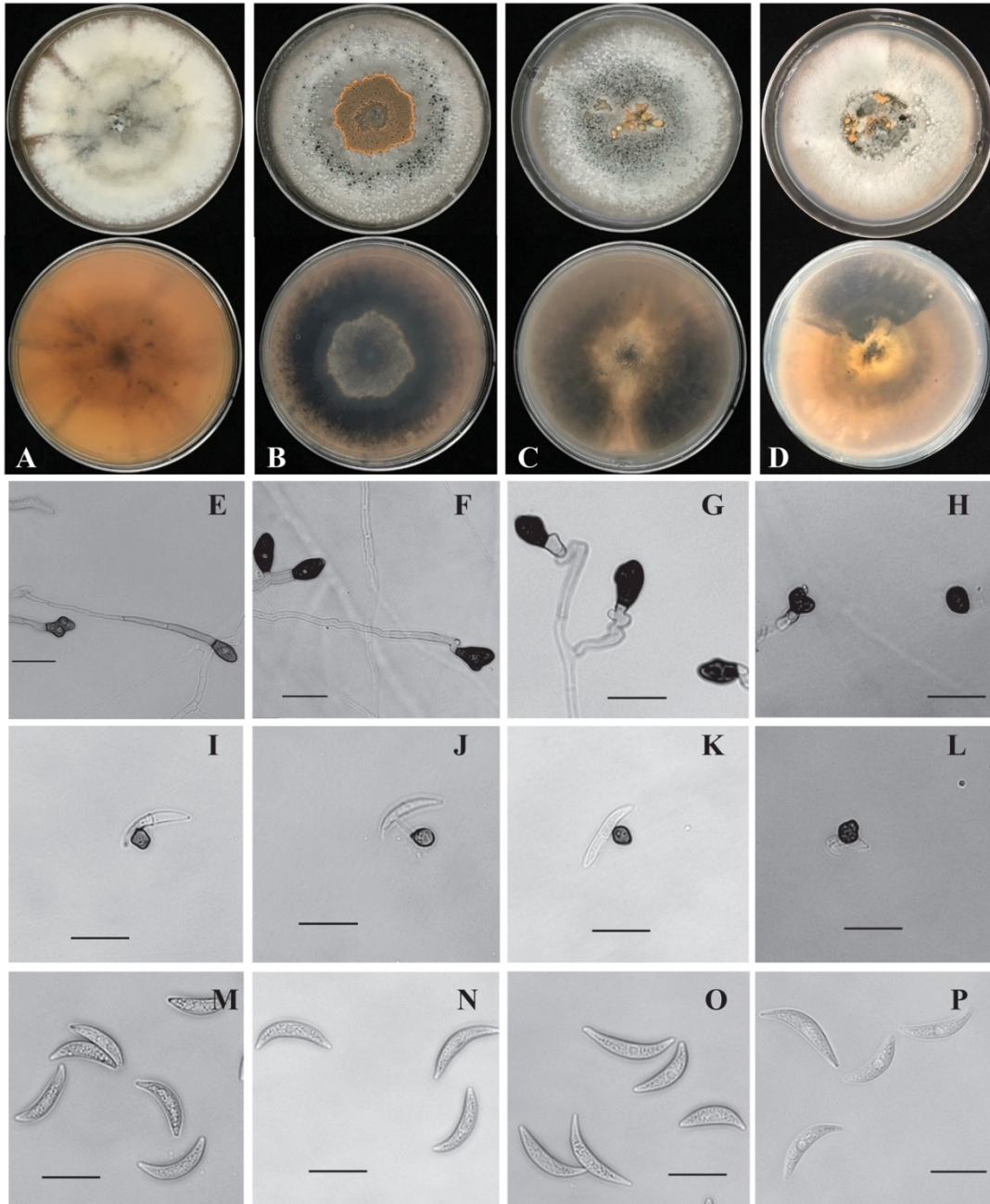


Figure 5.8. Morphological Features, Green clade (*Colletotrichum sublineola*). (A–D) Cultures on PDA, 15 d growth, from above and below; (E–H) Hyphopodia; (I–L) Appressoria; and (M–P) Conidia. Scale bars = 20  $\mu$ m. Isolates (A, E, I, M) JG34.00, (B, F, J, N) SS1.001, (C, G, K, O) JG7.001, (D, H, L, P) JG42.001.

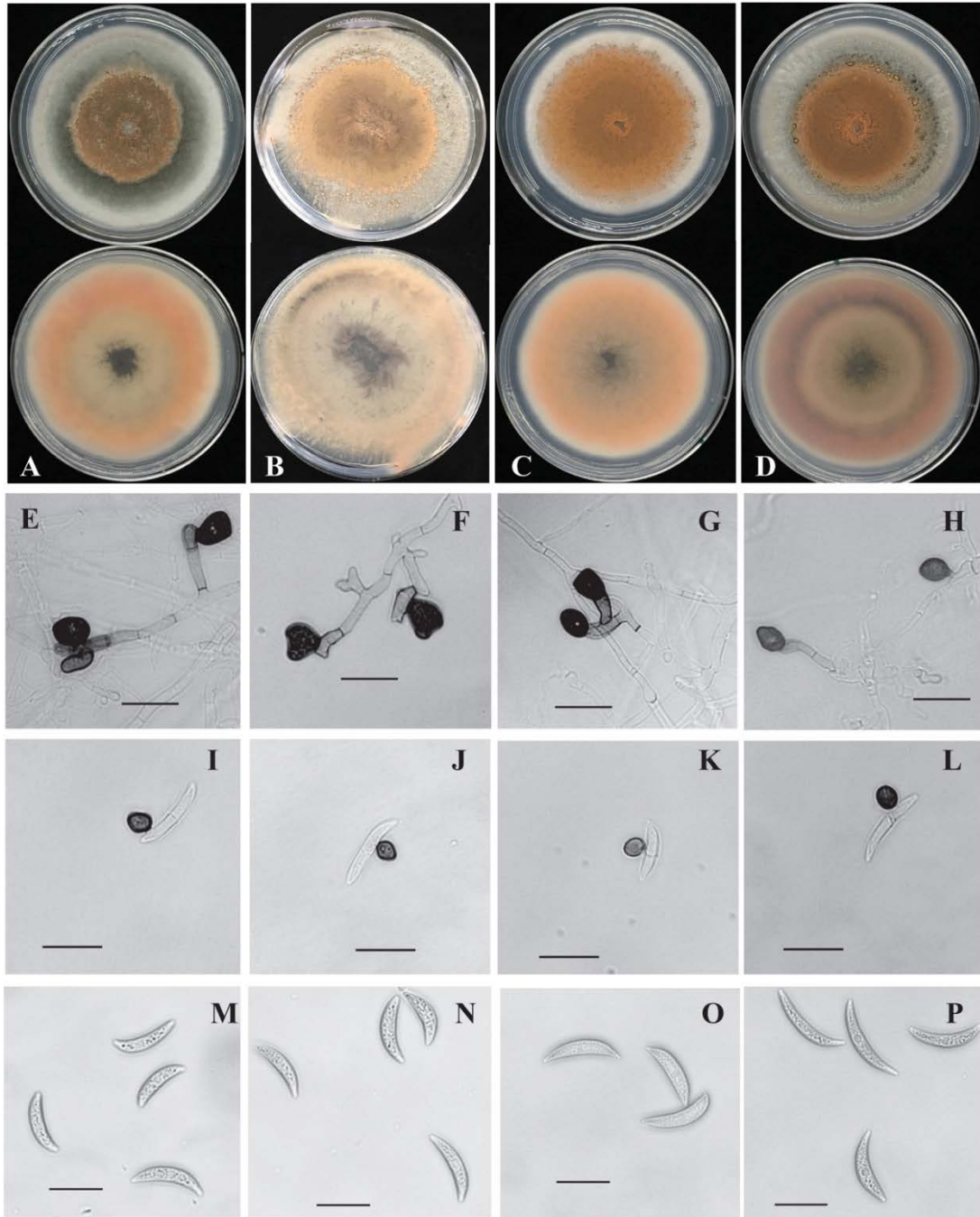
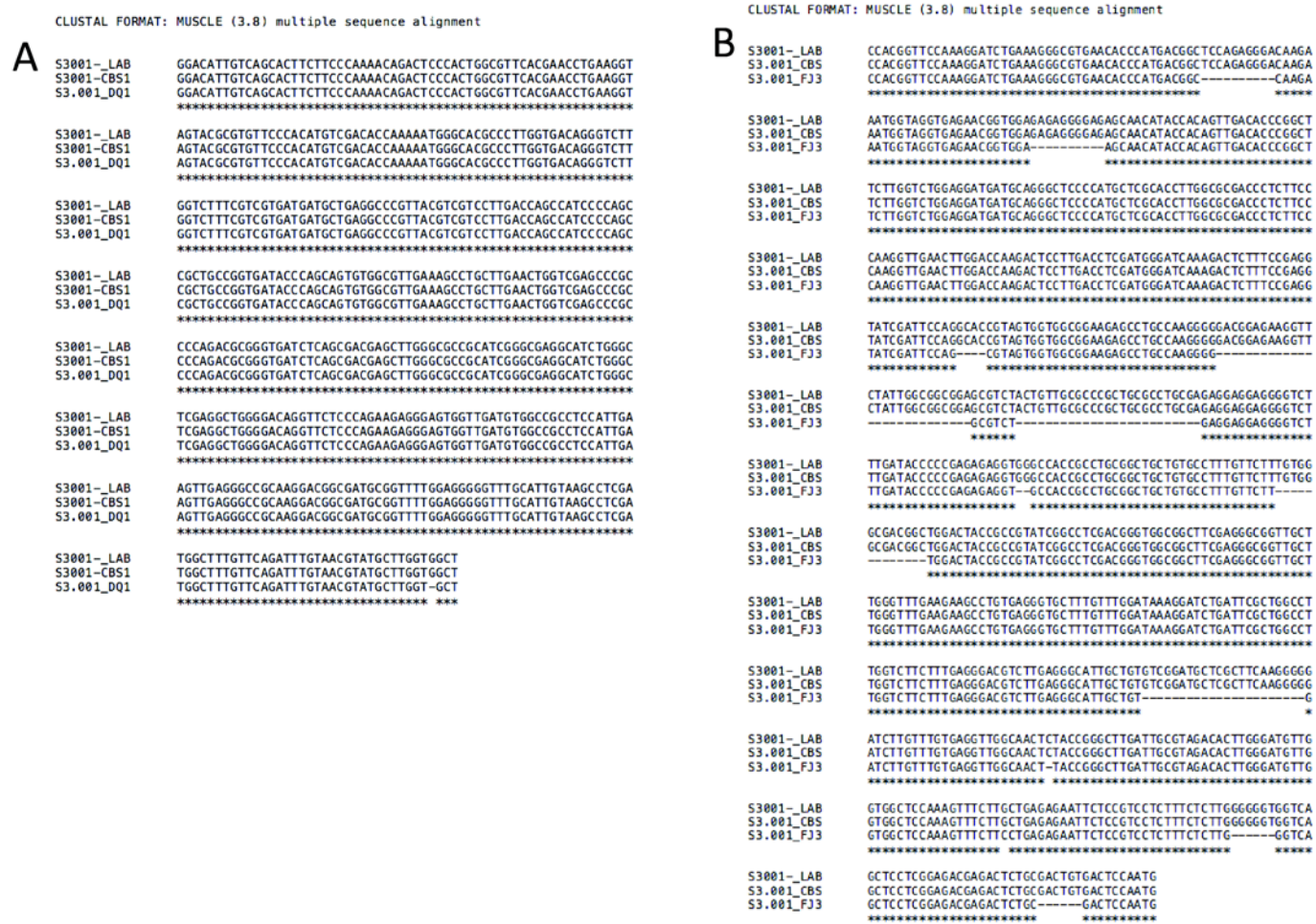


Figure 5. 9. Alignments; (A) *Sod2* and (B) *Mat1/Apn2*. S3.001-\_LAB, sequence obtained from our laboratory culture of S3.001. S3001\_CBS1 and S3001\_CBS, sequence retrieved from JGI, and S3.001\_DQ1 and S3.001\_FJ3, sequences retrieved from GenBank (accession number DQ132051.1 and FJ378029.1, respectively).



## Chapter 6 - Summary and Significance of the Study

Populations of *Colletotrichum sublineola* that cause anthracnose disease on grain sorghum have been extensively characterized both genetically and pathogenically, and a high level of diversity has been demonstrated worldwide. This diversity has been used to explain the lack of durability of resistance genes that have been deployed to manage anthracnose in this crop. In contrast, comparatively little was known about the *Colletotrichum* populations that infect sweet or wild sorghum, even though anthracnose is listed as one of most important diseases of sweet sorghum in Kentucky, and is growing in importance across the southeastern U.S. In my dissertation research, I have described the genetic and pathogenic diversity in the population of *Colletotrichum* on sweet and wild sorghum in Kentucky and the southeastern U.S. by using several different approaches. To support my work, I developed many new protocols, including greenhouse and laboratory pathogenicity assays, and novel molecular markers for fingerprinting and for RFLP analysis. I amassed and archived a strain collection containing more than 400 *Colletotrichum* isolates from across the region. This collection will provide a valuable resource for plant breeders who want to efficiently screen sorghum germplasms for new sources of resistance. The greenhouse and laboratory assays that I developed will be useful for pre-screening germplasms with a large number of strains, or with exotic strains that require containment. My work will enable plant breeders to screen sorghum germplasms more quickly and efficiently for new sources of resistance or to characterize new isolates. Indeed, this approach has already been used effectively by a sorghum breeding company that I worked with last year.

Results from my field, greenhouse, and laboratory pathogenicity assays were generally consistent in ranking pathogen aggressiveness and host resistance, and demonstrated that *C. sublineola* has a significant potential to cause anthracnose disease on sweet sorghum in Kentucky when conditions are favorable (i.e. with a combination of a susceptible host and favorable environmental conditions). I discovered that most johnsongrass isolates, despite being much less aggressive than most isolates from *S. bicolor*, were still able to complete their life cycles on sweet sorghum plants. And I also found that some johnsongrass isolates are highly aggressive on sweet sorghum. This implies that johnsongrass could serve as a reservoir for the pathogen during crop rotation schemes, and could also be a source of new races. This would complicate the implementation of an effective disease management program, and efforts to develop new resistant varieties of sweet sorghum.

My studies of genetic diversity using RAPDs and novel RFLP fingerprinting markers identified two distinct sub-populations, one consisting mostly of isolates from cultivated sorghum, and the other including primarily isolates from johnsongrass. The fingerprinting analysis provided strong evidence for relatively rare cross-infections between these hosts. It also revealed that there is a very high level of genetic and pathogenic diversity within both sub-populations. Thus, I anticipate that *Colletotrichum* across the southeastern U.S. has a significant genetic potential to overcome individual resistance genes, and may pose a serious threat to widespread sorghum monoculture in the future. Plant breeders should monitor the levels of genetic diversity in the pathogen population, because resistant varieties carrying major gene resistance against anthracnose may not last for long when deployed widely in regions with high levels of pathogen diversity. The fingerprinting markers that I developed may be particularly helpful for this purpose. A goal for the future

could be to develop a multiplexed RAPD primer protocol to facilitate individual strain identifications.

I confirmed that the closely related sibling species *C. sublineola* and *C. graminicola* are host-specific, and I also determined that the non-host reaction in both cases was associated with rapid cell death, a characteristic of ETI. Based on a genomic comparison of these two species, I identified putative pathogenicity genes that differed between them. I used some of these as probes to conduct an RFLP analysis of more than 80 representative *Colletotrichum* strains. Results of the analysis were consistent with the fingerprinting results, confirming that isolates from *S. bicolor* and from *S. halepense* mostly clustered into two distinct groups. However results of the RFLP analysis allowed further separation of the johnsongrass isolates into two groups, one containing isolates associated with *S. halepense* only, and another containing isolates from both *S. halepense* and *S. bicolor*. I identified several genes encoding putative effectors and specialized secondary metabolism proteins that showed allelic variation and presence-absence polymorphisms that differentiated the three groups of isolates. These allelic variants might play a role in pathogenicity and host determination: functional analyses of these genes should be performed to test this hypothesis. The RFLP analysis confirmed that there was a high degree of genetic diversity within these populations. High levels of genetic diversity among putative effectors and secondary metabolites suggests a possible threat to monoculture crops, because it increases the possibilities for rapid selection of new races able to overcome plant resistance genes, and of epidemics (“boom-bust” cycles). Pathogenicity assays revealed strong evidence of the existence of different races within the pathogen populations.



My phylogenetic analysis revealed that the three groups identified based on the molecular markers actually belonged to different species, two of which had not been previously described. I named these two new species *C. halepense* and *C. caselae*. My research findings suggest that *C. sublineola* and *C. caselae* have the ability to infect both *S. bicolor* and *S. halepense*, which has significant implications for sorghum breeding and anthracnose disease management in regions where johnsongrass is common. Identification of these species could be based on the inoculation of a differential host series. However, for more rapid diagnosis a better option would be to generate species-specific primers based on the putative pathogenicity genes. Accurate and rapid diagnosis will be an important tool for improved disease management.

My research findings have provided critical new information for plant breeders that develop and deploy resistant sweet sorghum varieties in areas where johnsongrass is also present. For the growers, my work implies that the pathogen has the potential to quickly overcome plant resistance based on major genes, increasing the risk of epidemics if those genes are widely deployed. The ability of two of the species to infect both *S. bicolor* and *S. halepense* complicates the use of crop rotation where johnsongrass is common, so alternative management strategies might be needed.

## Appendix A - Optimization of whole plant inoculation in the greenhouse

The goal of this work was to determine the age at which sweet sorghum plants were most susceptible to anthracnose caused by *C. sublineola* in order to optimize greenhouse pathogenicity assays.

**Material and methods:** Seeds of sweet sorghum (variety Sugar Drip) were sowed once per week for a period of four weeks. The experiment was conducted using plants of four different ages: (i) plants with eight fully developed leaves (V8); (ii) seven fully developed leaves (V7); (iii) six fully developed leaves (V6); and (iv) five fully developed leaves (V5). Four sorghum seeds were sowed in each 8' inch pot, in a mixture of three parts Pro-Mix BX (Premiere Horticulture, Ltd, Riviere du Loup, PQ, Canada) and two parts sterile topsoil. The pots were kept at the greenhouse under 14 hours of light and were watered daily to saturation and fertilized with a solution of 150 ppm of Peters 20-10-20 (Scotts-Sierra Horticultural Product Co., Marysville, OH), three times per week, beginning one week after seedling emergence.

Pots were arranged in a randomized complete block design (RCBD) with four blocks, with each experimental unit consisting of three pots, containing a total of 12 plants. The treatments were as follows: (1) V5 plants inoculated with SS1001; (2) V5 plants inoculated with GS1001; (3) V5 plants inoculated with JG9001; (4) V5 plants inoculated with JG18001, (5) V5 plants inoculated with water; (6) V6 plants inoculated with SS1001; (7) V6 plants inoculated with GS1001; (8) V6 plants inoculated with JG9001; (9) V6 plants inoculated with JG18001, (10) V6 plants inoculated with water; (11) V7 plants inoculated with SS1001; (12) V7 plants inoculated with GS1001; (13) V7 plants inoculated with

JG9001; (14) V7 plants inoculated with JG18001, (15) V7 plants inoculated with water; (16) V8 plants inoculated with SS1001; (17) V8 plants inoculated with GS1001; (18) V8 plants inoculated with JG9001; (19) V8 plants inoculated with JG18001, (20) V8 plants inoculated with water. The entire experiment was repeated twice, during the winter of 2013 and the summer of 2014. Inoculum preparation and plant inoculation was performed as described in chapter 2 of this dissertation

The number of plants that were infected (i.e. displayed lesions containing sporulating acervuli) was recorded weekly. Data were subjected to analysis of variance (ANOVA) using the PROC GLM procedure of the SAS software package (SAS Users Guide, SAS Institute, Cary, NC). Mean comparisons were conducted using Fisher's protected least significant difference (LSD) test at  $\alpha = 0.05$ . Pearson's correlation coefficients for different variables were computed using the PROC CORR command of the SAS program. The analyses were performed using SAS version 9.3.

**Results and Conclusions:** The results of the two experiments were similar. There were no statistical differences ( $p$  value  $> 0.10$ ) among the treatments within the blocks, or for the isolate X plant stage interaction ( $p$  value  $> 0.10$ ) at any time point in either of the two experiments. Therefore, data were pooled for plant stage and isolate, and reanalyzed for both experiments (Figure A1.1A, B).

The strain SS1001 was statistically more aggressive than all the other treatments across all time points ( $p$  value  $< 0.05$ ) (Figure A1.1A, B). The effect of the plant stage was not significant for most time points ( $p$  value  $> 0.01$ ). However, in the 2013 experiment, the V6 plants were statistically the most susceptible to the anthracnose ( $p$  value  $< 0.05$ ) in the second week of evaluation (Figure A1.2A). In the 2014 experiment, the V5 plants were

statistically more susceptible to the anthracnose ( $p$  value  $< 0.05$ ) from the second to the fourth week of disease evaluation (Figure A1.2B).

From the two experiments I concluded that the younger vegetative plant stages (V5 and V6) were the most susceptible to *Colletotrichum*. I also concluded that the most aggressive fungal strain was SS1001.

Figure A2.1. Pathogenicity assay of four strains of *Colletotrichum* on Sugar Drip. Disease evaluation was performed based on the number of infected (susceptible) plants (incidence), during four weeks after appearance of symptoms. (A) First repetition performed in the winter 2013 and (B) second repetition performed in the summer of 2014.

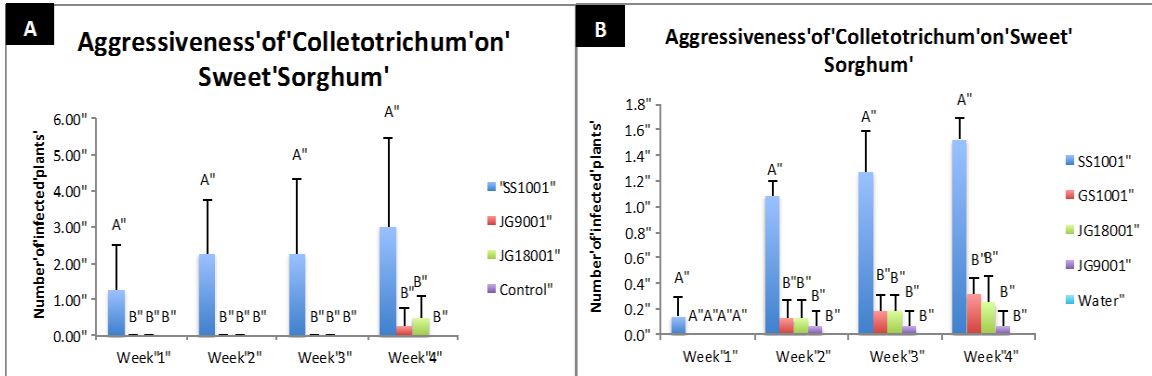
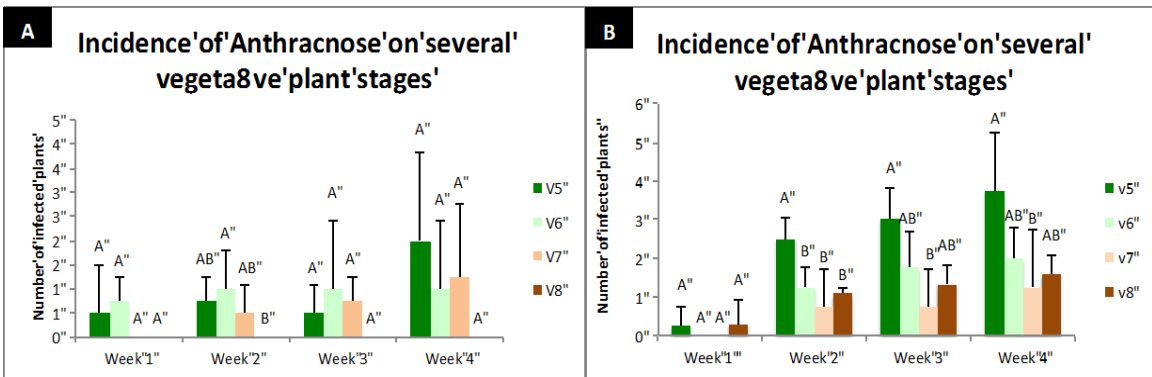


Figure A2.2. Pathogenicity assay of four strains of *Colletotrichum* on Sugar Drip. Disease evaluation was performed based on the number of infected (susceptible) plants (incidence), during four weeks after appearance of symptoms. (A) First repetition performed in the winter 2013 and (B) second repetition performed in the summer of 2014.



## **Appendix B - Optimization of sheath assay and rating system for sorghum anthracnose**

**Introduction:** A cytological study of compatible and incompatible interactions of grain sorghum with *C. sublineola* was performed by using a leaf sheath infection assay (195). This assay allowed detailed visualization of the pathogen growth into the plant cells, and of the plant response. The leaf sheaths were spray-inoculated, which made it relatively difficult to find and count the widely dispersed infection sites. Kankanala and her collaborators used detached leaf sheaths to study the interaction between rice and *Magnaporthe oryzae* (97). They applied fungal inoculum directly to the inner surfaces of the detached sheaths which made it more convenient to synchronize and locate infection sites. This method was successfully adapted to study the details of infection of maize by *C. graminicola* (97, 175). My goal for the work described in this appendix was to optimize this detached sheath assay to investigate interactions of sorghum and *C. sublineola*.

### **Effect of inoculation method**

In the protocol described by Torres et al. (2014), a 20 µl drop of a *C. graminicola* spore suspension was applied to the inner surface of a detached maize leaf sheath. Sorghum leaf sheaths are quite small compared with maize sheaths, and it is relatively difficult to apply drops without damaging them. In studies of infection of rice by *M. oryzae*, the entire detached rice leaf sheath was filled with a spore suspension (97). The aim of this experiment was to compare the effect of drops versus the more convenient filling method on the interaction between *C. sublineola* and sweet sorghum.

**Material and methods:** The fungal strain, CgS11 isolated from grain sorghum was used for these experiments. Sweet sorghum seedlings (var. Simon) at the V3 growth stage were cut at the soil line and brought to the laboratory. The sheaths were removed from the second leaves and cut into three-inch segments. The segments were inoculated by one of two methods: (i) applying a spore suspension ( $5 \times 10^5$  spores/mL) to fill up the whole leaf sheath; and (ii) applying a 20  $\mu$ L drop of the spore suspension ( $5 \times 10^5$  spores/mL) to the inner part of the leaf sheath just above the midrib. The inoculated sheath segments were supported inside a Petri dish with the midrib side downward. The Petri dishes were lined with moistened Whatman paper (Whatman No. 1, Whatman, Hillsboro, OR, USA) to maintain high humidity. Dishes were incubated at 23° C under continuous light. Fungal colonization was evaluated at 72 hours after inoculation (hpi).

To observe fungal colonization, the blade sections of the inoculated leaf sheaths were removed, leaving only the midrib section. This was then turned upside down on a wet slide, and a single-edged razor blade were used to remove excess tissue to leave a thin layer of mostly epidermal cells containing the fungal infection sites. A total of three sheaths, fifty infection sites per sheath, were observed for each treatment. The experiment was repeated twice.

**Results and Conclusions:** After 72 hours, only half of the propagules in drop-inoculated treatments had given rise to successful infections inside the host cells (Figure A2.1). In contrast, 90% of the propagules in filled treatments had produced infectious hyphae inside the host cells. Thus, for the compatible interaction filling the leaf sheath with the spore suspension resulted in faster development of the pathogen than the drop inoculation. However, in incompatible interaction *C. graminicola* was not able to colonize the cells

even in filled inoculations. For the interaction corn x *C. graminicola* we notice that the pathogen was able to colonize and sporulate faster by filling the leaf sheath with the spore suspension than by using a single drop. So it seems that filling leaf sheaths compromises the strength of the resistance response. Some possible reasons for this could be effects on the cuticle of being submerged or some kind of quorum issue related to having multiple infection sites across the entire leaf versus having infection sites concentrated in a single area.

For all the leaf inoculation experiments performed in this dissertation I used the more convenient fill inoculation methodology.

### **Time course of infection**

Torres et al. (2104) while studying the interaction between maize and *C. graminicola* observed that the fungus produced appressoria by 24 hpi, and that more than 90% of the infection sites consisted of hyphae that were colonizing at least two cells by 48 hpi. Secondary necrotrophic hyphae became evident by 60 hpi. Wharton and collaborators reported that *C. sublineola* formed secondary hyphae by 66 hpi. The goal of this experiment was to describe the time course of infection of detached sorghum leaf sheaths that were inoculated by filling with *C. sublineola* spore suspensions. The goal was to determine the best time point for the evaluation of fungal infection for my assay.

**Material and methods:** Fungal strains CgS11 (from grain sorghum); M1001 (from maize); and JG18001 (from johnsongrass); and V3 seedlings of sweet sorghum (Simon) and sweet corn (Golden Jubilee) were used for the experiments. The leaf sheaths were prepared, inoculated by filling with spore suspensions ( $5 \times 10^5$  spores/mL), and incubated in moist



chambers as described above. Fungal colonization was evaluated at 12, 24, 36, 48, 60, 72 and 108 hours post inoculation (hpi). I observed 50 infection sites per leaf sheath, and recorded the developmental stage of the infection for each. A total of 10 leaf sheaths were observed for each treatment, and the experiment was repeated three times.

**Results and Conclusions:** With the fill inoculation technique, *C. graminicola* colonized sweet corn much more quickly than had been reported by Torres et al., (2014), who had used the drop inoculation technique and a field corn inbred (Table A1.1). *C. sublineola* colonized sweet sorghum more slowly than *C. graminicola* colonized sweet corn. *C. sublineola* formed primary hyphae by 36 dpi, and didn't start to produce secondary hyphae until 72 dpi (Table A1.1). I decided to do my assay evaluations at between 60 and 72 hpi.

### **Effect of leaf sheath age**

The work of Ferreira and Warren (1982) showed that 35-day-old grain sorghum seedlings were more susceptible to *C. sublineola* than 15-day-old seedlings. My goal in these experiments was to discover whether *C. sublineola* aggressiveness varied according to age of the leaf sheath.

**Material and methods:** Two fungal strains (CgS11 and JG18001) were used for this experiment. Plant material used was the sweet sorghum variety Simon. Seedlings at the V5 stage of development were cut at the soil line and brought to the laboratory. Four leaf sheaths removed from each seedling, which were designated in order from oldest to youngest as (i) senescent; (ii) old; (iii) mature; and (iv) young, which was the youngest full-expanded leaf. The leaf sheaths were prepared, inoculated by filling with spore

suspensions ( $5 \times 10^5$  spores/mL), and incubated in moist chambers as described above. Observations were made at 60 hpi. The experiment was performed once with each strain.

**Results and Conclusions:** The youngest leaf sheaths were the most susceptible to CgS11 (Figure A2.2). I observed 15 infection sites in which the pathogen was growing in more than two cells (Figure A2.2). The old and mature leaf sheaths were more resistant: the pathogen was found colonizing more than two cells in only 3-5 infection sites on those sheaths. The senescent leaf sheaths were the most resistant to CgS11. The pathogen was not observed to colonize more than two cells at any infection sites in those sheaths (Figure A2.2). Similar results were found in inoculations with JG18.001, which was generally less aggressive than CgS11 (Figure A2.3). Based on these experiments I decided to use sheaths from the fourth fully expanded leaves of V5 plants for all subsequent experiments.

### **Disease evaluation scale**

Wharton and collaborators (195, 196) observed at a cellular level how *C. sublineola* attacks grain sorghum and how the plant defends itself. The pathogen produces primary hyphae that grow from one cell to another, invading cells that are alive but killing them rapidly once they have entered them. By 66 hpi, the cells appeared to be disorganized and the pathogen had produced narrower secondary hyphae branching from the primary hypha. These authors described how plant cells responded to invasion by the accumulation of a red pigment, which was distributed throughout the infected cells and which also accumulated in the epidermal plant cell walls. They reported that compatible interactions did not result in the accumulation of these red compounds (195, 196). However, in my own studies I observed production of red vesicles or pigments in incompatible interactions (in

which the fungus did not colonize significantly) as well as in compatible ones (in which the fungus colonized several cells). I could observe mixed infection types on the same leaf sheaths (Figure A2.4). The aim of the work described in this section was to evaluate the range of colonization and response types that occurred in the interaction of *C. sublineola* and sorghum, and to develop a uniform diagrammatic scale that I could use to evaluate fungal pathogenicity and plant response for the leaf sheath assay.

**Materials and Methods:** I used the inoculation to fill up the whole leaf sheath described appendix 1A.

**Results and Conclusions:** I developed a diagrammatic scale for evaluation of the interaction between *C. sublineola* and sweet sorghum var. sugar drip at a cellular level under the light microscope with no need to stain or fix the plant tissue. This scale includes all the interaction types that I observed in more than a thousand separate interactions. This diagrammatic scale was used to evaluate all leaf sheath assays performed in this dissertation.

Table A2.1. Summary of the time course of maize inoculated with *C. graminicola*, sweet sorghum inoculated with *C. sublineola* (CgSI1 and JG18001). Disease evaluation was performed every 12 hours.

| <b>Evaluation (hpi)</b> | <b>Maize/M1001</b> | <b>Sorghum/CgSI1</b> | <b>Sorghum/JG18001</b> |
|-------------------------|--------------------|----------------------|------------------------|
| 12                      | App                | App                  | App                    |
| 24                      | PH                 | App                  | App                    |
| 36                      | SH                 | PH                   | PH                     |
| 48                      | SH                 | PH                   | PH                     |
| 60                      | Acervuli           | PH                   | PH                     |
| 72                      | Acervuli           | SH                   | SH                     |
| 108                     | Acervuli           | Acervuli             | Acervuli               |

App, appressoria; PH, primary hyphae; and SH, secondary hyphae.

Figure A2.1. Development of CgS11 when inoculated on sweet sorghum leaf sheaths by drop or fill inoculation at 72 hpi. Number of infection sites with appressoria, first picture; or has grown in one cell (second picture); or has grown in more than 2 cells (third picture). Error bars represent standard deviation.

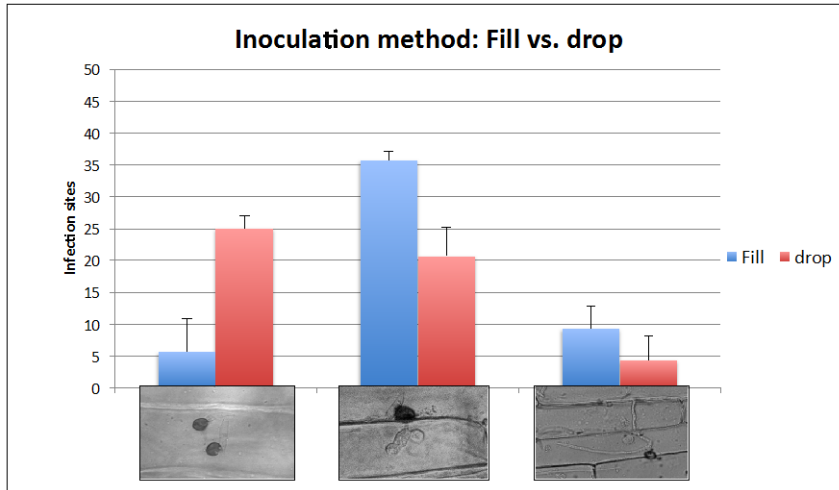


Figure A2.2. Development of CgS11 on sweet sorghum leaf sheaths at different stages. Senescent leaf sheaths (dark orange bar); old leaf sheaths (light orange bar); mature leaf sheaths (light green bar); young leaf sheaths (dark green bar). Number of infection sites with ungerminated spores, appressoria, hyphae growing in one cell, or more then 2 cells; infection site surrounded by reddish, vesicles, cells entirely red, and red hyphae. Error bars represent standard deviation.

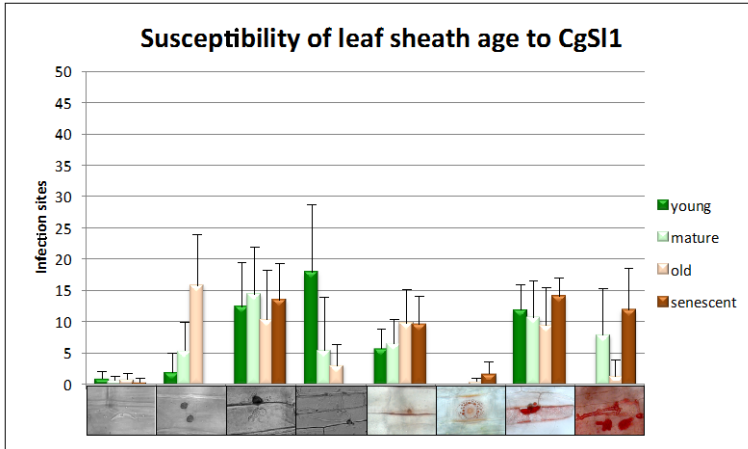


Figure A2.3. Development of JG18001 on sweet sorghum leaf sheaths at different stages. Senescent leaf sheaths (dark orange bar); old leaf sheaths (light orange bar); mature leaf sheaths (light green bar); young leaf sheaths (dark green bar). Number of infection sites with ungerminated spores, appressoria, hyphae growing in one cell, or more than 2 cells; infection site surrounded by reddish, vesicles, and cells entirely red. Error bars represent standard deviation.

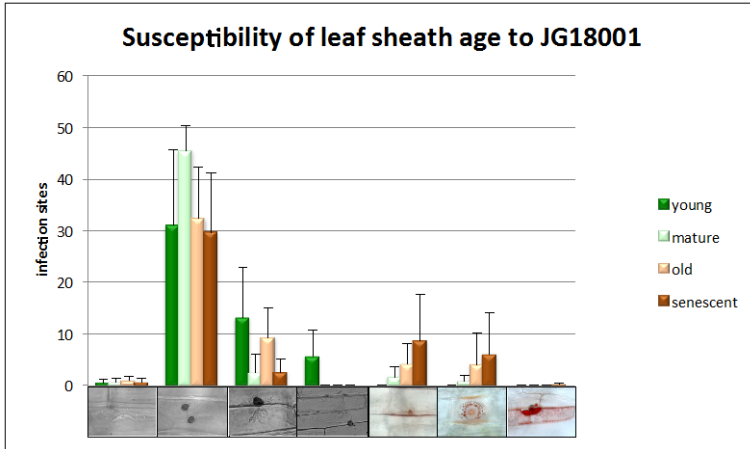


Figure A2.4. Pattern of CgSI1 development on sweet sorghum leaf sheaths at 106 hpi. On the top of the picture there are red cells which a plant defense strategy in a incompatible. In the bottom of the picture we there are acervuli, which are structures produce by the pathogen in a compatible interaction.

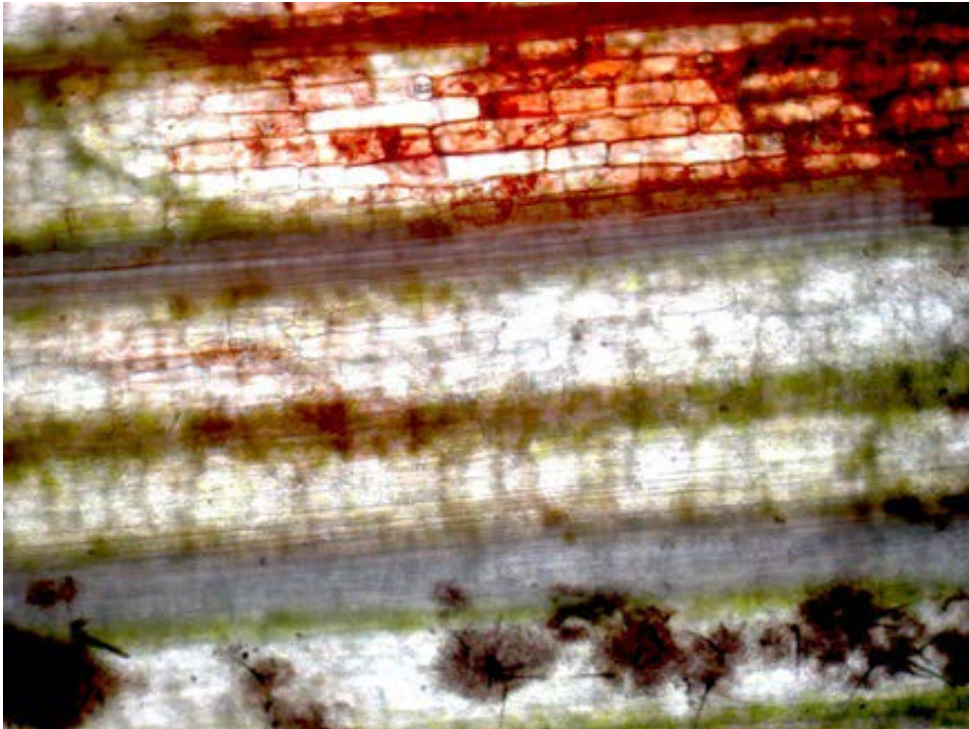
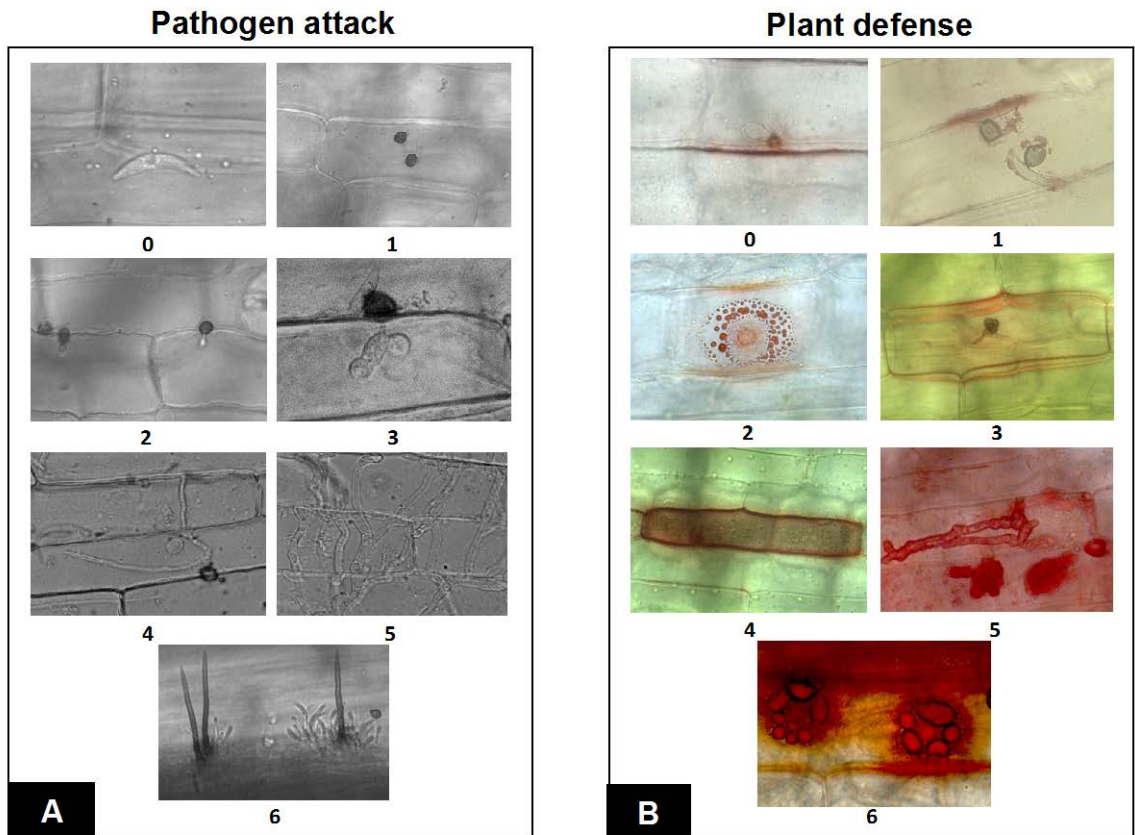




Figure A2.5. Diagrammatic scale for evaluation of the interaction between *C. sublineola* and sweet sorghum. A. Pathogen Attack; (0) spore did not germinate, (1) Appressoria has been formed, (2) Hyphae smaller than appressoria, (3) Hyphae bigger than appressorium but restricted in one cell, (4) Hypha growing in more than two cells, (5) Hypha growing in more than three cells, and (6) Acervuli has been formed. B. Plant Defense; (0) Reddish surround infection site, (1) Reddish surround appressoria, (2) Red vesicles around the infection site, (3) Plant cell is light red, (4) Plant cell is very red, (5) Red compound has covered the pathogen hyphae, and (6) Big red vesicles has accumulated.



## Appendix C - Alignments used to create the unrooted Secondary Metabolites

### Phylogenetic Trees in Chapter 4

#### PART 1: DMAT protein sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

```
CH063_0589 -----
GLRG_01200 -----MAQLIPPSHNALVVRPHIPITCQEREEVLRVSEYNVFAFPAA
CS1 -----MARLIPPSHNALVVRPHIPITAEEEREEVLRVSEYTVFAFPAA
CH063_0902 -----MGEMIMSHPEWDFLNSVLRFRD-----
GLRG_07878 -----MGEMIMSYEPWDYLNLSILRFRD-----
CS.DMAT -----MGEMIMSYEPWDFLNSILRFRD-----
CS7 -----MPGLTASRTQAEKS----LVVWQS
CH063_0738 -----MEATSTDQFPLWDSFPRGKTKSWMYKGAGATESKSKSTSKPEHDGTLVFPS
GLRG_11574 -----MHNGDIVADSKPKQYLT---GNLILSS
CS6 -----MKPARDKTKSWMHEGDIVTESKAKQNST---GIPVLPS
CH063_0204 -----MVAATNGSDLARQ-----
GLRG_11853 -----MVMSTSGPEPERQ-----
CS8 -----MVIATSGPEPSRQ-----
CS5 -----MPSMERMEIYRLNDVRDDHSGTVTKN----PTYNEA
GLRG_11423 -----MEIYRLTVVNDANGSI-TEK----SIYKDA
CH063_1280 -----MEIYRLSAVHDASGGIDTKR----NIYNDA
CH063_0144 -----MSKTVLIRPRGPVLSAASLDTGP-----
CH063_0491 -----MQATASIPGPLPAEHGHAAVGRPGLSAQTQS-----SN
CB2 MAPIIATPTYTATTRSPHPLQLSSRCVRSPMAFGQTEILSPRAMAKQEK----DLCLPA
CH063_0654 ----MATSTYTTSHRPPFLQLSPRALRSPMAFGQSEILSPRALAKQEK----DLCLPA
GLRG_02500 -----MAFSQADILSPRAMAKQEK----DLCLPA
CS4 -----MALMPVRTTS-----
GLRG_07955 -----MTPETISNDFQTNVDAPRPPQNFKE-----FKSL
CH063_1116 -----
CB.DMAT -----MGEISSGSYNLN-----
CH063_0401 -----

CH063_0589 -----MMYEANYPDETSNRFLTF-----
GLRG_01200 LLTCDFLSDSGTSAMTDVQWAAMLRGDESYGRNSGYCLLEAFRDIFE-----RDV
CS1 LLTCDFLSDSGTSAMTDVQWAAMLRGDESYGRNSGYCLLEAFRDIFERGEDRKYVFRD
CH063_0902 -----NDSQFWWDKTGRMFASKLLKYAG-YSASEQYRELNF-----
GLRG_07878 -----DDSQFWWDKTGMMFASKLLKYAG-YSTSEQYRELNF-----
CS.DMAT -----DDSQFWWDKTGRMFASKLLKYAG-YSTSEQYRELNF-----
CS7 LAKKCLPPAGN--KNTEFWWNLTGYHLAVMIDAAG-YSTQLQYETLLF-----
CH063_0738 VSKWLPPRD---EHSYWWNMAGPHFATLFRNAG-YSLLEEYEAALLF-----
GLRG_11574 VTKWLPSRD---EHSYWWNMAGPHFSALLQNAG-YSSQAQYETLLF-----
CS6 VSKWLPSRD---EHSYWWNVAGPHFSALLENAG-YSIQAQYEILLF-----
CH063_0204 -----EDSRFWWDELAHPLLSLMKSAG-YSAEEQEYVEF-----
GLRG_11853 -----EELQFWQDEISHSLLSLMQSAG-YSLKEQEHYLEF-----
CS8 -----DESQFWRDELSHSLLSLMQSAG-YSLKEQQHYLEF-----
CS5 IDPSTSSSKGSPRPLVSSWLPPIHNSLSLLRWAGPYPAEVQESHAF-----
GLRG_11423 VASSP-SSKGS--PLFSSWLPLIHNSLTSLLRWTGTYPAEVQESHAF-----
CH063_1280 VDSSPSSKGS--PLYSSWLPLIHNSLSLLRWGTYPADVQESHAF-----
CH063_0144 -----EDVDFWSSYLVPFRHAYLSEAGSYTPEQQAHLSC-----
CH063_0491 ALYVGMNPSPPANPDHRFWWDHFAPFLSWLCASGCYSESIDIAAQLTI-----
CB2 QESIYIHK----PDQDFWAARCESLDCLMQAAGSYTPAQRAAHLQF-----
CH063_0654 QESIYIHR----PDQDFWTVRCAEILDGLMQAAGSYTPAQRAAHRL-----
GLRG_02500 QESIYIHR----PHQDFWAVRCAESLDGLMQAAGSYTPAQRAAHLQF-----
CS4 -----PL-----
GLRG_07955 VNNNEVPIDPLNPEHRWWDRCAPLLNSLLNSAESYTSEEKADHLRV-----
CH063_1116 -----MAPAVTLNHDTDRPLSLLTSAGSYTPEEKADHLRV-----
```

CB.DMAT -----PDQQYWWQFVEPALSSMLVYAGRYTLEEQESHKAW-----  
 CH063\_0401 -----  
  
 CH063\_0589 -----  
 GLRG\_01200 LAGTADGIFYRQTFPKEVGGGFVNGGPLQLIRPNFFILPQGRCAESLLFSTMSEILRNES  
 CS1 LAGTADSTLYRQTFLKEVA-----VAPSL-----ARQLSSAM-----  
 CH063\_0902 -----  
 GLRG\_07878 -----  
 CS.DMAT -----  
 CS7 -----  
 CH063\_0738 -----  
 GLRG\_11574 -----  
 CS6 -----  
 CH063\_0204 -----  
 GLRG\_11853 -----  
 CS8 -----  
 CS5 -----  
 GLRG\_11423 -----  
 CH063\_1280 -----  
 CH063\_0144 -----  
 CH063\_0491 -----  
 CB2 -----  
 CH063\_0654 -----  
 GLRG\_02500 -----  
 CS4 -----  
 GLRG\_07955 -----  
 CH063\_1116 -----  
 CB.DMAT -----  
 CH063\_0401 -----

CH063\_0589 -----YRDVVCPLLG-GRPKPXSL-----  
 GLRG\_01200 ILNNDTTDPPALISNGFFDITGANAAATGFQLHTLTQPGLTDPFPFDQVEKSNPFKGNLD  
 CS1 -----GFFDITGAKAAATGFQLHTFTQPGLTDPFPVEPVGRSSPFKGNLD  
 CH063\_0902 -----YSLFVAPELG---PSPDCHGNV---RRW-  
 GLRG\_07878 -----YSLFVAPELG---PSPDSQGNV---RRW-  
 CS.DMAT -----YSLFVAPELG---PSPDSHGNV---RRW-  
 CS7 -----HYHWIVPRLG---PAPYPDGN---PKF-  
 CH063\_0738 -----VYHQVIPRLG---AAPGSSDSAAAAAAGG  
 GLRG\_11574 -----IYHQITPRLG---PSPLLSNA---DRL-  
 CS6 -----IYHHISPKMG---PSPIISNP---DRP-  
 CH063\_0204 -----VNQFVVPSLG---PRPMTTKTGVRPLPHF-  
 GLRG\_11853 -----TKQHVFSSLG---PPPITTKSGARIPHF-  
 CS8 -----TNQHVVSSLG---PPPITTKSGVRIPRF-  
 CS5 -----MRDVVVPCLK---PPAAA-----DRL-  
 GLRG\_11423 -----VRDVVVPRLK---PPAAA-----DRL-  
 CH063\_1280 -----VRDVVVPRLK---PPAAA-----DRL-  
 CH063\_0144 -----VRAAAV-ALGPKHPPHPV-----  
 CH063\_0491 -----LRDVVIPAFG---PPSVV-----  
 CB2 -----LSEIIVPAMG---PHPST-----APT-  
 CH063\_0654 -----LTEIIVPAMG---PHPSA-----APT-  
 GLRG\_02500 -----LSEIIVPAMG---PHPSA-----APA-  
 CS4 -----ARTVVVPSSG-----F-  
 GLRG\_07955 -----FRDVVVPSFG---TPTPR-----AKV-  
 CH063\_1116 -----FRDVVVPSFG---TPTPE-----AKV-  
 CB.DMAT -----YATQVTPIFG---PRPSE-----GNP-  
 CH063\_0401 -----

CH063\_0589 ---PTAVGWDGN-----PFEYSF-----  
 GLRG\_01200 IEKTSTFLSCNGNAARSVMLLLTITNNWAGAQPVS MANIKATAALAQT HDIPLFFDACRF

CS1 IEKTS AFLSRDGN AARSAM LLLTITNNWAGAQPVS MANIKAASAL AQT HDIPLFFDACRF  
 CH063\_0902 ---RSPGTPDST-----PIDFSW-----  
 GLRG\_07878 ---KSPGTPDST-----PIDFSW-----  
 CS.DMAT ---RSPGTPDST-----PIDFSW-----  
 CS7 ---KSLIAYDGS-----PFEYSW-----  
 CH063\_0738 ---DSGLSLDNT-----HIEYSW-----  
 GLRG\_11574 ---DSGLSLDGT-----RFEYSW-----  
 CS6 ---DSGLSIDGT-----RFEYSW-----  
 CH063\_0204 ---DSFCSDDFS-----PAELSW-----  
 GLRG\_11853 ---DSFCSDDFS-----PAELSW-----  
 CS8 ---DSFCSDDFS-----PAELSW-----  
 CS5 ---SYVANHNHS-----TYEASL-----  
 GLRG\_11423 ---YYIGTHSHG-----IYEASL-----  
 CH063\_1280 ---HYIGTHSHG-----IYEASL-----  
 CH063\_0144 ---KSALTKNGS-----PIELSF-----  
 CH063\_0491 ---QHALKPTGS-----PFELSW-----  
 CB2 ---KSLLTADGS-----PFKPSW-----  
 CH063\_0654 ---KPLLTADGS-----PFEP SW-----  
 GLRG\_02500 ---KPLLTADGS-----PFEP AW-----  
 CS4 ---QHRRQKSGP-----SRTM-----  
 GLRG\_07955 ---RPLLYDGS-----TFEP SW-----  
 CH063\_1116 ---KPLLYDGS-----TFEP SW-----  
 CB.DMAT ---DPIFTHDSS-----PCHISI-----  
 CH063\_0401 -----  
  
 CH063\_0589 -----EFKGSTKKAGVRFVLDLSEVRPA-----NRDYPL-SL  
 GLRG\_01200 AENAKFIQDFEDGYSNMTPQVVREMF SHAGGFTISLKKDGLATMGGALCFRDEGMFSRK  
 CS1 AENAKFIQDFEDGCGNKTIQIVQEMFSQVDGFTISLKKDGLANMGGALCFRDEGVFSRK  
 CH063\_0902 -----EWGH-DNRAVIRYSFEPIGLHAG-----TDL DPL-NR  
 GLRG\_07878 -----EWGC-DNRAVIRYSFEPIGLHAG-----TDL DPL-NQ  
 CS.DMAT -----EWGH-DNRAVIRYSFEPIGLHAG-----TDL DPL-NQ  
 CS7 -----KWNTTAGEPDIRYSWEAINSSG-----TTADPL-NH  
 CH063\_0738 -----RWNXADTKPEIRVMMEPFSRFAG-----TYLDPL-NL  
 GLRG\_11574 -----RWNQSYTKPEVRMVVEPFSRFAG-----TYMDPL-NI  
 CS6 -----RWNQPDTKPEVRMVLEPFSRFAG-----THMDPL-NI  
 CH063\_0204 -----NVGP--ARSKIRVGSEPIGPFAG-----TSKDPF-NQ  
 GLRG\_11853 -----NIGP--KRSKIRVGSEPIGPYAG-----TAKDPF-NQ  
 CS8 -----NIRP--KQSKIRVGSEPIGPFAG-----TTRDPF-NQ  
 CS5 -----AFSS-YRPPKVRYSVQPLVDP-----SPGDPL-GQ  
 GLRG\_11423 -----AFAT-HKPARVRFTVQPLVDPSA-----QGDLL-GQ  
 CH063\_1280 -----AFGS-HKPARVRFTVQPLVDPSA-----QRGDPL-GQ  
 CH063\_0144 -----NLSE-DRLPTARFYIEPLGPETG-----TENDPF-GE  
 CH063\_0491 -----NFTP--HGNTIRYTFQPMGSRAG-----TDEDPFGSS  
 CB2 -----TITD-SGSSFIRFSFEP MGRHGG-----SASDPF-AQ  
 CH063\_0654 -----NITD-SGSSIRFSFEP MGRHGG-----SESDPF-AQ  
 GLRG\_02500 -----NITD-SGSSLVRF SFEP MGRHGG-----STSDPF-AQ  
 CS4 -----NFTK-GDDGVIWYTFEPLCDTTG-----SADDPF-AG  
 GLRG\_07955 -----NFTK-GNDGVVRYTFEPLGDTAG-----SEEDPF-AG  
 CH063\_1116 -----NFTE-GDDGVIWYTFEPLGDDAG-----SEEDPF-AG  
 CB.DMAT -----NWCS-KTKPTVRSGMT-----RPHDVFN SQ  
 CH063\_0401 -----  
  
 CH063\_0589 ATVEKVLVDLSTK--SPL-YDDH-----WHRSL-ERW-----FVYS  
 GLRG\_01200 FGADV GIRLKERQIMCYG-NDCSGMSGRDVM AAAVGLYEVTKEAYLADRIGQVKRFAEG  
 CS1 FGADV GIRLKERQIMCYG-NDSYGGMSGRDVM AAAVGLYEVTKESYLADRINQVKRFAEG  
 CH063\_0902 HATNDWIFKLQQQKMVPG-LDLE-----WYNHFTQOI-----LPHG  
 GLRG\_07878 YATNDWIFKLQQQNMVPG-LDLD-----WYNHFTDQI-----LPRG  
 CS.DMAT YATNDWIFKLQQQNMVPG-LDLD-----WYNHFTNQI-----LPRG  
 CS7 DPTLDYMEKVPVI--LPE-VDFS-----WYRHFLAEL-----Y--N  
 CH063\_0738 RPATEMLYSMTPQ--VPS-LDMS-----LFNHVAKF-----Y--

GLRG\_11574 RPSMETLYSMKSQ--VPS-LDMT-----LFNHFIACL-----Y---  
 CS6 KPAMETLYSMMPQ--IPS-LDMT-----LFHHFVAKL-----Y---  
 CH063\_0204 DESQVVMRLLRQ--GRGPVDGE-----LWEFFKKHL-----HVEA  
 GLRG\_11853 DESEVVMRLLRQ--GRGPVDGE-----LWEFFKKHL-----HVEA  
 CS8 DEPQVVMRLLRQ--GRGPIDEE-----LWEFFKKHL-----HVEA  
 CS5 KALRNILEGIASK--C-G-ADRK-----WLDADFIDSS-----FLTT  
 GLRG\_11423 KALRNTLEGMASA--C-G-ADRA-----WLD AFLDSV-----FLTA  
 CH063\_1280 KALRETLDLASA--C-G-ADRA-----WLD AFVDSV-----FLTA  
 CH063\_0144 SWAARGFSCLASQ--MTS-MDTS-----WYEH LGQAF-----RLKG  
 CH063\_0491 NLDDEVLP L LERH--SAN-VDLR-----WFCQFRDAW-----MMHR  
 CB2 KIVPAILPALRMV--SHG-ADTR-----WFEQFMSVL-----FLTE  
 CH063\_0654 KIVPAILPALRMV--AHG-ADTR-----WFEQFMSAL-----FLTE  
 GLRG\_02500 KILPAILPALRMV--SHG-ADTR-----WFEQLMSAL-----FLTE  
 CS4 EIRRS LIPILSQA--PSD-IDLQ-----WYKQVMNEW-----FVTP  
 GLRG\_07955 EIGRSLIPILS QV--SSD-VDLQ-----WYKQVVNEW-----FVTP  
 CH063\_1116 EIGRSMMPVLSRV--SSD-VDLR-----WYEQIVNAW-----FVTP  
 CB.DMAT AFVDELRTAIET--SHE-PDLT-----LFDALAKSL-----FVHD  
 CH063\_0401 ----MLSQMKEE--IRD-IDLH-----WADQFIENL-----FPNN  
 \*  
 CH063\_0589 HKPSERQRELVAKVGY-----QTPTI-----LGF  
 GLRG\_01200 LIQEGIPVLLPPGGHAIFLDMNGFFAGCARSYGEFASVGF TLELLKDYEIRACEAGPFGW  
 CS1 LIEEGIPVLLPPGG-----CARSYGEFASIGFTPELLKDYGIRACEAGPFGW  
 CH063\_0902 TRTKTVDRFVEETTPK-----AGTV-----VAL  
 GLRG\_07878 NRTKTVDRFIEETTPK-----AGTV-----VAL  
 CS.DMAT NRTKTVDRFIEETTPK-----AGTV-----VAL  
 CS7 PDRSVYAKEIEEGEPP-----AT TLM-----HAV  
 CH063\_0738 --DAAH HKYLETNERP-----VMTNVC-----LGF  
 GLRG\_11574 --DSEWHRYLDTNERP-----IVTNVC-----LGF  
 CS6 --DSEWHRYLETKERP-----IVTNVC-----LGF  
 CH063\_0204 KNAHDIVAKMATNEHM-----TTNT-----ISF  
 GLRG\_11853 KHANEIVSKMAPNEHM-----TTNT-----ISF  
 CS8 KHANEIVSKMAPNEHM-----TTNT-----ISF  
 CS5 EEQAALVSKGAVAAAAS----DASHPLPQSAL-----VAF  
 GLRG\_11423 EEELTLVGKRGMDAAN-GDAGAAGGPLRQNGF-----VAF  
 CH063\_1280 EEEASLVGKAAAAAAGGGGPGAAGGPLRQNGF-----VAF  
 CH063\_0144 QVEVDTANSQSRPGMW-----LPKVF-----LGV  
 CH063\_0491 HHADAAA AKEAMKQHA-----NVKIPSQLF-----LGY  
 CB2 SETRAALARIPKGTRA-----PTSF-----LAF  
 CH063\_0654 SETKAALAKLPKGTRA-----PTSF-----LAF  
 GLRG\_02500 SETRAALARLPEGTRP-----PTSF-----LAF  
 CS4 EGAVAAACQNMPPHV KR-----IPQLF-----LAC  
 GLRG\_07955 EEAVAAARQNMPPHIKR-----IPQLF-----LAY  
 CH063\_1116 DEAAAARQNMPASVQR-----IPQVF-----LAY  
 CB.DMAT PQEVAKVKAVVPLHLH-----TLIPNIA-----IAW  
 CH063\_0401 EAEVELALKRAQTELP-----PPLDHALTFN-----MAL  
 .  
 CH063\_0589 DINPKITELAPALLPVMVKSYFPPCFVTE DRG----FTRF--RSLALGVR-----  
 GLRG\_01200 EWDKKGAI AQDR-DSIP-NLVRFAVPRYVMSD----RHIEYTATAISALHKRRHTIPGAR  
 CS1 EWDKKGAI AQDR-DSIP-NLVRFAVHRYVMSD----RHIEYTVAAISALHKRRQNIPGVR  
 CH063\_0902 DIE-----KS-GPVM-KIYIYPGLKAEELG---ITNL--ELVEQSIR-----  
 GLRG\_07878 DIE-----KS-GPVM-KIYIYPGLKAEELG---ITNL--ELVEQSIR-----  
 CS.DMAT DIE-----KS-GPVM-KIYIYPGLKAEELG---ITNL--ELVEQSIR-----  
 CS7 EYN-----RH-ASFGLKSYFLP--RKLFG---GDPATLEEWDA AIV-----  
 CH063\_0738 EFL-----GH-DILP-KAYFFPR-KLGHVG---ITPM--AVWEDAIA-----  
 GLRG\_11574 EFK-----GS-EILP-KAYFFPR-KLGQSG---LTPM--RVWEDAIL-----  
 CS6 EFQ-----DTNDILP-KAYFFPR-KLGQAG---LTPM--EVWEDAIA-----  
 CH063\_0204 DLE-----GE-LPAP-KVYFYPIPI SLLQN---NHAG--EIITDVIA-----  
 GLRG\_11853 DLD-----DE-QPAP-KVYFYPIPI SLLAN---NHAG--EIITDVVA-----  
 CS8 DLD-----GE-QPVP-KVYFYPIPI SLLKN---NHAG--EIITDVIA-----  
 CS5 DLHVNTDKGGKA-ANTM-KTYLFPQYKALITG---QKTV--DTTDSIVR-----

GLRG\_11423 DLEADMDENEKA-VTVM-KTYLFPQLKAIATG---QKTV--NTTDSIVK-----  
 CH063\_1280 DLEANMDESCKA-ATVM-KTYLFPQLKAIATG---RKTV--DTTDSLVR-----  
 CH063\_0144 DFK-----GA-DRML-KCGFCPLLKLSAMGAKWDRDLADHNKFLVDVVR-----  
 CH063\_0491 DLV-----GP-KAQL-KAYFLPIFKHFATG---RSTD--ALAAEMIR-----  
 CB2 DLD-----GD-KTIF-KAYFFPTLKHAIATG---ESAE--NITFDAIR-----  
 CH063\_0654 DLD-----GD-KAVF-KAYFFPILKHIATG---ESPE--NITFNIR-----  
 GLRG\_02500 GLD-----GD-KTVF-RAYFSPILKHFATG---DSTE--SITFNIR-----  
 CS4 DVK-----RS-KRIL-KAYLFPALKHSMVG---ATTS--DFVDMIS-----  
 GLRG\_07955 DMI-----RS-KRML-KAYLFPVLKHFATG---ATTS--DLVFDLIP-----  
 CH063\_1116 DMK-----RA-KRVL-KAYLFPVLKHFATG---AATS--DLVFDLIP-----  
 CB.DMAT DLV-----GP-IKKL-KLYHNPQAKKLATG---RTGN--EIVISSIR-----  
 CH063\_0401 DLS-----GA-TRKM-KAYMFPMAKNLATG---RHRDARDAGFDAIR-----  
  
 CH063\_0589 -----QLPDIGSHP-NILLGLK-----MIEDFVA  
 GLRG\_01200 ITRGEELRMRVFSQGLEPVPVTSTADDSATSPMPVSTFLSEAKKDVKKLHATLELRREAW  
 CS1 ITRGKELRMRVFSQGLEPVPVKTSTN-----LHAALKLDREVW  
 CH063\_0902 -----SLPAEQFQSLQAEPLFD-----FLRE--  
 GLRG\_07878 -----NLPAEQFQSLSAEPLFE-----FLRE--  
 CS.DMAT -----NLPAEQYQSLNAEPLFE-----FLRE--  
 CS7 -----KLSPDSKHP-GRDALMH-----FLAT--  
 CH063\_0738 -----TAVPGS--P-SMXTVFS-----  
 GLRG\_11574 -----TAIPQS--P-TVATVFS-----FVQ---  
 CS6 -----TAIPQS--T-TMENVFS-----FVK---  
 CH063\_0204 -----QLP---V-NLEPSFN-----PVRDFVY  
 GLRG\_11853 -----QLP---V-NLEPAFN-----YVREFVY  
 CS8 -----QLP---V-NLEPAFN-----YVRDFVY  
 CS5 -----VLAEGN--Q-EMLAAWE-----LIKTFLN  
 GLRG\_11423 -----RLAGGD--K-DMLAAWE-----LVKTFLI  
 CH063\_1280 -----RLAQGD--K-QMLASWE-----LLKTFLV  
 CH063\_0144 -----GLPGCG--A-NMSAALG-----MLERYLV  
 CH063\_0491 -----SLEPFG--P-ELEDQVR-----QMEQFL-  
 CB2 -----SLSPGG--A-DLTSSAN-----AAEYF-  
 CH063\_0654 -----SLSPGG--A-DLARSAN-----ATEAYF-  
 GLRG\_02500 -----SLSPGG--P-DLAPSAH-----ATEEYF-  
 CS4 -----KLQSFQ-----  
 GLRG\_07955 -----KLQPGF--D-KLAMPAR-----KLQKYL-  
 CH063\_1116 -----KLQPGF--D-KLAAPAA-----KLRKYL-  
 CB.DMAT -----ALAKYG--H-DFTEAMD-----ILERYVM  
 CH063\_0401 -----NLKPHG--D-KLVPAVD-----FLDRY-W  
  
 CH063\_0589 -----DNPQYEGCGRG--LSTDFVPAG--Q-----  
 GLRG\_01200 ELIEAALDISMQGWGKLTVLDVDFSPGWRPDVCLNLCPIEYSVAIERNTGKPKQ-----  
 CS1 ELLQAALDVSMQGWGNWTVSECPNGWRSASLSLCPFEYSVAIERDTGKPKQ-----  
 CH063\_0902 -----GTKKYDFETGI--LAIDCLAPR--D-----  
 GLRG\_07878 -----GTKKYDFETGI--LAIDCLAPK--D-----  
 CS.DMAT -----GTKNYDFETGI--LAIDCLAPK--D-----  
 CS7 -----SPEGQLMKPNV--MGMDNVTPS--K-----  
 CH063\_0738 -----  
 GLRG\_11574 -----NDAPRLGLTLAPLWLGIDVVNPV--E-----  
 CS6 -----NDAPTLGLTLAPLWLAIIDVVNPA--K-----  
 CH063\_0204 RYKRQ-----RDNEYILRLEL--IAFDAIRPA--D-----  
 GLRG\_11853 RYKKQ-----RNNESILRLEL--IAFDAVRPV--E-----  
 CS8 RYKKQ-----RNNESILRLEL--IAFDAVRPV--D-----  
 CS5 -----TNSGKKVMLDF--LAIDCLAPS--KEARSKR  
 GLRG\_11423 -----TDGGEKINLDF--LAIDCLAPC--KE-----  
 CH063\_1280 -----NDGGEKMNIDF--LAIDCLAPS--KE-----  
 CH063\_0144 QDREHSAGAQQGDGVDGGRKDCASSMCQACAKANRPKPFNLVSDCADPSNGK-----  
 CH063\_0491 -----AGCSYPHFIDM--VGIDCCDPA--K-----  
 CB2 -----KNAPFPIPVEM--ISLDCIDPAAG-----  
 CH063\_0654 -----RNAPFSIPVEM--IALDCIDPAAG-----

GLRG\_02500 -----KNASFPIPVEM--IALDCIDPAAG-----  
 CS4 -----DKLPCIVES--MAIDCFDPS--K-----  
 GLRG\_07955 -----ATCKEPCLEVEM--MAIDCVDPDPS--K-----  
 CH063\_1116 -----AGCREPCLVEM--MAIDCVDPG--K-----  
 CB.DMAT -----KLNAEQLELII--IGLDAADPNLPT-----  
 CH063\_0401 -----DTCPERLILDM--IGMDCIDPS--K-----

CH063\_0589 ARLKV-----YLRWYG-DSFDEIWDYY----TLGGRI  
 GLRG\_01200 LRFLVEAQADAATLEAHQKSALQLTADIQSH-YPQTVSLGRLNAVKDLFFPPASQVEGIF  
 CS1 LRFMVEAQADAATLEAHQKSALHLTADIQSG-YSQIVSLDRFNVVKDLFFPPA-QAEGTF  
 CH063\_0902 ARIKV-----YIRAKH-TTFDYMMDCI----TLGGRL  
 GLRG\_07878 ARIKV-----YVRAKH-TTFNYMMDCI----TLGGRL  
 CS.DMAT ARIKV-----YVRAKH-TTFDYMMDCI----TLGGRL  
 CS7 SRLKM-----YFTSRH-TCFNSVREII----TMGGRL  
 CH063\_0738 -----FVRTTP-----XXSG--  
 GLRG\_11574 ARLKF-----YCVESR-TSFDSVKSIM----TMGGII  
 CS6 ARLKF-----YCVPEQ-TCFNSVKSIL----TMGGII  
 CH063\_0204 SRFKL-----YLRTKE-TCMARVEEVY----SLGGAL  
 GLRG\_11853 SRFKL-----YLRTKE-TCLARVEEVY----SLGGAL  
 CS8 SRFKV-----YLRTKE-TCLARVEEVY----SLGGAL  
 CS5 PRLKI-----YFSTNF-KSLAAVREVV----TLGGLL  
 GLRG\_11423 PRFKV-----YVSTRF-KSLAAVREAF----TLGGLL  
 CH063\_1280 PRFKV-----YVSSH- KSLAGVREAF----TLGGLL  
 CH063\_0144 GRVKL-----YTRTSC-NAFACIRDAV----TLGGRL  
 CH063\_0491 ARIKV-----YVRVNN-VSRACVKYFL----TMGGRL  
 CB2 ACAKL-----YARTRS-NAFSVVRHVM----TLGGQI  
 CH063\_0654 ARAKL-----YARTRS-NAFNVVRDVM----TLGGQI  
 GLRG\_02500 ARAKL-----YARTRS-NAFSVVRHVV----TLGGQI  
 CS4 ARVQV-----YARRIS-NSKCVLVDVF----TLGGTQ  
 GLRG\_07955 ARVKV-----YARTTS-NSKSVLADVF----TLGGTQ  
 CH063\_1116 ARVKV-----YARTAS-NSRSVLADVF----TLGGAQ  
 CB.DMAT TRVKVCHFSKSFHSDGPRTPESFLADTLHLQPYGIVSEANSWETVKNIIY----TLGGQV  
 CH063\_0401 ARIKI-----YAHVSTRNSWDLIRHVS----TFGGQA

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CH063\_0589 PIADLEDDKEKLRDLIQLSRGSE-----  
 GLRG\_01200 AAWHSFVAGHDQRPEWKIYLNPNARGKLQAAAVVREAFERLGMPPQAWILLEKVVLLRGGRL  
 CS1 AAWHSFAADNDRRPEWKIYLTNPVRGKSNATGVVREALERLEMPQAWTHLDKVVLLRGGRL  
 CH063\_0902 --DMSGEAIEDLKDFWRIFL-----  
 GLRG\_07878 --DMSGEAIDDLKDFWRTFL-----  
 CS.DMAT --DMSGEAIDDLKDFWQAF-----  
 CS7 --HVSESSLQDLRSLILAILGLPADYPEEDEF-----  
 CH063\_0738 -----  
 GLRG\_11574 ----EIASDMLAMIWDLMKAVCDLPDD-----  
 CS6 ----DIAPNMLAMIWDLMKAVCDLPDD-----  
 CH063\_0204 KGPEIDNGLDLIRSFYMHVLT-----  
 GLRG\_11853 KGPEIDHGLGLIRSFYLHVLGT-----  
 CS8 KGPEIDHGLNLRISFYLQVLGT-----  
 CS5 --PRSSANMDFLSEAWPLLMDMEDIP-----  
 GLRG\_11423 ---PSSADFLSQVWPQLDMEDIP-----  
 CH063\_1280 ---PRSSADFLPQVWPQLMDMEDVP-----  
 CH063\_0144 TDEDTEGLRRLRSVWHLLLNDP-----  
 CH063\_0491 DDERTRRMLADMDCRWHHVIDEE-----  
 CB2 NDEATLESLDALRSIWHLLHNEP-----  
 CH063\_0654 HDETTLEGLDILRSIWHLLHNEP-----  
 GLRG\_02500 SDEATLEGLRGLRCIWHLLHNEP-----  
 CS4 TDEATLKGVEAAEKVWHLLLDGP-----  
 GLRG\_07955 TDEATLKGVEAAEKVWHLLLDGP-----  
 CH063\_1116 TDEATLRGVETAEKVWHLLLDGP-----  
 CB.DMAT VNKERMKGLEILHSIWDLMRCHRGRDP-----  
 CH063\_0401 TDPDRLRGLGLEILHSLWDVLRNEQSNP-----

CH063\_0589 -----YP--ADKIRPETDAEKKRRA-  
 GLRG\_01200 TFLALDLCPGPQARVKAYVQYDNVGAELAEAAALVAPDTASASEIRRFCSTLSGGSEGP  
 CS1 MYLSLDLSPSSQARVKVYVQYDNVGAELAEAAALVAPETATAPEIQKFCSTMSGGSEGP  
 CH063\_0902 -----ADAPDVLPEEAPGRAS-  
 GLRG\_07878 -----ADAPDILPADAPGRAS-  
 CS.DMAT -----ADAPDILPADAPGRAS-  
 CS7 -----TPTSAKSGDKWDDFDSLCEG-  
 CH063\_0738 -----  
 GLRG\_11574 -----FPQDKDLPRAPQYNPSTNGID  
 CS6 -----FPQDKSLPRAPRYNPSTDGID  
 CH063\_0204 -----TAAEEDLPRSTHRTAG-  
 GLRG\_11853 -----TAPEEDLPHSTHRTAG-  
 CS8 -----TAPEEDLPHSTHRTAG-  
 CS5 -----QAEIEDLEKPLNDPTSHYQG-  
 GLRG\_11423 -----QAEMEDLEKPLNPNDSHHQG-  
 CH063\_1280 -----PAAMEDMEKPLSNPDSHYKG-  
 CH063\_0144 -----VSQHDDEYSRTVAVESHRRG-  
 CH063\_0491 -----AGYPDEQDKKPKDPATFHKG-  
 CB2 -----EPYGDDFDKQPRLEGTLHKG-  
 CH063\_0654 -----EPYGDDFDKQPKLLQTLHKG-  
 GLRG\_02500 -----EPYSDDFDKQPKMENTIHKG-  
 CS4 -----  
 GLRG\_07955 -----QGMAPDQRKDARDMKNLHKG-  
 CH063\_1116 -----EGMAPDQRKECRDARTLHKG-  
 CB.DMAT -----LP--DDYHKPKNDASSTRGV-  
 CH063\_0401 -----G--EDYDKPMRHPTSFLGS-

CH063\_0589 -IFGTKPSTLYFSLTPEKP--YPIPKLYFYPAFQAPNDEAIAQGIDAWLKRYDWYDGGK-  
 GLRG\_01200 YKKGAPMTCFGFTASDHGNGITAESAVYFPVHDYANDDAEVRLRLEKYLSDTSGSGESGI-  
 CS1 YKKGPMTCFGFTASGHGHGVTTESTVYFPVHDYATNDAEIRLRLEKYLSDTSGSGESGI-  
 CH063\_0902 -----PGFYITLGCGR---ISPKVYISPNYFSKNDVDVLRRLRFFATRRSDSMMD-  
 GLRG\_07878 -----PGFYITLGNGRE---ISPKVYISPNYFSKNDVDVLRRLRQFFSSRRTDSMMD-  
 CS.DMAT -----PGFYITLGSGRQ---ISPKVYISPNYFSKNDVDVLRRLRQFFSSRRTDSMMD-  
 CS7 -----FTYFFDIAPTSG--VPDVKFYLPTRKYGADDLTIARNLVAWLRERGRDAYCN-  
 CH063\_0738 -----  
 GLRG\_11574 AIGLWGTFAYYFDIGLGRG--LPDVKFYIPVCHYGTNDQTIASATANWMKKNRSGQYVD-  
 CS6 TVGLWGTFAYYFDIGIGRE--LPDIKFYIPVCHYGTNDQAIASATANWMKRNRRGQFVN-  
 CH063\_0204 -----IIFNMELKHNSP--EPLPKVYIPVRHYGGTDLRIAQSLSNFFRACGLTHMAD-  
 GLRG\_11853 -----IIFNMELKHNSP--APVPKVYIPVRHYGGTDLRIAQSLSNFFRACGLTHMAD-  
 CS8 -----IIFNMELKHNSP--APVPKVYIPVRHYGGTDLRIAQSLSNFFRACGLTHMAD-  
 CS5 -----VGFTIIVAPGEA--IPQIKTCVPIWQFARDEARIIACCQRLSQKHGP--TGKF  
 GLRG\_11423 -----VGFAFSVVPQA--VPQIKMYVPMWQFARDEARIVECYRRVLQTQGT--MGDY  
 CH063\_1280 -----VGFAFSVVPQA--VPQIKMYVPMWQFAHDEAKIVECYRRVLQTQGT--MGDY  
 CH063\_0144 -----IDINWEISDQLP--APQAKVYVPMWQFARDEARIVECYRRVLQTQGT--MGDY  
 CH063\_0491 -----IVMAITLSPALNDGVVTRPYCSWSNYQSSDWGAVGNFAMILKELGMEKEAE-  
 CB2 -----VCYGFELKPGAK--WPEVKAYVPLWQYADSDAVIASNLANAFHLRGW-PVAE-  
 CH063\_0654 -----ICYGFELKPGAE--WPEVKAYVPLWQYADSDAVIASNLAKAFRRSRGW-PVAE-  
 GLRG\_02500 -----VCYGFELKPGAE--WPEVMVYVPLWQYADSDAVIASNLADVFRSRGW-PVAE-  
 CS4 -----  
 GLRG\_07955 -----ICFAFELKQGV--RIDIKAHLPWQGTARSDFQTIENFAQVLRNVGLDSAEEK-  
 CH063\_1116 -----ICFAFELRPGAE--RIDIKAHLPWQGTARSDFQTIENFAQVLRNVGLDSAEEK-  
 CB.DMAT -----LTPSFEVAPGQT--LPNVKLYLSQWQFGKSDREIAECTVEIFRKLGWQREAD-  
 CH063\_0401 -----IMFSFEIVPGRQ--IPEVKIYVPMWQYSPSDGHVAKNLISAFQKLGWHDVAE-

CH063\_0589 TLEQMVANVL-----  
 GLRG\_01200 QTLRAYQGAT-----EAAANRPLQDGRGI-HAWVGLKTTRHRGS  
 CS1 QTLRTYQRGI-----DAAANRPLRDGQGI-HAWVGLKMTKNRGS  
 CH063\_0902 NYEQALKDIF-----GSKTLESRCGS-HYYYVGCALHK-GQL



GLRG\_07878 NYEQALKDIF-----GSKTLESRCGS-HYYVGCALHK-GHL  
 CS.DMAT NYEQALEDIF-----GRKTLESRCGS-HYYVGCALHK-GQL  
 CS7 RYLDMLDKLA-----VHRGLENKGM-HAYISYQCTEKGEP  
 CH063\_0738 -----  
 GLRG\_11574 AFWDTMHKII-----SHRKLDESRGA-QMWLSMMVKQ-GEL  
 CS6 AFWDSMHKII-----SHRKLDESRGA-QMWLSMMVKR-GEL  
 CH063\_0204 TYVDAVQKAF-----PTQDFSTTIGR-HSYVGLSYNK-NGP  
 GLRG\_11853 T-----PNQDFSTTIGR-HSYVGLSYNK-NGP  
 CS8 TYVDAVQKAFLPVQSLCHSVYEHKLTSLLTVPSPSQDFSTTIGR-HSYVGLSYDK-NGP  
 CS5 NFGAAIQDAL-----  
 GLRG\_11423 DINAAIQAL-----  
 CH063\_1280 DIGAALEGA-----  
 CH063\_0144 RYEKMLGRVF-----PEADFATSHV--NTWISYCYKGRGS  
 CH063\_0491 TYVRG-----HTATASSV-----  
 CB2 QYEDTDSKLI-----MDVVSPRSNMEKTTGT-HAYISFAFSKKNGA  
 CH063\_0654 TYEDAMPCCF-----PRSNLGKTTGT-HSYISFAFSKQKGA  
 GLRG\_02500 KYEETIRRSF-----PRSKLEKTTGT-HSYISFAFSKKNGA  
 CS4 -----  
 GLRG\_07955 FLKGALETAK-----LPGRDYTKPGL--SYVSYNYNQ-NGP  
 CH063\_1116 KFYRGASAAA-----K-----  
 CB.DMAT SYFDFLRDAL-----  
 CH063\_0401 NYLPNLRRTF-----PGADLDSPPSVLHSNLSYSYPATGA

CH063\_0589 -----  
 GLRG\_01200 VITFYLAASEILVHCL-----  
 CS1 VITFYLAASEVLTHCL-----  
 CH063\_0902 RVVTYLSPQSFDCCKDAIQDRKP-----  
 GLRG\_07878 RVVTYLSPQSFDCCKDRIQGVKS-----  
 CS.DMAT RVVTYLSPQSFDCCKDRIQAGGA-----  
 CS7 DIKSYISPELYHKARYSAA-----  
 CH063\_0738 -----  
 GLRG\_11574 QVTYYIAPEGFHPKRGQGHQLSHRAVVERVTGSC---  
 CS6 QVTYYISPEGFHPNRVQRQDISHRAIAERTSSH---  
 CH063\_0204 YITMYNTMTYSAGDERNADGKLVGPAAMKQRHLLD-  
 GLRG\_11853 YITMYNTMTFSAGNERDVNGKLVGPAAMKQRHLLD-  
 CS8 YITMYNTMTFSAGNERDITGKLVGPAAMKQRHLLDQ  
 CS5 -----  
 GLRG\_11423 -----  
 CH063\_1280 -----  
 CH063\_0144 YMTMYHSPPW-----  
 CH063\_0491 -----  
 CB2 YMTMHYSIRPQTSLAF-----  
 CH063\_0654 YLTIYYSIRPQDSLMAF-----  
 GLRG\_02500 HMTMYYSIRPQDSLAV-----  
 CS4 -----  
 GLRG\_07955 YLSSYFSPKIQDN-----  
 CH063\_1116 -----  
 CB.DMAT -----  
 CH063\_0401 YISVYYAVSGNATTATGQAE-----

## PART 2: A-domains of NRPS and Hybrids sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

```
GLRG_09842 -----IAVIGIGCRFPGASSFP-ELADALH-----EPAD-----
CH030B_PKS -----IAIIGTGCRFPGGSDSPSKLWELLK-----DPRD-----
CSP24 ----WAQRTPDSVAVKDMNGEILSYSQLMDRAGSVSIALS-N---AGV---EASA-----
GLRG_09715 -VARSAAQFSDSIAIKDDEGNELKYSDMIKTTNQCIRGLQ-A---SKI---QPGS-----
CSP6 ---DHAAESHPDHIAVKD GARAYTYEDMMRSVDSIATSLR-A---MGVPRSARP-----
CH063_0617 ----EAYPDRVALRDGSR--SYTYREVA AETDRIAAVLL-E---ATA---VSK-----
CH063_0392 -IDRIAAQQPNVSAISNGFGSTATY GAMQRCIQTIAQTILGN--TQGR---SAG-----
CS.P.NRPS IEALAEHPHSLALKSPHGGPSMTYRDMI AKSRAIGAALV-A---VGC---VPGA-----
GLRG_10367 IEALAEHPHSLALKSPHGGPSMTYRDMI AKSRAIGAALV-A---VGC---APGA-----
GLRG_07434 -----EKVAVTDSAGASMTYAQLSSRMSTIASSELL-R---QGV---QPGS-----
CSP1 -FEVSAEKHKRNTALIHADGTSLY AQMQQRIDTIAAALE-E---NSI---KPGD-----
CSP10 -----YPGKLAIKDGNNGTILTYADMSNRVAAISSGIS-R---LQV---RPGS-----
CSP20 ----ADKYASKAALRDGHGDGLTY AQMVARVEAIAAYLI-K---EGI---GRRK-----
CH063_0306 -IDDMADKHASKAALRDGQGGGLTY AQMAARVNAIAARLE-E---AGI---GSSN-----
GLRG_05027 -----TYAELDQETQALDRLRR-H---YGV---GRDG-----
CH.NRPS1 -----TYAELDQETQALADRLRRH---YGV---GRDS-----
GLRG_08225 -----TYRQVCLVSRLATFLV-Q---WGV---RPGV-----
CHN24A -----TYAELHKRSDKLASWLR-Q---RRL---PAGS-----
CSN9D -----TYAQLNQSDRLAAWLR-R---REL---APGS-----
G0043A -----TYTQLDEQSDCLAAWLR-E---RTF---APGT-----
CSN9G -----TYAQLDRQSDDELADWLR-R---RQHRLPGPEAGGRGP
CH.NRPS2 -----TYAELDRRSDDLARWLR-R---RRL---DPET-----
CHN29A -----TYRELDRESSRIAAYLQRY--HRQM---APES-----
CSN9I -----TYSQLDQQANRIAAYLG-N---RNM---APET-----
G0046A -----TYRQLYQQSNQIASYLG-Q---RQM---APET-----
CSN9C -----TYAELDRQSDQLASWLVHD---RGL---APET-----
CHN23A -----TYSELDRQSDQLASSLV-R---RGF---APET-----
CHN210A -----TYGQLDEKSDRLAAWLR-K---KHM---STET-----
G0047A -----TYAQLSETSDRLAAWLR-L---RKM---EPET-----
CS.NRPS -----TYAQLNQTSDH LAHWLR-L---QQM---ETET-----
CSN9H -----TYAQLDEQSDRLAAWLR-RD GQQHHRQRPAES-----
CHN28A -----TYAELDEQSDRVASWLR-R---RRL---APET-----
CHN22A -----TYTEL DQQSDRLSGWLR-Q---RRL---APET-----
CSN9B -----TYAELDRESDTLAGWLR-R---RRL---APET-----
GLRG_00469 -----TYAELDRQSDKLAGWLR-Q---RRL---APET-----
G0044A -----TYSQLDEKSSILAAWLR-N---QHL---AAET-----
CSN9E -----TYSQLDYESGVLAAWLR----KQRQKQEGHSTAAT
CHN25A -----SYAQLDRQSDILASWLQ-K---QKL---AAET-----
G0045A -----TYSELDRQSDKFAAWLR-L---RGL---PDET-----
CHN26A -----SYSQLDQQSDTLAAWLLRR---RDM---ADEA-----
CSN9F -----TYSELDRRSDTLAAWLR-A---RGM---ADEA-----
CH.NRPS3 -----TFRELNERGNQVAVLA----PHA---KVGQ-----
CSN1C -----TFRELNERGNQVANVLA----PYA---QTGQ-----
GLRG_06542 -----TFRELNERGNQVANVLG----PYA---QTGH-----
CHN32A -----SFGELNSVSNRIARFLR-G---QGA---REGD-----
CSN1B -----SFNTLNLSNRIARYLQ-E---QGA---KEGY-----
G0652A -----SFSTLNLSNRIARYLQ-E---QGA---KEGD-----
CSN1A -----SYSQLHHAADRLASRIICSVDKVST---SEDF-----
G0651A -----SYSQLHHAADKLASRVVCSVEKASA---SEDF-----
CHN31A -----SYSQLHRAVDSLASRISRS---LGKASTLEHF-----
CHN41A -----TYAELDDASDLRDLRLR-Q---AGL---APGD-----
CSN7 -----SYARLNRRANHVATMLV-Q---RGL---RPGD-----
CHN62A -----SYAQVERYSNLFSQHLL-A---AGV---NKGD-----
CSN2A -----SFKGLEDASNHLGKQLI-Q---KGV---SPAS-----
CH.NRPS9 -----TFSQIDGLSTSLCQHLVHR---LGL---SRGD-----
CHN91A -----TYRDLGKYSGVICSRLSQNIEMRS AFATGEEDRRHP-
CH.NRPS5 -----
CH.NRPS6 -----TYGELERLSSALAVSLR-H---LGV---RREV-----
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CHN21A -----LS----LKL--DAGH----  
 CSN9A -----SHGELDAVSSRLAGYIQ-S--LKL--DAGH----  
 G0041A -----SHAEMDGVSSRLAGHIQ-S--LKL--DPGH----  
 CSN4B -----SFSELDIMASRLARILV-E--LCV--VPET----  
 CHN102A -----SFAELDVMASRLARVLV-E--LQV--GPEV----  
 CH.NRPS7 -----SYSQLQCTKQLAHHLS-N--MGV--GPEV----  
 CSN6A -----SYSQLQNHADKLANHLR-S--IGA--GPEA----  
 G0681A -----SYTQLQDHADKLANHLS-S--IGV--GPEV----  
 CSN2B -----TYRELDDLSRNLAAHHLK-S--LGI--GPEK----  
 CH.NRPS4 -----TYGELDRATGCVAQYLRDS---CGV---VPDG----  
 CH.NRPS11 -----AVRLQ-S--LGA--GPGE----  
 CSN8B -----SYAELKNLTMRLASRLR-I--LGA--RSGA----  
 GLRG\_00920 -----SYTELNDLSRRLVSRQLQ-T--LGT--RPGA----  
 CSN2D -----TYAELDFASSFLAQKLQ-E--IGV--GPEK----  
 CHN44A -----TYAELTAASVRLASRLRDK--FGD--CRGR----  
 CSN2C -----SYAEMDLWANQISQIIAT--NLV---KPGD----  
 CHN43A -----  
 CHN111A -----TYRELDEASTAVARRIL-A--LGI--GPGA----  
 CSN8A -----SYRELDEAADVVATRIS-S--FGI--GPGA----  
 G0091A -----SYRELDEAADVVSHIT-S--LGI--GPGA----  
 CSN4A -----SYAELGETSDMVAHELFI-Q--HGV--GPEC----  
 CH.NRPS10 -----SYAELGEASDKVAHELIC-Q--RGV--SPES----  
 CHN72A -----SYATLESMASALGQRLV-D--SGV--SPGD----  
 CSN6B -----E--CGV--SPGE----  
 GLRG\_06838 -----SYAALESMSALATHLT-K--SGV--SPGE----  
 CHN87A -----TYGQLEAEASRFSHRLR-R--AGVV--GLGA----  
 CH063\_1253 -----TYGDVERLSTRLARHLV-H--RGV--KVGS----  
 GLRG\_08065 -----TYGDVERLSTRLARHLV-H--RGV--KVGS----  
 CSN5 -----TYGDVERLSTRLARHLV-R--RGV--KVGS----  
 CHN84A -----TCGELDGLVGRSLRHLVRQ---YGV--GPGM----  
 CHN85A -----SYGELDSLAEERLAGDIV----AALD--TAGS----  
 CHN81A -----SYGALDRLSDQLAIEII-R--RGV--NAGD----  
 CH.NRPS8 -----TYRELDQLAAELSYSLVSR--RGI--GPGS----  
 CHN86A -----TFAELDRLTDSLASHLVHH--LGV--EPET----  
 CHN83A -----TYGELDALSRLMSSRLA-Q---LGV---GPEV----  
  
 GLRG\_09842 -----LSQTIPSDRFSIDGFHHEDGKYHG----NTNVRKSYFLRDGIRK  
 CH030B\_PKS -----LLKEIPEDRFSVEGFYHPKNSHHG----TTNVRHSYLLDEDLRR  
 CSP24 ---P-----VGVLLDPNVDTIASMLGILRIGAAVPLDVR-NPDERLLDIL-QESG-  
 GLRG\_09715 ---R-----VAVLLTPDVWSICTLLAALQQLVWVPLDTR-NARQLAAML-TDCK-  
 CSP6 ---F-----CCLLIEPSADYIFAYLAVLKAGAVVLSLDTT-NHRERLAAIV-SDCK-  
 CH063\_0617 ---A-----AASTSPPVFCCLLFEPSADYV FALLAVLKVGLVAVSLDTT-NHAERLASIV-ADCK-  
 CH063\_0392 ---L-----IGVFCEPSIDAICSLLAAMRVGFAYVPLDPT-LPSERLAALV-ESAR-  
 CS.P.NRPS ---S-----VVVYQDSTPDWICSVLGTF SINGVCVPCDAG-TPVKRLADMV-ASSK-  
 GLRG\_10367 ---S-----VVVYQESTPDWICSVLGTF SINGVCVPCDAG-TVVKRLADMV-ASSK-  
 GLRG\_07434 ---K-----VAVFQHPVCWIPSLAILRIGAVVPLDVN-TPVARLSSIV-RDCQ-  
 CSP1 ---S-----VGVFQESTTDWVCSMMALWKRGA VFPVPLDPG-TKTERLAMVV-KDCK-  
 CSP10 ---V-----IGVFQEP TANFVCSMLAIWHAGCVYLPDPA-TPMPRLESQV-YHCS-  
 CSP20 ---V-----VGVMQASTLDFVCSILAIWKVGATYTPLDPRLNSIDRLAAII-RECQ-  
 CH063\_0306 ---I-----VGVMQASTLDFVCSILAIWKVGATYTPLDPRLNSTDRLLAAIV-RECR-  
 GLRG\_05027 ---L-----VGVLMNRSADYVIASLAALRAGGAFLVLELA-YPSGLLRDVI-QDAK-  
 CH.NRPS1 ---L-----VGVLMNRSADYVVASLAALRAGGAFLVLELA-YPSGLLRDVI-QDAK-  
 GLRG\_08225 ---A-----VPVILDKN-----KAGGCFVPIDAE--DRANVETFI-RQLA-  
 CHN24A ---L-----VGVLSRSCETIV ALFAILKADLAYLPFD TN-VPTARLEDILSEVSG-  
 CSN9D ---L-----VGVLPVRSCEAVVTLAVLKANLAYLPFDN N-VPTARLQDVLAEVAAG  
 G0043A ---L-----VGV LAPRSCEAVVTLAILKANLAYLPFDN N-VPTARLEDILSEVPG-  
 CSN9G ---L-----VGV LAPRSCEAVVTLAILKANLAYLPFDN N-VPTARLEDILSEVPG-  
 CH.NRPS2 ---L-----VGV LAPRSCEAVVTLAILKANLAYLPFDN N-VPTARLEDILSEVPG-  
 CHN29A ---L-----VGV LAPRSCEAVVTLAILKANLAYLPFDN N-VPTARLEDILSEVPG-  
 CSN9I ---I-----VGVLSHRSCQTIATFLGILKANMAYLPD VN-VPDARVEAILS AVPG-  
 G0046A ---M-----VAVLAPRSCEAVVTLAILKANLAYLPD VN-VPAARVEAIL-SSV--

CSN9C ---L-----VGV LAPRSCEAIV ALLGIMKANLAYLPLDVN-IPLGRLESILTSVEG-  
 CHN23A ---M-----IGVLAPRSCEAIV ALMGILKAGLAYLPLDVN-VPLGRLESIL-SSIEY  
 CHN210A ---I-----VGV LAPRSCQTTVAFMAVLKSSLAYLPLDVN-VPAARLESILSAVPG-  
 G0047A ---L-----VAVLAPRSCQTVVAFILAILKAGLAYLPLDIN-VPAARLESILSAVPR-  
 CS.NRPS ---L-----VAVLAPRSCQTVVAFILAILKAGLAYLPLDVN-VPAARLESILSAVPG-  
 CSN9H ---L-----IGVLAPRSCETIVAFILGILKANLAYLPLDVN-VPAARNETILSTVPG-  
 CHN28A ---L-----IGVLAPRSCQTVAFILGILKANLAYLPLDVN-VPAARNETILSTVPG-  
 CHN22A ---L-----VAVLSPRSSLTILTFILAILKAGLAYLPLDVN-MPLARVEAIL-SAVK-  
 CSN9B ---L-----VAVLAPRCCRTILTFILAILKANLAYLPLDVN-MPAGRIEILSAVQG-  
 GLRG\_00469 ---P-----VAVFAPRCCQTTVTFILAILKANLAYLPLDVN-MPAGRIETILSAVQG-  
 G0044A ---P-----VALFSPRSCETIVAILGILKANMAYLPM DVK-MPAARLNSIL-STLE-  
 CSN9E TTPV ---VAVFSPRSCETIVAFILGILKADMA YLPLDVN-SPVARLTSILSAVEG-  
 CHN25A ---P-----VAVFAPRSCETIVAFILGILKANLAYLPLDVN-VPAARLTSILSAVDD-  
 G0045A ---I-----VGV LAPRSCETTIV ALLGILKANLAYLPLDIK-VPLPRLEAILSSLQG-  
 CHN26A ---L-----VGV LAPRSCDTIVAFILGILKANMAYLPLDIN-APVARLNAIFSTRPG-  
 CSN9F ---L-----VGV LSPRSCDTIVAFILGIMKANMAYLPLDVN-VPAARLEAIL-SAV--  
 CH.NRPS3 ---T-----IAIHFDKCPEACFSILGILKAGCAFLALDPS-APKARKEFIL-KDSG-  
 CSN1C ---T-----IAIHFDKCPEAIFSILGILKAGCAFLALDPS-APKTRKEFIL-KDSG-  
 GLRG\_06542 ---T-----IAIHFDKCAEAFSILGILKAGCAFLALDPS-APKTRKEFIL-KDSG-  
 CHN32A ---I-----VGLIMEKSISLYAAMLGILKAGCAYLPLLPT-TPAERTKVIL-TQAG-  
 CSN1B ---I-----VGLVMEKSISLYAAMLGILKAGCAYLPLLPT-TPAERVRVIL-TQAK-  
 G0652A ---I-----VGLVMEKSIDLYAAMLGILKAGCAYLPLLPT-TPAERIRVIL-AQAE-  
 CSN1A ---V-----VPLLVPQSPHLYIAILAILKAGGAF CPLNLD-APLERVRFIL-QDVS-  
 G0651A ---V-----VPLLVPQSPHLYIAILAILKAGGAF CPLNLD-APPERVRFIL-QDVS-  
 CHN31A ---V-----VPLLVPQCPHLYIAILAILKAGGAF CPLHLD-APPERVRFIL-QDVS-  
 CHN41A ---G-----VLLCFEKSVMWSVAMLA VLKTGAFCSLDPD-YPEHRVTQIA-EATE-  
 CSN7 ---C-----VALLLEKSVEAVACILGVLKAGCTYVPLSPD-NSAERNAFIC-RDPS-  
 CHN62A ---F-----VPLLLERSPWAPVLM LAVLKLGAA FALDLS-HPVQRLRTMC-TVLS-  
 CSN2A ---F-----VLLSFTHSLWAVVSWLA VLKAGAACVFLDQR-QPAERTKQII-GTTS-  
 CH.NRPS9 ---H-----VTLLCDKSAWVVVAMLA VL RAGAACAFLSPA-DGLNRLRN MVTEQMR-  
 CHN91A ---F-----VAICLEKSVWTTIPIMMGVWQAGAAVLLDPE-LPRYRLRKA V-QQVN-  
 CH.NRPS5 -----MKAGNAYVGLSAE-TPLSFLQECS-CIAD-  
 CH.NRPS6 ---F-----VPLVFDKSKWAIVALLGVLKAGGAYFFLNPT-HPVEYNKTL C-KPFD-  
 CHN21A ---A-----VPLCFEKS KWMVAGVLA VLKAGHAFALVDPA-HPAARVAQIC-RQTS-  
 CSN9A ---A-----VPLCFEKS KWMVAGVLA VLKAGHAFALVDPA-HPAARVAQIC-RQTS-  
 G0041A ---A-----VPLCFEKS KWMVA AVLA VLKAGHAFALVDPA-HPAARVAQIC-RQTS-  
 CSN4B ---K-----VAYCFEKS MYTVVSM LAILKAGGTIVPLDPA-HPPERKAFII-QSIA-  
 CHN102A ---N-----VAYCFEKS MYTVVSM LAILKAGGTIVPLDPA-HPPERKSFVI-RKIS-  
 CH.NRPS7 ---L-----VPVIFEKSLWSVVA ILA VVRAGGAVVALDPS-LPAARIRMIV-QDVK-  
 CSN6A ---L-----VPVIFEKSLWSIVAM LGVIRAGGAVVALDPS-LPTARIRMIV-QDVK-  
 G0681A ---F-----VPVIFEKSVWSVVA ILG VIRAGGAVVALDPS-LPTTRICMIV-QDVN-  
 CSN2B ---I-----VPLCFEKS KWA VVSM LA VL RAGACFVMLDPA-HPNVRVMSIA-EDVE-  
 CH.NRPS4 ---V-----VPICSEKSIWAVVAM LGVMRAGGCFVMLDPG-HPDSRLLSIV-EQVR-  
 CH.NRPS11 ---I-----VVLCFEKSLWAMVAM LA VAKGAA FVHIDIQ-GAPKRTE SVI-MQTK-  
 CSN8B ---V-----VVLCFEKSLWAIVSMLA VARS GAA FVHIDPA-APFKRIESIV-GQTR-  
 GLRG\_00920 ---V-----VVLCFEKSLWAVVSM LA VARS GAA FVHIDPA-APSKRIESIV-GQTK-  
 CSN2D ---T-----TPFMFDKSKWAIVSMLA IWKAGGSFVPLDPK-SPNQRLQHII-QATS-  
 CHN44A ---A-----FPILCRKSKWAIVGMLA IWHAGGYLVPMEPT-HPIQRLRLVA-GTVA-  
 CSN2C ---F-----VGLCFDKSASAILAMIA VLKAGGAFPLNPD-HPPARLRALL-KEAN-  
 CHN43A -----  
 CHN111A ---F-----VALCFEKS I WYSVAM IGV LKSGNSFVPIDVS-NPTS RREEIL-QQLGI  
 CSN8A ---Y-----VALCFDKSRWYSV ALLGVLKSGNAFVPIDAA-NPISRRTEIL-QQLGI  
 G0091A ---Y-----VALCFDKSLWYSV ALLGVLKSGNAFVPIDVK-NPTS RLEIL-QQLGI  
 CSN4A ---T-----VALFFEKSIWNTVATVA VLKASGAFVPLDPS-QPNNRISYIL-DQVK-  
 CH.NRPS10 ---T-----VALFFDKSIWNTVATVA VLRSSGAFVPLDPS-QPDSRISYIL-GQVK-  
 CHN72A ---M-----IPMCFEKS VWTVV ALLGALKAGAVFVPLDPS-QPISRLREV V-AQVR-  
 CSN6B ---M-----VPLCFEKS VWA VVAM LGV LKAGAA FVPLDPS-QPISRLRDVA-AQVK-  
 GLRG\_06838 ---M-----VPLCFEKS VWTVIAM LGV LKAGAA FVPLDPS-QPISRLRGVA-AQVK-  
 CHN87A ---M-----VPLCFEKS KWA VVAM LA VLKAGAA FVPLDPSQAPDRRERVLVRTRAR-  
 CH063\_1253 ---T-----VPMCFEKS R WTTV ALLAVMKAGAA FALTDPS-QPEARLRTIV-EQTG-  
 GLRG\_08065 ---T-----VPMCFEKS R WTTV ALLAVMKAGAA FALTDPS-QPEARLRTIV-EQTG-  
 CSN5 ---T-----VPMCFEKS R WTTV ALLAVMKAGAA FALTDPS-QPEARLRTIV-EQTG-

CHN84A ---V-----LPLCFEKSMTVVAFLAVLEAGAGFVLLDPA-LPEARLRMLV-SQVN-  
 CHN85A ---V-----VPVCSEKSAWAVVSLAVLKSNTFVLLDPS-QPESRLSAVV-RQTG-  
 CHN81A ---L-----VPLCFEKSAWTVVAILAVLKAGGAFVLLDSS-HPVERLRITV-AQAS-  
 CH.NRPS8 ---L-----VPLCFEKS KWAVVAILSVLRTGAAFVLM DPS-QPEERLRSIV-RQLD-  
 CHN86A ---L-----VPICFDRSKWAVVAILGILKAGGAFVPLDPA-QPPARREQVL-SQTG-  
 CHN83A ---L-----VPLCFEKS LWAIVAMLA VLRAGGAFVPI DDPD-AARGRRD GIL-KQTD-

GLRG\_09842 FDNNF--FNITAV-----EASAMDPQQRLL-----LET  
 CH030B\_PKS FDFASF--FGIKAT-----EANSIDPQQRML-----MET  
 CSP24 ---AT--IVLFHS--PTAK-----RAKTLLGGLTNT-----QHM  
 GLRG\_09715 ---PR--HIIHSDATGVLAE-----ELVAAARPENPELEPLRL-----AEL  
 CSP6 ---PQ--AIHCK--AMSN-----LAQWLGQSCDVA-----SVM  
 CH063\_0617 ---PM--VILHHG--ATKD-----LAV-RLGEPHEG-----IS--TLI  
 CH063\_0392 ---PL--LILAHDETIDRAK-----EPSSFVGHIPLI-----NVS  
 CS.P.NRPS ---AT--VLLVDA--STAE-----QAAGALSTTISRERVRILRV----DRI  
 GLRG\_10367 ---AT--ILLVDA--STAE-----QAAGALGTTISDEERVRILRV----DRI  
 GLRG\_07434 ---PA--SILVHE--FTIG-----EAEKLDTEPNQIFI-----NVS  
 CSP1 ---PA--AILIDD--ATKG-----LHTELGATETTVFI-----DVS  
 CSP10 ---PH--LILVDN--RFAD-----AAASFGTPVFNV-----PLW  
 CSP20 ---PA--CVLIDA--SSRS-----LFDQLDSA AAVLI-----DVE  
 CH063\_0306 ---PT--HVLVDA--STRS-----LFAQLESTAVPV-----DVT  
 GLRG\_05027 ---PQ--VVITQN--AHVS-----HIKADVPIIVY-----DEP  
 CH.NRPS1 ---PT--VVITQN--AHVN-----HIKADVPIIVY-----DEP  
 GLRG\_08225 ---PK--IVVATD--LGWK-----SLEFIVEGVIVV-----DDS  
 CHN24A ---EK--LVLVGA--SVPM-----PDLGVPDVKLVRIV----DAM  
 CSN9D HGRSD--LPLLVG--ATVP-----MPDLKMPGVQLVRI-----AEA  
 G0043A ---HK--LLL VGS--TVS-----MPDLKMPDVQLVRIVEA----TNG  
 CSN9G ---RK--LVLVGA--GVPT-----DEVGLLPDVELVRI-----RAA  
 CH.NRPS2 ---RK--LVLVGP--DVST-----DTT--LSDVEFVRIGA----ALN  
 CHN29A ---HK--LVLVGA--GVPT-----LPAESRDVEQV-----QIS  
 CSN9I ---HK--LVLLGD--NVPV-----VTLTTSSTSGSAGEIEVQVQISHALGQKAAAA  
 G0046A ---PGHK-LVLLGT--GVSP-----LPTESAEME QVQI-----SHA  
 CSN9C ---EK--LLLGA--GVPV-----PATHLPDVEFLTIA-----EAR  
 CHN23A ---KK--LLLGA--GVPV-----PATQLDDVEFL-----TIA  
 CHN210A ---LT--LVLLGA--DVAT-----PNVRLPAINFVPISE----ALA  
 G0047A ---LT--LVLLGD--DVST-----PNVRLSNLRFVPLGG----AIA  
 CS.NRPS ---LT--LVLFGS--EVSA-----PDVRLPDLQFVSLGD----AIA  
 CSN9H ---RK--LVLLGT--DVPA-----PEPPLVDVELVSIRDILTSVVDHH  
 CHN28A ---RK--LVLLGS--DGAA-----APERPFADVELVPLA----DVL  
 CHN22A ---GS--TVLFLG--EDTP-----RPEIKVADVEIVSITDALN--DNA  
 CSN9B ---HK--LVLLGP--GTQK-----PPINVTDVEMVPI-----TDA  
 GLRG\_00469 ---HK--LMLLGA--DAPK-----LPIKVADVEMVVS-----ITD  
 G0044A ---GR--VIVLLG--SAVT-----RPALDVPNVSMVL-----ICD  
 CSN9E ---QE--VIVLLG--STVT-----RPAFHLPHVSMVP-----IRD  
 CHN25A ---GK--VLLLGA--STVT-----RPSLDLPHVMTMVP-----ISD  
 G0045A ---RK--LLFLGA--GVPV-----PESPLPDVELVR-----VGE  
 CHN26A ---RK--LLLGN--DTPV-----PDSLAE DVETV-----RLG  
 CSN9F ---PGRKLLFLGTS--VDAPV-----SDSSPLVDVEMVRRISEALKEEEARN  
 CH.NRPS3 ---AL--ALMTNT--DDID-----FVVEQPLIRV-----DDT  
 CSN1C ---A--LALLSN--TNID-----FTVEQPHIHV-----DDA  
 GLRG\_06542 ---A--LALLTN--TDID-----FAVEQPHIHV-----DDA  
 CHN32A ---VK--LCVADV--EVYT-----NLEDVSSCKFV-----DTR  
 CSN1B ---VK--LCVADA--KVAT-----TLEGLSDCKFM-----DIQ  
 G0652A ---VQ--LCVADA--KVST-----TLEGLSN SKFM-----DIQ  
 CSN1A ---AK--VVVSR--DMVS-----KIPTDEPDRVVLIV-----DED  
 G0651A ---AK--VVVSR--DMVS-----KIPADEPDRVVLIV-----DED  
 CHN31A ---AK--IVVSR--DLVS-----KIPQDEPDRILIV-----DQD  
 CHN41A ---AA--FLVASQ--AQLK-----KLRGYEISQQLGLVQVPSNIGDEHELD  
 CSN7 ---AR--LVILQR--EQGS-----FASHIEGIEIL-----PVE  
 CHN62A ---AK--RVVCFP--HHQT-----MVENDLLLPEVVF-----DPE  
 CSN2A ---AT--HALADS--EAVA-----ATLGELGINVMQVPS-----QAA

CH.NRPS9 ---AN--VIIASP--AHED---KAMALLGDCHRNDNRSSRVVVLDP-----DSD  
 CHN91A ---AV--LVLVSRATRYLWD-----ELDDGYAMDGNITSLLM-----TLP  
 CH.NRPS5 ---VP--LIITSP--QQKH-----LAEKIGRPVLVL-----DQD  
 CH.NRPS6 ---IK--LAVCSP--KHHT-----LAQNFAPRFVELGSEAGE---DLK  
 CHN21A ---AT--FALASR--LHAG-----TFSGLVSRITIVL-----EDA  
 CSN9A ---AT--FALTSR--LHAD-----TFRGLVPTTVVL-----EDD  
 G0041A ---AT--FALASR--LHAD-----TFRGLVPTTVVL-----EDD  
 CSN4B ---AD--VVLASA--SNAH-----MFEGLDTNVVIV-----DSH  
 CHN102A ---AN--VVVASA--ANAA-----MFLDMDVHVVVVV-----DAG  
 CH.NRPS7 ---AS--VVISSP--GSQYR-----IPEEILPGRNVIV-----DES  
 CSN6A ---AS--IILSSA--SNRH-----KIPEHITQENRVIV-----GEP  
 G0681A ---AS--IVLSSV--SSQD-----KIPEHILQENRVIV-----NER  
 CSN2B ---AE--ILLCSP--RTRP-----KFEAIKDKVLT-----ETG  
 CH.NRPS4 ---PR--VMLSSL--GQRARLERWARDLDSKSKDDGPPSPISVQVLAITDDWIKRRM  
 CH.NRPS11 ---SR--LGLTST--AQHA-----KLASVVETVMIV-----HKT  
 CSN8B ---PA--LGLGSA--AGHE-----KLSHHISPAIVV-----DQS  
 GLRG\_00920 ---PA--LGLGSA--AGYE-----RLSPYIRPAIV-----DRS  
 CSN2D ---AT--MILAPT--HYCG-----RCRDLNCTPVLI-----DGD  
 CHN44A ---AS--AVLVSS--DCLS-----TGLETGFPCIVIDEDGDGSGTGIRCV  
 CSN2C ---VK--LVFASL--GRIA-----SLREALNSEVL-----PIG  
 CHN43A -----  
 CHN111A SALAG--LIVCSR--DQAP-----SLRPFSRHLEL-----DGE  
 CSN8A AGDSG--LIICSS--SQFS-----SLQQLGRHVLQL-----PCR  
 G0091A GGNSG--LVICSS--SQAS-----SLKHLSGHMLQL-----PCT  
 CSN4A ---PK--VILCSK--EHEQ-----RLKISQNVVAL-----DEA  
 CH.NRPS10 ---AK--VILCSR--VHEQ-----RLGLSDNVVAL-----DEA  
 CHN72A ---AR--TVLMSA--FQAK-----ATDLGTLERIV-----DRE  
 CSN6B ---AK--FVLVSK--FQAK-----TTDLGVSTRIIV-----DRD  
 GLRG\_06838 ---AK--IVLMSK--FQSK-----ATDLGIDTRIIV-----DRD  
 CHN87A -----VVLVSA--RYSN-----LDVGAGRRVVVVVDGDNND---DDD  
 CH063\_1253 ---AT--IIVTSQ--RQSE-----LGRRIAPEGVLVVA-----SDE  
 GLRG\_08065 ---AT--VIVTSQ--KQSE-----LGRRIAPDGALIA-----SDD  
 CSN5 ---AN--IIVTSQ--KQSE-----LGRRIAPDGMLVIA-----SDD  
 CHN84A ---FS--VLLASP--ATHE-----LGRRLSGTVFAVGP-----EMD  
 CHN85A ---SR--LVLLCSGPQSAAS-----DDADAAAAGPFRDFGGRTWHIDSG  
 CHN81A ---SG--LVLTSR--ACES-----LGRCLEKDVLVVCP-----DAS  
 CH.NRPS8 ---AG--LILTSE--TSYL-----VSKRLVDESLGVVVVV-----EAE  
 CHN86A ---AS--FVLTSV--RHSS-----SSMDFGGRDAVPVGA-----DTE  
 CHN83A ---AK--IILVSE--RYHA-----AEKLELEQDVLVLPVGPSSHSHSRR  
  
 GLRG\_09842 VYEAFFEN-----ADVSTPQLKG---SDTAVYVGLMCGDYENILLRDIDSA  
 CH030B\_PKS VYEGLES-----AGLSIKQLQG---SDTAVYVGVMSADYTEMLARDVEKY  
 CSP24 RLVSLDE-----VPWSSTQDIQDVSTLEGLAMMLYTSG-----  
 GLRG\_09715 MAPTAPS-----ASVPTPNLSS---RDADAVLLYTSG-----  
 CSP6 DISSIP-----RERQHELNRSSD---PQAPAVLFYTSG-----  
 CH063\_0617 TLSSSS-----SSSSSMGNRSL---PDTPGVLFYTSG-----  
 CH063\_0392 RQRYLPR-----DPVPISAR---SQDTMAVHFTSG-----  
 CS.P.NRPS EAATLGH-----ASSTARGRPPPG---PQDPAMMLYTSG-----  
 GLRG\_10367 EAATLGH-----ASSTARGRLPPG---PQDPAMMLYTSG-----  
 GLRG\_07434 GLKDTSE-----EAIPNLAR---PDAPAILYTSG-----  
 CSP1 ALTTKT-----DAPSSKILAS---PDDLAMVYYTSG-----  
 CSP10 IDDQNPT-----EIMLEVPETK---STDPAAVLYTSG-----  
 CSP20 KVPLLSV-----KTSTQAK---ADDAAVVLYTSG-----  
 CH063\_0306 KNSSPDA-----KVPTQAR---AGSAAAVLYTSG-----  
 GLRG\_05027 SKTTING-----HANGELPPLPASNDLERLAFVSYSSG-----  
 CH.NRPS1 SKTTVNG-----HANGELSPLPASDDLRLAFVSYSSG-----  
 GLRG\_08225 LFEVNF-----PPQAPLMAA---PDDAACMLLSPR-----  
 CHN24A TASHQGQ-----QNGFVAAPPQPK---ATSLAYVIFTSG-----  
 CSN9D MSHPLKG-----QPRADAAPSRPG---ATSLAYVMFTSG-----  
 G0043A SRHRQRQ-----DGDGEDATPGPQ---ATSLAYVMFTSG-----  
 CSN9G LGGHAGPNGTTDGVTVNGVTRNSTARSVTRPG---PDSLAVHIFTSG-----

CH.NRPS2 EVAGSNG-----S---AKEPATSSPQPG---ASSLACVIFTSG-----  
 CHN29A DALRLGA-----AVNPRDLHPRPAATASSLAHVIFTSG-----  
 CSN9I ATVVVSS-----GDARQRGERYKKPT---ATSLAYVMFTSG-----  
 G0046A LSQGETA-----STSSARKYTQVA---ATNLACVIFTSG-----  
 CSN9C DSHRQSD-----ATTVVDRLPKPT---ATSLAYVMFTSG-----  
 CHN23A EARDGDA-----KTAVKQLPSPT---ATSLAYVMFTSG-----  
 CHN210A AFRPASS-----EAPARVSLPS---PTNLAYVIFTSG-----  
 G0047A SSRQPST-----KPPASVSFPS---ATSLAYVIFTSG-----  
 CS.NRPS SSRHSLT-----ASSSVNPSLPS---ATNLAYVIFTSG-----  
 CSN9H KYADGDD-----DDDDAAVRPH---AKSLAYVMFTSG-----  
 CHN28A AANIVDR-----DDARVDAPPRPH---AKSLAYVMFTSG-----  
 CHN22A TTTADAR-----HAGDASAGPT---ATSVAYVMFTSG-----  
 CSN9B LSEAVAD-----ARHQVDGVSARPQ---ATSLAYVMFTSG-----  
 GLRG\_00469 ALGEAAE-----IHHQTDGSAGPR---ATSLAYVMFTSG-----  
 G0044A TLRNYPGD-----KSSQDIAGPT---ATSLAYIIFTSG-----  
 CSN9E ALRSAPD-----RASSQHVLPGPT---ATSLAYIIFTSG-----  
 CHN25A TLRDAPS-----KSSIQHLPKPT---ATSLAYIIFTSG-----  
 G0045A ALKGKHA-----TQGAAGSQPS---ARSLAYVVFTSG-----  
 CHN26A EALKTSL-----AIPPTASKPS---ATSLAYVMYFTSG-----  
 CSN9F TATNTND-----GATTNGAITNGTVSQPS---ATSLAYVMFTSG-----  
 CH.NRPS3 LLDGASR-----VFNSPESLS---IQDNSYCLYFTSG-----  
 CSN1C LLDSASP-----IFTSPASLS---VQDNSYCLYFTSG-----  
 GLRG\_06542 LLNSASP-----IFTSPKSLs---VQDNSYCLYFTSG-----  
 CHN32A SLDMSDL-----ADDDLRLTAE---PSRPAYVIYFTSG-----  
 CSN1B SIDMSGF-----ADANLCLPPD---PSRPAYVIYFTSG-----  
 G0652A SIDMSGF-----ANANLRLPPN---PSRPAYVIYFTSG-----  
 CSN1A EVEKSTS-----STYDSCHDTE---PHSLAYVMYFTSG-----  
 G0651A EVEKSAS-----STHGSFRDPE---PRSLAYVMYFTSG-----  
 CHN31A DVEEPTS-----PHSGPVRDSK---PHDLAYVMYFTSG-----  
 CHN41A TILQNESRRTKQK-----QYQQQTRPS---PDSTAFVVFTSG-----  
 CSN7 DISLSEG-----EAPSVALT---PKHIAYIIFTSG-----  
 CHN62A VVTDVSK-----STRDAASPAGLAEID---MDAPACVVFTSG-----  
 CSN2A VSHGFPD-----DGGQTPWRTR---PEDAAFVVFTSG-----  
 CH.NRPS9 VFIRDPC-----INAAKGLLDRSQ---PDGVAFLVFTSG-----  
 CHN91A LEEEDDS-----YSQEETKLRESR---PENACYCVFTSG-----  
 CH.NRPS5 LLGTLGS-----PANIADFTSPAI---PSDLAYLVFTSG-----  
 CH.NRPS6 VLSPADE-----DPPEEEGQETPS---PSQAMYATFTSC-----  
 CHN21A LLESIPA-----ATAAAPFTPVAK---PHDLAYIIFTSG-----  
 CSN9A LLQSLSG-----PRAGGFFTPVAK---PHDLAYIIFTSG-----  
 G0041A LLRSLSG-----SRAGFTPVAK---PQDLAYIIFTSG-----  
 CSN4B LFRLEE-----LKVAFSSTDVQ---PHNAATVLFTSG-----  
 CHN102A LFRLEE-----LQVAFSSTDVQ---PHNAATVLFTSG-----  
 CH.NRPS7 FCRRLPA-----RTAATSLPPTKPPWTPDNALYVVYFTSG-----  
 CSN6A LFTTLST-----RATSWAVRPMVT---SHNALYVVYFTSG-----  
 G0681A LFPTLST-----RAASWPARLVTS---NNALYVVYFTSG-----  
 CSN2B FAHTLPL-----TDPTQPVCPEVS---PDNAMYVVFTSG-----  
 CH.NRPS4 AMTEISG-----LAICICEVR---PRHAMYVQFTSG-----  
 CH.NRPS11 SVEELPS-----PESRDQPVASAD---PSSVLYVIFTSG-----  
 CSN8B LVDGITS-----VGNNSDATVE---PGSVLYICFTSG-----  
 GLRG\_00920 LVEGIPS-----VSNIYDVAVD---PGSVLYICFTSG-----  
 CSN2D SVPALSK-----IPVTKQSTVG---KHNLAYILFTSG-----  
 CHN44A DMERCQGNPEEEGETPDHDPNPNPTSSSTPT---PSDLAYILFTSG-----  
 CSN2C DFRPSEE-----TAAPLDVAVT---PANAA YLLFTSG-----  
 CHN43A -----DAAYLLFTSG-----  
 CHN111A TLAHIAS-----ADCSSSLPSAS---LTDPAYIIFTSG-----  
 CSN8A KLDA-----EIRPPSLPVS---VDDPAYVIFTSG-----  
 G0091A KLDA-----KLRPSLTVS---VDHPAYVIFTSG-----  
 CSN4A WIQSVPS-----SSLPHPSFPR---PWNLAYIMFTSG-----  
 CH.NRPS10 WIQSALN-----TPLPHPLSPQ---PWNLAYIMFTSG-----  
 CHN72A SLERPGS-----SDDPEMLATRST---PDSPAYVIFTSG-----  
 CSN6B SLEGLTS-----SSPSRLTRHSE---PDCPAYVIFTSG-----  
 GLRG\_06838 SLEAFTS-----WSSFETLAPHSE---PDRPAYVILTSG-----

CHN87A DDEPLPE-----HDEPGMAPQA---PSSTAYVLFTSG-----  
 CH063\_1253 TLNQDTL-----ELAPELPYIP---PSSPLYIQFTSG-----  
 GLRG\_08065 TLTQDTL-----ELAPELPYIP---PSSPLYIQFTSG-----  
 CSN5 TLTQDTL-----ELAPELPYIP---PSSPLYIQFTSG-----  
 CHN84A TSVTVATAAAVVSSPRPTTLNTPAAAAAAGFDPSFPMYVIFTSG-----  
 CHN85A YFDNNKP-----AGLRSPPPPPPRRSSAPPAVDPDSNMYTVFTSG-----  
 CHN81A VWHSDPQ-----KQKHDLPQTA---PESTMVYVFTSG-----  
 CH.NRPS8 LESLSEP-----NKRDSLPRVD---PSSTMVYVFTSG-----  
 CHN86A SMRARPE-----SFVQPRVP---VDAAYILFTSG-----  
 CHN83A LASHGEEDR-----PSSRTTNHAS---PSSAAYMIFTSG-----

GLRG\_09842 PRYQAT-GV-GRSIMANRISYI-----WDLHGPSM--TIDT-A---CSSSLVALHQ  
 CH030B\_PKS PTYFAT-GT-ARSILSNRLSYA-----FDWRGPSM--TIDT-A---CSSSLIAMHQ  
 CSP24 ---ST-GK-PKGIPLTNANIR-----TPILGASE--KISL-----GQEVVLQQSG  
 GLRG\_09715 ---ST-GV-PKGVRVTHLGLL-----NQIYVCKD--IIG-----ERQVVLLQQT  
 CSP6 ---TT-GV-PKGAILKHSRYA-----NLIEASSR--RLQVRP---QKEVVLLQQT  
 CH063\_0617 ---TT-GV-PKGALLTQGNK-----HIIAAASR--RLGIRPA--TQEVVLQQT  
 CH063\_0392 ---TT-GT-PKGVVLCCHDTV-----NVVEACAD--IYRT-----ASNVLQQA  
 CS.P.NRPS ---ST-GT-PKGIRLKHEGFTNWAEFTVPRLLTDTCT--GSGG-G---GDLVVLLQSS  
 GLRG\_10367 ---ST-GT-PKGIRLKHEGFTNWAEFTVPRLLTDTCT--GSGG-G---GDLVVLLQSS  
 GLRG\_07434 ---ST-GT-PKGVILRHSSLK-----HEFDHCAA--TYGM-N---EHDVVLLQSA  
 CSP1 ---ST-GN-PKGIAVKHEGVR-----DVFESACW--LYGV-N---EKVVTLQSS  
 CSP10 ---ST-GV-PKGIVLSHQNLI-----HELEFSSK--TYDF-----EFENVLCQSA  
 CSP20 ---TT-GT-PKGILLSHRSVR-----NNIEIATH--HLWGFK-----GSDIMLQQA  
 CH063\_0306 ---ST-GT-PKGISLSHASLR-----NNIELATR--QFGFK-----GSDVMLQQA  
 GLRG\_05027 ---TT-GQ-PKGIANPHRAAV-----LSYDLRFG--LSDL-Q---PGDRVA--CN  
 CH.NRPS1 ---TT-GQ-PKGIANPHRAAV-----LSYDLRFG--LSDL-Q---PGDRVA--CN  
 GLRG\_08225 ---SSRGKDAKGIIFFHHAALS-----SAFLTQGR--ALNL-N---RDSRVMQLSS  
 CHN24A ---ST-GR-PKGVMEHRAIV-----RLVKNTNK--HLQI-G---AGSSMPHVS  
 CSN9D ---ST-GR-PKGVQIEHRSIV-----RLVKNTHP--HLQP-G---VGVSMHLSN  
 G0043A ---ST-GR-PKGVMEHRSIV-----RLVKNTSP--HLQA-G---VGVGLAHVSN  
 CSN9G ---ST-GK-PKGVMIHRAIV-----RLVMETNT--VPRK-Q---ALAVVAHMSN  
 CH.NRPS2 ---ST-GK-PKGVMEHRGIV-----RLVKETNT--HPPT-ETNPSATATVSHVSN  
 CHN29A ---ST-GK-PKGVMEHRSII-----RLVKSTNF--MTQS-Q---TAQPVAHVS  
 CSN9I ---ST-GR-PKGVMEHRGIM-----RLVKSTNL--MTQSQA---ATKPFHMSN  
 G0046A ---ST-GR-PKGVLEHRGIV-----RLVKSSNV--THPL-----KAMTVFHISN  
 CSN9C ---ST-GR-PKGVMEHRGIV-----RLVKNTNI--QSAA-Q---SAVPVAHIAN  
 CHN23A ---ST-GR-PKGVMEHRGIV-----RLVKNTNI--QTQA-Q---SAVPVGHIAN  
 CHN210A ---ST-GK-PKGVMEHRGIV-----RLVKKSNI--VREL-P---DATTISHVSN  
 G0047A ---ST-GR-PKGVMEHRGIV-----RLVKQSNII--VREL-P---EETTIAHVSN  
 CS.NRPS ---ST-GR-PKGVMIKHRGIV-----RLVKQSNII--VSEL-P---EATTIAHVSN  
 CSN9H ---ST-GK-PKGVMEHRGIV-----RLVRQSRL--TYKL-P---KTPRVAHLSN  
 CHN28A ---ST-GL-PKGVMEQRGIN-----RLVRQSSK--MYKM-P---QHPRVAHLSN  
 CHN22A ---ST-GQ-PKGVMEHRGIV-----RLVRQSNV--STKL-P---ESPRVAHMSN  
 CSN9B ---ST-GQ-PKGVMEHRGII-----RLVKQSGS--VNSDL-P---PAARVAHLSN  
 GLRG\_00469 ---ST-GQ-PKGVMEHRGII-----RLVRESNA--HSKL-P---AAARVAHMSN  
 G0044A ---ST-GK-PKGVMEHRGIV-----RLVKNSNV--VAKL-P---PTARLAHLSN  
 CSN9E ---ST-GK-PKGVMEHRGIT-----RAVKNTNV--NAKL-P---PAARVAHLSN  
 CHN25A ---ST-GR-PKGVMEHRGII-----RLVKNSNV--VAKL-P---PAARVAHLSN  
 G0045A ---ST-GK-PKGVMEHRGIV-----RLVRNTNC--VADL-P---PAARVAHMSN  
 CHN26A ---ST-GT-PKGVMEHRGIV-----RLAKNTNA--LARL-P---PSARMAHMSN  
 CSN9F ---ST-GK-PKGVMEHRGIV-----RLVKNSNV--VAKL-P---PAARVAHLSN  
 CH.NRPS3 ---TT-GT-PKGCEITHENAV-----QAMKAFQRLFAGHW-D---ETSRWMQFAS  
 CSN1C ---TT-GT-PKGCEITHENAV-----QAMKAFQRLFDGHW-N---ENSRWMQFAS  
 GLRG\_06542 ---TT-GT-PKGCEITHENAV-----QAMKAFQRLFDGHW-N---EDSRWMQFAS  
 CHN32A ---TT-GT-PKGVVVTQGNIV-----SNLDVLSR--IYPHGG---NRSRLQACS  
 CSN1B ---TT-GT-PKGVVVTQGNIV-----SNLDVLSK--LYPH-S---NRSRLQACS  
 G0652A ---TT-GT-PKGVVVTQGNVM-----SNLNVLK--LYPH-S---NRSRLQACS  
 CSN1A ---ST-GT-PKGVGVSHDAAA-----QSLLAHNR---HV-P---PFKRFLQFAS  
 G0651A ---ST-GT-PKGVGVSHDAAA-----QSLLAHNR---HI-P---PFKRFLQFAS  
 CHN31A ---ST-GT-PKGVGVSHDAAA-----QSLLAHNR---HI-P---SFRRFLQFAS



CHN41A ----ST-GV-PKGIVVSHANVC-----TAVAALGD--AFGV-D---GATRTVQFAA  
 CSN7 ----ST-GQ-PKGIKVPHQAVT-----AAVKSMCE--VEGRTK---GEQRVLQLSN  
 CHN62A ----ST-GL-PKAIQLPHRALS-----TSAYHLST--TGHL-S---QCSRVLQFAS  
 CSN2A ----ST-GI-PKGVVLSHSCIY-----TRAKDMT--LLDV-K---SDSRVLQFGS  
 CH.NRPS9 ----ST-GM-PKGIRLTHSSLC-----LSILNYTR--RLGI-S---HRSRLQFSA  
 CHN91A ----ST-AE-PKGVIVQHRAIS-----TSAVYHGL--EAAL-D---HTSRVLQFSS  
 CH.NRPS5 ----ST-GV-PKAVMTEHRAYV-----TDLAQQQ--SALL-D---AASRVLHFAS  
 CH.NRPS6 ----TT-GT-PKSITSHDAFY-----HMAIATASPCALHI-S---PSTRMLQFAS  
 CHN21A ----ST-GE-PKGAMLEHRAFA-----SCALEFSP--GLDI-D---RDTRSLQFSS  
 CSN9A ----ST-GE-PKGAMMEHRAFA-----SCALQFSP--GLGI-D---KDTRSLQFSS  
 G0041A ----ST-GE-PKGAMLEHRAFA-----SCALQFSP--SLGI-D---QNTRSLQFSS  
 CSN4B ----ST-GE-PKGVVQEHKTL-----SAAAHSG--AMQM-S---ESSRILQFSA  
 CHN102A ----ST-GE-PKGVVQEHKTL-----SAAAHAG--AMDM-S---GSSRVLQFSA  
 CH.NRPS7 ----ST-GK-PKGVVPHAAFC-----SSAKGFSK--AIRLDS---PSARVLQYSS  
 CSN6A ----ST-GK-PKGVVTHSAFC-----SSSRGFSK--AIHLDS---PKARVLQYSS  
 G0681A ----ST-GK-PKGVVVTHTSFF-----SSSRGFSK--AIHLDS---PKARVLQYSS  
 CSN2B ----TT-GV-PKGSITSHRAYC-----TGFKEHAW--AIEV-G---PETRSLQFWA  
 CH.NRPS4 ----TT-GA-PKGVVISHRNFA-----TGFRRHCA--ATEV-W---PHTRALQFSA  
 CH.NRPS11 ----TT-GV-PKGVVVIQHSFC-----SAVASNRS--WLQI-K---AESRVLQFTN  
 CSN8B ----TT-GT-PKGVVIPHESFC-----SAVASNSR--ELQI-T---ADSRVLQFTH  
 GLRG\_00920 ----TT-GT-PKGVVIPHESFC-----SAVASNSP--ELQI-T---AKSRVLQFTH  
 CSN2D ----TT-GT-PKGVMIHGSLS-----SSITALGK--GMGL-D---TETRFLQYSA  
 CHN44A ----ST-GT-PKGVVMEHRSL-----SSLVALGS--SHDM-T---PATRMLQFAS  
 CSN2C ----ST-GK-PKGVVMEHRAWA-----SAITPLSK--AIGI-E---PDTRTLQFSS  
 CHN43A ----ST-GE-PKGVVVQHRALC-----SGMAAQAA--AIRC-T---SETRMLQCAN  
 CHN111A ----ST-GK-PKGVVVQHGAYS-----YAAQAHSP--GIHI-N---EDSRVLQFAS  
 CSN8A ----ST-GT-PKGVIVNHGAYS-----YAAQAHSG--GIRI-N---QDSRVLQFAS  
 G0091A ----ST-GT-PKGVAVNHRAYT-----YALQAHSG--GIRI-D---QDSRVLQFAS  
 CSN4A ----ST-GE-PKGVVMIHSAACA-----AAVSCHGN--ATGF-G---RKTRALQYAR  
 CH.NRPS10 ----ST-GE-PKGVVMIHSAACA-----AAVISHGK--ATGF-N---QKTRALQYAR  
 CHN72A ----ST-GT-PKGVVSHAAFA-----SSAAHGS--VFGM-S---RDNRVLQFSA  
 CSN6B ----ST-GT-PKGVVISHSAFA-----SSGASHGS--AFGM-S---CNNRVLQFSA  
 GLRG\_06838 ----ST-GT-PKGVVSHSAFA-----SSAASHGS--AFGM-S---CTNRVLQFSA  
 CHN87A ----ST-GQ-PKGVVSHRAIS-----SSCYHHGL--RMGH-G---PETRVLQFAA  
 CH063\_1253 ----ST-GK-PKGVVMVSHENFT-----SGAVPRGW--AVGY-R---AHSRCDFAS  
 GLRG\_08065 ----ST-GK-PKGVVMISHENFT-----SGAIPRGW--AVGY-R---AHSRCDFAS  
 CSN5 ----ST-GK-PKGVVMISHENFT-----SGAIPRGW--AVGY-R---SHSRCDFAS  
 CHN84A ----ST-GV-PKGAIIPHRVA-----SALHHQLP--RLGY-G---PGSRHCDFSA  
 CHN85A ----ST-GT-PKGVVMPHRSA-----AALAHQTR--LFGF-G---PGSRHYDFMS  
 CHN81A ----ST-GT-PKGVVISHASFY-----SGLHHQLD--RLGF-K---PGCRHYDFMA  
 CH.NRPS8 ----ST-GN-PKGVVIPHSSFR-----AALHHQLP--RMGF-T---SATRHYDFLS  
 CHN86A ----ST-GH-PKGVVLIHRAAS-----TSCTYYGD--RVGF-D---ETSRLFFSS  
 CHN83A ----ST-GE-PKGILVEHRAIS-----TSCYHHGT--RIGY-T---RDSRVLQFAS

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GLRG\_09842  
 GVQALRLGETQLAVVAGSNLLGPEWYVTESNLNMLSPTGTSRMWDADANGYARGEGVAA  
 CH030B\_PKS AVQALRSGESKVAIAAGTNLLGPEQYIAESKLQMLSPSGRSRMWDADADGYARGEGVAA  
 CSP24 QGFDAAVFQIFIALANGGTLV-----A--DNRIDPAELAALMSRERVTC  
 GLRG\_09715 HGFDIALGQIFDALTRGGKLIV-----VGQHGRGDPKHLKMLTESVTY  
 CSP6 VGFDVCVFEIFTALANAGTLV-----ASKDQRRDPLAIANLIKDEKVTF  
 CH063\_0617 VGFDLCPFEIFMALSNAAGTLVV-----ASKEQRQEPSAVVGLIKSEGVTF  
 CH063\_0392 LNFDMALWQVLVTLCSGGKLIV-----VPQDKRLDMTTVTRLIRQEEVTL  
 CS.P.NRPS IGFDMAYLQAFALAYGGALCI-----VPRAQRVDAGAITRIMAAEGVTV  
 GLRG\_10367 IGFDMAYLQAFALAYGGTLCI-----VPRAQRVDAGAITRIMAAEGVTV  
 GLRG\_07434 WSFDLSVTQIFLALGVGAQLHT-----VSHTMRADSHAMAEIQRQGVTA  
 CSP1 LGFDITLIQMFISWVPGGTVCM-----ISRDMRGDSVALMEFMTKHNVT  
 CSP10 FGFDMSLTQIFTALAFGGCVHM-----VTLDERGDALSITKRIVDDGITF  
 CSP20 FFDMSLAQTLTALANGGTLVV-----APSQLRGDAAGLTRLAAEGVTF  
 CH063\_0306 FFDMSLSQTLTALANGGTLVV-----VPAQLRGDAAGLTRLVAENVTF  
 GLRG\_05027 VFF--VWEMLRPLLRGATVFA-----IPDSASYDPSALVSLDLSRQITD  
 CH.NRPS1 VFF--VWEMLRPLLRGAAVFA-----VPDSASYDPSALVNLLDSRQITD

GLRG\_08225 LNVDTALVELLSTLVHGGCVCI-----PSSVET--TSDIVGAACRMDVNW  
 CHN24A LAFDAATWEIYAPLLNGGTLVC-----LDQMTVLSPLLLEAFRVNKIAY  
 CSN9D LAFDATTWEMYAPLLNGGTVVC-----LDQMTVLNPSHLLNALRTNNITM  
 G0043A LAFDATTWEIYGPLLNGGTVVC-----LDQMTVLSPLSLLDAFRANDIKM  
 CSN9G LAFDAATWEMYAALLNGGTLVC-----VNHLTVLNPADLGRVLSNRVSA  
 CH.NRPS2 LAFDAATWEIYAPLLNGGTLVC-----IDRMTVLNNSSELARVLSQRVTT  
 CHN29A LAFDAATWELFTALLNGGTVVC-----IDHMTVLDQSAALGQALRANGVTA  
 CSN9I LAFDAATWEMYAPLLNGGTVVC-----IDHMTVLNPTALADTLAAHGVRV  
 G0046A LAFDAATWEIYTPLLNGGTVVC-----IDHMSVLNPTALGQAFVANGATA  
 CSN9C LAFDAATWEIYTALLNGGSLVC-----VDHTTVLDVAMLGRVFEKEGVKA  
 CHN23A LAFDAATWEIYTALLNGGSLIC-----IDHTTVLDVAALGRVFEQEKVKA  
 CHN210A VAFDASAWEMYTALLNGATLVC-----IDYFTTLDRALEAVFAREHIRV  
 G0047A IAFDASAWEMYTALLNGATLVC-----IDYFTTLDRAALGAVFARESIRV  
 CS.NRPS IAFDASAWEMYTALLNGATLVC-----IDYFTTLDRALEAVFARENVRV  
 CSN9H TAFDNSTWEIYIALLNGGTVVC-----IDYTTIDSKALESVLVQERVQA  
 CHN28A TAFDNSTWEIYMALLNGGTVVC-----IDYTTIDSKALESVFTREGVQA  
 CHN22A TAFDASTWEIWSALLHGGLVC-----IDYFTALDSKALAASFTEHRIQA  
 CSN9B VAFDASTWEVWSALVNGGSVVC-----IDYFSTLDSKTLEAAFAEHKIQA  
 GLRG\_00469 IAFDASTWEIWVALLNGGTAVC-----IDYFTTLDSEALGAFAQHGHIHV  
 G0044A LAFDASTWEIYTALLNGGTLVC-----VDYFTTLDPAALNTLFAEQIQV  
 CSN9E VAFDASTWEIYSALLNGGTLVC-----VDYFTTLDPTALNTVFLERIQA  
 CHN25A VAFDASTWEIYSALLNGGTLVC-----IDYFTTLDPAALNTLFLRERIQA  
 G0045A LAFDISVWEIFGSLNGGTMIC-----VDYFTLLDPKALAALFIRERVEA  
 CHN26A IAFDAATWEIYCALLNGGTLVC-----VDYFTAIDVKTLQALFASRRVEA  
 CSN9F VAFDAATWEIYAALLNGGVVVC-----VDYFTLLDTKALQTLFEHHRVNA  
 CH.NRPS3 FHFDDVSVLEQYWSWSVGIPLVG-----APRDLI--LDDLAGTIRRLNITH  
 CSN1C FHFDDVSVLEQYWSWSVGIPLVA-----APRDLI--LDDLAGAIRRLRITH  
 GLRG\_06542 FHFDDVSVLEQYWSWSVGIPLVA-----APRDLI--LDDLAGAIRRLNITH  
 CHN32A QAFDVSVEIFFTWKVGACLS-----GTNDTL--FEDLERAIRFLGCTH  
 CSN1B QAFDVSVEIFFTWIVGACLS-----GTNDTL--FEDLERAIRFLKCTH  
 G0652A QAFDVSVEIFFTWKVGACLS-----GTNDTL--FEDLERAIRFLECTH  
 CSN1A PTFDVSVEIFFPLFRASLTVS-----CDRGRM--LNDLPAVIRTLQVDA  
 G0651A PTFDVSVEIFFPLFRGSLTVL-----CDRGRM--LNDLPAVIRTLQVDA  
 CHN31A PTFDVSVEIFFPLFRGSLTVL-----CDRGRM--LNDLPAVIRMLQVDA  
 CHN41A HSWDVSVDYLGSLLRGATVCV-----PSDNER--WNDLEGYMVRTAVNW  
 CSN7 YVFDASIIDIFNTLSTGGTLCM-----APTAEL--LSDMAGYIRSMDVRQ  
 CHN62A FAFDLSIGELLFSLGATVVCV-----PTEEER--RANPSKAAGDLGVTW  
 CSN2A YAFDMSIAEMVIGLLTGACLCI-----PSEEDR--LDRLQDYVQEAHATW  
 CH.NRPS9 YTFDVGIGDMLASLATGAVLCV-----PCEQDR--LGDLNGAIDGYRATH  
 CHN91A YSFDVAIDEMITTLVHGGCVCV-----VGEEDRKSPADLVDAMREMEVNV  
 CH.NRPS5 YNFDATNFDILSTLIAGGTICV-----PSEFDR--INRLAGAINDLGANV  
 CH.NRPS6 YTFDVANRDMLIPLMFGGCVCV-----PSESER--VEDLGGFIERTGVTL  
 CHN21A YAFGSSLAETMCTLMQGGCVCI-----PSDHDR--MNDVAGFIRRARVNW  
 CSN9A YAFGSSLAETLCTLMQGGCVCI-----PSDHDR--MNDIAGFIQRARVNW  
 G0041A YAFGSSLAETMCTLMHGGCVCI-----PSDHDR--MNDIAGFIKRARVNW  
 CSN4B HVFDVSIIEIVNSLVGACICI-----PSDEDR--MNNLASFVSKSRADW  
 CHN102A HVFDVSVIEVNSFILGACICI-----PSDEER--MNDLAGFITRSRADW  
 CH.NRPS7 FSDISMLEILSTLMVAGACLCI-----PSEEER--MNNLARCISMGVNV  
 CSN6A FSDISMLEILSTLMAGACICI-----PSEEER--MNNLAGCITGMGVNV  
 G0681A FSDISMLEILSTLMAGACICI-----PSEEER--MNNLAGCITGMDVNV  
 CSN2B YSFDASVGDIMTLLVGGCICI-----PSEGDL--NAEIFNFVARSATW  
 CH.NRPS4 YSFDSSVEILTTLAVGGCLCS-----PSDEDR--SMAIVAFIARSGANW  
 CH.NRPS11 YCFDASLEEIFTVLVAGGCICI-----PSETDR--LSDIPGFVARHQVNW  
 CSN8B FCFDASLEETFTVLVAGGCICI-----PSENER--ISDVAGFAARKSVNW  
 GLRG\_00920 FCFDASLEETFTVLVAGGCICI-----PSEEER--MSDLAGFVTRKSVNW  
 CSN2D FTFDGMLLDTFALVHGGCVCV-----PSEHQK--MNDLAGFIQNFQINT  
 CHN44A YAFDAMLVEIWSLAFGGCVCV-----PSDQQR--MNDITGFITARVNH  
 CSN2C YTFDVMMLLDIFATLISGGCVCT-----PSESER--MNDVGGYIARENVTVA  
 CHN43A FAFDASILEIFAPLSVGGCVCL-----PDDAER--MADLAGFIRRHVDNA  
 CHN111A YGFDTSMEDHLTTFAVGACLCV-----PSEEDRLNLQNLASFTSKSGANW  
 CSN8A YGFDTSMEDHLTTFAVGACLCV-----PTDEGRHTLQGLASVHSSGANW  
 G0091A YGFDTSMEDHLTTFAVGACLCV-----PSDEDRHTLPGLASFARSSGANW

CSN4A HTFDASIAEILTTLAFGGCVVV-----PSEYER--LNNIAAVIQEKKVNW  
 CH.NRPS10 HTFDASIAEILTTLAFGGCVVV-----PSADER--LNNITAVIQEKRNVW  
 CHN72A YSFDASLFEILTTLIHGGTVCV-----AMEAER--LESIGGFMRMRNVDL  
 CSN6B YSFDASLFEIFTTLIHGGTVCV-----PTEKER--IEAIEEFIERHHVNF  
 GLRG\_06838 YSFDASLFEILTTLIHGGTIFV-----PTEKER--LEAIGEFIEGHRVNF  
 CHN87A YTFDASCYDIFTLVWGGVAT-----PSEDQR--LGRLSEAMDEMKVNT  
 CH063\_1253 YAFDVAIDCMLCTLAAGGCLCI-----PSDEDR--MNDLSGAIIRNSKCNM  
 GLRG\_08065 YAFDVAIDCMLCTLAAGGCLCI-----PSDEDR--MNDLSGAIIRNSKCNM  
 CSN5 YAFDVAIDCMLCTLAAGGCLCI-----PSDEDR--MNNLSGAIIRNSKCNM  
 CHN84A YSFDISIHNAIGMLATGGTLLI-----PSDRDR--KSNVRGALVSMQATS  
 CHN85A YSFDASIWNVTVAPLVAGGCLCI-----PSEHER--RNNLAGSIAAMKATS  
 CHN81A YSFDIAIHNTIATLAVGGCLCI-----PSESER--KNNLRGSMMLAMGATS  
 CH.NRPS8 YAFDASIWNVTVATLAAGGCLCI-----PSEHDR--KNNLTRSVELSRATS  
 CHN86A FVFDALILEVITPFVRGSCICI-----PSEDAR--LDDLTGCINELDVNI  
 CHN83A YVFDVSLQEILTTLVFGGVCV-----PAEAER--LSNVAHCISSMSVNM

GLRG\_09842 VVL----KRLSDAVT-DGD-----FIE--CVVRETGVCQDGRNTNGITPSSSEQA  
 CH030B\_PKS IVMKPL--SQALADGD-----SIE--CVIRETGLNQDGKTPGITMPSSSAQA  
 CSP24 SVFIVSEMQVMLRFGY-EEL-RRC--P--SWR--FAVVA-----GETFT-VNLLD  
 GLRG\_09715 TCIVPSEFHMHQHGL-EYL-KQC--R--QWR--TAHVA-----GEKVT-HQLRR  
 CSP6 MAGTPVEFKHIFQHAS-GILKTC--T-SFR--TVAVG-----GEIFT-AQLAA  
 CH063\_0617 MSGTPAEFKHIFRHDA-LGTLRTC--G--GFR--TVVVG-----GEVFT-PQLAG  
 CH063\_0392 TIATPSEYSAWLSHGA-EDL-RRN--R--QWQ--IAVIG-----GEQYS-AQVDN  
 CS.P.NRPS TCAVPSEYKNWLTYGD-RAL-LAAGLS--SWR--TAVSG-----GEPGS-DAVVE  
 GLRG\_10367 TCAVPSEYKNWLTYGD-RAL-LAAGVS--SWR--TAVSG-----GEPGS-DAVVE  
 GLRG\_07434 TYATPTEYKSWLRRES-QDG-LRS--S--AWR--LALVA-----GEPVT-EPLLQ  
 CSP1 TVGTPSEYNSWLRFGD-QAK-LRS--S--AWR--VAICG-----AEAFP-PTLLE  
 CSP10 TGATPSEYLSWL VYGV-PTLGRSE--S--SWR--RAISG-----GEPIT-PALLN  
 CSP20 TVATPTEYASWIGAGS-QHL--KS--S--KWR--VAASG-----GEKMT-QSLLL  
 CH063\_0306 TQATPTEYASWIGAGA-QHL--KS--S--KWR--VAASG-----GEKMT-QSLLL  
 GLRG\_05027 TLMTPTLLATVLSRHP-DLSKRL--P--KLR--SLWLN-----GEVVT-TDLCR  
 CH.NRPS1 TLMTPTLLATVLSRHP-DLSKRL--P--KLR--SLWLN-----GEVVX-TDLCR  
 GLRG\_08225 TYMTPVL-ARKMT--P-AAI-----P--SLK--TVCFR-----TRRLD-EDTCA  
 CHN24A VFFTTALMRQLEEMP-SII-----G--SLD--VLFTG-----GETMRPQDAFK  
 CSN9D AFFTTALLRQYLEEMP-SIV-----A--SLE--VLLAG-----GEAIRPQDAFK  
 G0043A AFFTTALMRQFLEEMP-SII-----G--SLD--VLLVG-----GETMRSKDAFK  
 CSN9G ALLTTALKQILQEAP-AVI-----S--GLD--LLFSG-----GEWMRPQDAQK  
 CH.NRPS2 AFFTTALLKQVLEEAP-SII-----S--DLD--YVFAG-----SEWMRPQDAQK  
 CHN29A ALFTTALLNQFLNEMP-SVI-----A--GLD--LLISG-----GEAMRPKDAFR  
 CSN9I AFFTAALLRQFLEDAP-FLI-----S--RLD--LLFVG-----GEAMRPKDAFK  
 G0046A AFLTTALLRQVLEEAP-SVI-----S--GLD--LLLSG-----GETMRPKDAFR  
 CSN9C AMFTPALLKQCLAESP-STI-----E--GLE--LLFSS-----GDRLDVRDALS  
 CHN23A VMFTPALLKQCLAESP-STI-----K--GLE--LLFAS-----GDRLDVQDALT  
 CHN210A AMLSPALLKQCIANIP-DTL-----A--ALE--ILYVG-----GDRLNPSDARD  
 G0047A AMLSPALLKQCIINTP-STL-----S--DLE--ILYVG-----GDRLNPLDATD  
 CS.NRPS AMLSPALLKQCIANIP-ATL-----A--ALK--VLYVG-----GDRLNPSDAAD  
 CSN9H AMLLPSLLKQCLANAP-AAV-----A--ALD--IILAA-----GDRFDGRDAVE  
 CHN28A TMMLPSLLKQCLAHAP-GAV-----A--ALD--IILAA-----GDRFDGRDAVE  
 CHN22A ALLPPALLKQCLSNAP-DML-----R--GLA--LLFVG-----GDRFDGRDAIE  
 CSN9B AMLPPALLKQCLANAP-DML-----R--GLR--LLFVA-----GDRFDSRDAIE  
 GLRG\_00469 LLLPPALLKQCLANTP-DTL-----R--GLD--MLFVG-----GERFDGRDAIE  
 G0044A TILAPALLKQCIKPMs-AAI-----G--ALD--VLLVG-----GDRFDGHDAAA  
 CSN9E AMLPPALLKQCLKPMa-SAL-----S--ALD--LLFAA-----GDRFDGHDAAA  
 CHN25A TMLPPALLKQCLKPMa-ATL-----G--ALS--LLFAA-----GDRFDGPDAAA  
 G0045A MILPSLLKQYLATVP-EML-----A--SLK--LVLSG-----GDRFDAHDAIR  
 CHN26A AVFPPALLKQCLALAP-ATL-----A--SMQ--FLLTA-----GDRCDPRDAAR  
 CSN9F AMFPPALLKQCLAHAP-AML-----G--PME--LLFAA-----GDRFDARDAVK  
 CH.NRPS3 LDLTPSL-ARLLD--P-SEV-----P--SLSRGVFTG-----GEALK-QEILD  
 CSN1C LDLTPSL-ARLLD--P-SEV-----P--SLSRGVFTG-----GEALK-QEILD  
 GLRG\_06542 LDLTPSL-ARLLD--P-SEV-----P--SLSKGVFTG-----GEALK-QEILD  
 CHN32A LSMTPTV-ASLVD--P-RNV-----P--TVE--FLVTA-----GEPMT-AAVAE

CSN1B LSMTPTV-ASLVD--P-RNV-----P--TVE--FLVTA-----GEPMT-AVVAE  
 G0652A LSMTPTV-ASLVD--P-RNV-----P--TVE--FLVTA-----GEPMT-AVVAE  
 CSN1A CELTPTVAGSLLR--SREKV-----P--CLK--LLLTI-----GEMLT-ESVIR  
 G0651A CELTPTVAGSLLR--SRKNV-----P--CLN--LLLTI-----GEMLT-ESVIR  
 CHN31A CELTPTVAGSLLR--SRKNA-----P--CLK--LLLTI-----GEMLT-EPVIR  
 CHN41A AHLTPTV-ARTLR--P-ERT-----S-KSLR--TLLLV-----GEPMG-DADIA  
 CSN7 AAITPTV-ASILQ--P-DHV-----P--SLE--TLCMV-----GEPLS-RCVRD  
 CHN62A ALLTPSV-IGLLD--P-EEV-----P--TLQ--VLASA-----GEQLT-TQIVE  
 CSN2A AILTPTV-ARLLK--P-SML-----R--HMR--TLILG-----GELVG-ESDIR  
 CH.NRPS9 VILTPTV-AAFLR--P-ERLEN---G--GLR--TMVLI-----GEVAT-SELYK  
 CHN91A ALLTPAV-LQTLD--P-YSV-----P--ACLK--TLMVG-----GSPLN-SSLLT  
 CH.NRPS5 LGVTATL-AQTLD--P-DDV-----P--LLK--VVILC-----GEANS-TEL VH  
 CH.NRPS6 ALLTPSM-AGTLR--P-ASV-----P--SLK--GLVVG-----GEQMT-ESHVQ  
 CHN21A STLTPSF-VATIP--P-ESL-----S--GLK--TLLSG-----GESFS-AYQRD  
 CSN9A STLTPSF-VATVP--P-ESL-----S--GLR--TLLSG-----GEAFS-AYQRD  
 G0041A STLTPSF-VSTIP--P-ESL-----S--GLR--TLLSG-----GESFS-AYQRD  
 CSN4B AFFTPSF-ARTLD--P-RDL-----A--SLK--TVCLG-----GEAMT-LDNIQ  
 CHN102A AFFTPSF-ARTLD--P-RDL-----P--TMK--TVCMG-----GEAMT-LDAIQ  
 CH.NRPS7 AMLTPSV-ASLMS--P-EEA-----P--GLE--VLCFV-----GEALP-QAVAD  
 CSN6A AMLTPSV-ASLMS--P-EEV-----P--SLE--VLCFV-----GEALP-QNVAD  
 G0681A AMLTPSV-ASLMS--P-EEV-----P--SLE--VLCSV-----GETLP-QDVAE  
 CSN2B AGWTPSF-ASLID--P-DTV-----P--TLT--VLLMA-----GEPLP-ASQVD  
 CH.NRPS4 AAWTPSF-ASLID--P-DDV-----P--SLD--AMCLA-----GEPLS-ASLVD  
 CH.NRPS11 AAFTPSY-LRTL D--P-DEL-----E--SLE--FITVH-----AEPMS-QDLVA  
 CSN8B AAFTPSL-LRILN--P-DEL-----MF-SLR--FITVH-----AEPMC-GDLVS  
 GLRG\_00920 AAFTPSL-LRTL N--P-DEL-----A--SSVC--FITVH-----AEPMC-ADLVS  
 CSN2D CFTFPSL-YRIID--P-DSV-----P--SLK--VVMIG-----GEPVL-QSDVD  
 CHN44A LFCTPSL-SRTIP--P-DAV-----MPWPLQ--KVSLG-----GEAMS-QGDVD  
 CSN2C LFSTPSV-IRLID--P-AQV-----P--SLK--TILSL-----GEPLA-QSDVE  
 CHN43A CTFTFSL-LRLLD--P-ADV-----P--GLR--TIVSG-----GEPLT-RTDIE  
 CHN111A AHLTPSF-AELLT--P-TLM-----P--TMK--TMVLG-----GEAMT-ARNIR  
 CSN8A AHLTPSL-AAVLS--P-SLV-----P--TIR--YMVLG-----GEPMT-ADNIR  
 G0091A AHLTPSF-AAMLS--P-SLL-----P--TIR--YMVLG-----GEPMT-ADNIR  
 CSN4A AFFTPSV-IRLLE--P-FEV-----P--GLT--TIVLG-----GEAIG-QDNIE  
 CH.NRPS10 AFFTPSL-IRLLE--P-FEV-----P--GLA--TIVLG-----GEPIG-QDNIE  
 CHN72A ALLTPSV-ARIVD--P-AEV-----P--SLR--TLILG-----GEAPD-GVLVK  
 CSN6B ALLTPSV-ARIID--P-AEV-----P--SMQ--TLVVG-----GEEP D-QLVVK  
 GLRG\_06838 ALLTPSV-ARIIE--P-TEV-----P--SIK--TLILG-----GEEP D-RLVVK  
 CHN87A AAL TASV-SRLIE--P-SRV-----P--SFH--TLTLA-----GEAPL-MTDFT  
 CH063\_1253 AHMTPSV-ARVLD--P-DVI-----P--SLE--VLGLG-----GEAVS-AGDAS  
 GLRG\_08065 AHMTPSV-ARVLD--P-DVI-----P--SLE--VLGLG-----GEAVS-AGDAS  
 CSN5 AHMTPSV-ARVLD--S-DVI-----P--SLE--VLGLG-----GEAVS-AGDAS  
 CHN84A TVLTPSVARLVLR--E-GIP-----P--SSLN--AIGLV-----GEALT-RDDVQ  
 CHN85A VSLTPSV-ARLVS---GAL-----DVD--TLVLL-----GEGVSVEDTRL  
 CHN81A TSLTPSV-ARLLG--D-DLP-----A--TLE--SLVLI-----GEAVTLADTKR  
 CH.NRPS8 VSLTPSVLGLLLL--P-DFP-----P--SLQ--TVVLL-----GEAVT-AEHAR  
 CHN86A IFFTPTV-ARLIE--P-SEV-----P--SLR--TILMG-----GEP PS-QTDLG  
 CHN83A AFLTPTV-ARVVD--P-DHV-----P--SLK--TIMLG-----GEKAS-RQDFS  
  
 GLRG\_09842 SL-----IRH--VYA-KAGLDVVKD--GCQYF-----E  
 CH030B\_PKS AL-----IRS--TYA-RAGLDLSKPSDRPQFF-----E  
 CSP24 QF----RALS R---SDLTIIN---AYG-PTEASICSS--IWQVS-Y-----ADLE  
 GLRG\_09715 AF----RDLAL---PELRLWN---AYG-PTEASVTCG--RGMP-----YQTE  
 CSP6 QF----KQAMP---PGARVFD---AYG-PTEACIFAT--IGEVN-----YLE  
 CH063\_0617 QF----KRSMS---SNVKVFN---VYG-PTETCIFCS--VEEV PYS-----RGDD  
 CH063\_0392 GL----RTLQL---PHLRLMN---FYG-PSEVSFVSH--YMEVF-----PGM  
 CS.P.NRPS LLAVRGPRQSKGQ--QLPRVMH---IYG-PTEITFFAT--FGALG-----PDD  
 GLRG\_10367 LLAVRGPRQSKG---QLPRVMH---IYG-PTEITFFAT--FGVLG-----PDD  
 GLRG\_07434 LF----REVDR---DGLR LFN---VYG-PTETTCGST--KTELS-Y-----RIPG  
 CSP1 KL----RELNK---PDLQLFH---MYG-TTETTVYAT--QQELNWK-----QESF  
 CSP10 AF----ASVAN---PQLR LFN---AYG-PTETTC SVT--RIEVPYR-----DLVQ

CSP20 SF----LSLGK---PDLDLVN---AYG-PTETSFASN--TRKMPYQ-----DLGS  
 CH063\_0306 AF----RSLGK---PDLGLFN--GYG-PTETSFTSN--TREMP-----YHDTE  
 GLRG\_05027 RA----MKAL---PETKLLN--VYS-ASETHEVAAGDIRTFV-----D  
 CH.NRPS1 RA----IKAL---PETRLLN--VYS-ASETHEVAAGDIRTFV-----D  
 GLRG\_08225 PW-----M---AKTKVLL---AYGAPDTCPLGVS--VLEVT-----  
 CHN24A AR-----GL---VRKALCH--VYG-PTENTVFST--MYTMR-----SDE  
 CSN9D AR-----GL---VRKAFCN--IYG-PTENTTYST--LYEMR-----PDE  
 G0043A AR-----GL---VRKAFCH--VYG-PTENTTFST--LYEMG-----PDD  
 CSN9G AA-----RL---VRHSFFH--AYG-PSENATYST--VYQVR-----AEED  
 CH.NRPS2 AA-----RL---VRRSFRH--MYG-PTENTTYST--VFQMPTAAAASEKDDEQRE  
 CHN29A AC-----QL---VKQALYN--AYG-PTENTTIST--AYRID-----ADE  
 CSN9I AR-----DL---VRQGLLN--GYG-PTENTTFST--VYAVG-----RDE  
 G0046A AR-----EL---VRQRFYH--VYG-PTENTTYST--LYHMG-----EDE  
 CSN9C AQ-----AL---AKGGIIN--ALG-HTENTVYST--AF-----  
 CHN23A AQ-----AL---AKGGIIN--ALG-HTENTVYST--AFHMR-----PAE  
 CHN210A AQ-----AL---VKTGVYN--AYG-PTENSVAST--IYKVQ-----PGD  
 G0047A AK-----AL---VKTGVYN--AYG-PTENSVAST--IYKVK-----EGD  
 CS.NRPS AQ---ALVSTD---GGGVVYN--AYG-PTENTVAST--IYKIN-----KGD  
 CSN9H AQ-----EL---VRVNVYN--AYG-PTENTVTST--VYAVR-----GDE  
 CHN28A AQ-----GL---ARINVYN--AYG-PTENTVTST--VYAVR-----GDE  
 CHN22A TK-----ALV---RNGGVYN--AYG-PTENTVMAT--VYEVY-----DEDT  
 CSN9B TK-----ALL---ARGDVYN--AYG-PTENTILST--VYQVA-----END  
 GLRG\_00469 AK-----AL---VRGSIYN--AYG-PTENTVIST--FYEVY-----DGDA  
 G0044A VQ-----AL---VRGSVYN--AYG-PSENTVIST--IYELS-----EMD  
 CSN9E AR-----ALV---APGSVYN--AYG-PTENTILSS--IYEVY-----DAD  
 CHN25A AS-----RAL---VSGSVYN--AYG-PTENTILST--IYEVY-----GQD  
 G0045A TR-----SLIS---ASGSVYN--AYG-PTENSVLST--MHKIS-----KKA  
 CHN26A IK-----SI---VPGTFIN--AYG-PTENTVLST--FHEVT-----DE  
 CSN9F VA-----SI---ISGSVYN--AYG-PTENTILST--IHEVS-----SQD  
 CH.NRPS3 VW-----G---PVGVIYN--AYG-PTEATIGVT--MYQRV-----P  
 CSN1C VW-----G---PVGVIYN--AYG-PTEATIGVT--MYQRV-----P  
 GLRG\_06542 VW-----G---PVGVIYN--AYG-PTEATIGVT--MYQRV-----P  
 CHN32A KW-----L---TGCLFQ---GYG-PAETTNICT--VKKMR-----  
 CSN1B KWGYLFGQGVSSWHRNDNYRVLTDLAGYGP-PAETTNICT--VKKMQ-----  
 G0652A KW-----L---TGCLFQ---GYG-PAETTNICT--VKKMK-----  
 CSN1A EF---GGTKE---SEGILWG--MYG-PTEAAIHCT--LRPQY-----P  
 G0651A EF---GGTED---SEGILWG--MYG-PTEAAIHCT--VRPEY-----P  
 CHN31A EF---GGTGE---SGSILWG--MYG-PTEAAIH-----  
 CHN41A GW-----VE---AGTRAFN--VYG-CTEATWVQV--SRPKT-----GA  
 CSN7 KW-----L---PFCRLN---SYG-PTECSVVVC--TKEVQ-----S  
 CHN62A RW-----A---GRVRLFN--MYA-PAECTVISH--ISRIL-----PG  
 CSN2A PW-----ID---AGIRVYN--GYG-PAEVTFVAT--IAQAS-----  
 CH.NRPS9 TW-----H---GRVRLFN--AYG-PAECSVLTT--VHEVE-----S  
 CHN91A QW-----E---SRVRLLV--AYG-PSECSITAT--MSSDV-----  
 CH.NRPS5 KW---TRPGA---GPRDVIN--GYG-PSEASCAFS--YNVYT-----R  
 CH.NRPS6 SW---TGDBG---NKVGLFN--AYG-VSESTGIAA--LARDV-----QPG  
 CHN21A AF-----A---GRIRVIN--AYG-QSESSTMAG--AARVY-----A  
 CSN9A AF-----A---SRLRVIN--AYG-QSESSTMCG--AARVY-----E  
 G0041A SF-----S---SRIQVIN--SYG-QSESSTICG--GSRVY-----P  
 CSN4B RW-----S---DQVQLIN--GYG-PCEGS-VCT--TANFS-----N  
 CHN102A TW-----S---SRVQLIN--SYG-PCEGS-VCT--TANLS-----S  
 CH.NRPS7 TW-----A---GHVQAIN--AYG-PAECSAITI--VSEPR-----T  
 CSN6A TW-----A---DRVKTIN--AYG-PAECSAVTI--VSQPR-----T  
 G0681A TW-----A---GHVKTIN--AYG-PAECSAVTV--VSKPR-----T  
 CSN2B AW-----V---DRLKLLN--IYG-PSECSVACV--VNKNV-----T  
 CH.NRPS4 RW-----A---DRVRMVN--IYG-PSECSMAST--VNNPV-----T  
 CH.NRPS11 RW-----A---GKIRMRP--SYG-PTECSVTST--VGAPF-----T  
 CSN8B RW-----A---GKVHMRS--SYG-PTECSVTST--VGARM-----T  
 GLRG\_00920 RW-----A---SKVHMRS--SYG-PTECSVTST--VGARM-----T  
 CSN2D RW-----A---PKVRLIA--GYG-PTEACIATL--AGELT-----  
 CHN44A RW-----CS---RGAWLSN--GYG-PTEACIAST--VQVMT-----  
 CSN2C PW-----LGQ---PGICCIN--GYG-PTETCILST--CRKLT-----

CHN43A AW-----LGRLPHGEERHMYN---VYG-VTEACVVSA--AMPMA-----  
 CHN111A NW-----AAS---PDTELIQ---VYG-PSECCVTST--ISPAL-----S  
 CSN8A TW-----SCSS---PGPQLIQ---VYG-PSECCVTST--ITRPV-----L  
 G0091A TW-----SCPS---PGPQLIQ---VYG-PSECCVTST--ITQPV-----L  
 CSN4A KW-----A---PGRKLVV---GYG-PTENTVFST--MHPLL-----  
 CH.NRPS10 KW-----A---PGRNLVA---AYG-PTENTVFST--MHPLP-----  
 CHN72A KW-----RD---AGTRLFN---AYG-PSECSVIAA--CHCYS-----  
 CSN6B KW-----LD---AGIRLFN---AYG-PSECSIIAA--CYSCS-----  
 GLRG\_06838 KW-----LD---AGTRLFN---AYG-PSECSVIAA--CYSCS-----  
 CHN87A RW-----S---HLPRVMN---CFG-PTETAIVST--IYDL-----F  
 CH063\_1253 TW-----S---KTTSVII---AYG-PSECTVGCT--INSNV-----  
 GLRG\_08065 TW-----S---KTTSVII---AYG-PSECTVGCT--INSNV-----  
 CSN5 TW-----S---KTTSVII---AYG-PSECTVGCT--INSNV-----  
 CHN84A GW-----LD---AGVRVTN---LYG-PSECTSIST--VARSA-----VVVDDDA  
 CHN85A LW-----G---RARVIIN---AYG-PAECTPNSV--ISYAS-----E  
 CHN81A IW-----NKTRLIN---SYG-PAECTPYST--TNDQA-----A  
 CH.NRPS8 LF-----RG---KKVAIVN---AYG-PAECTPNSV--IGTSS-----SS  
 CHN86A RW--GWGRGPG---PDRTVFN---GYG-PTECVVFCFA--LQKID-----P  
 CHN83A RW-----SG---RVGVVLN---GYG-PAECAVCCC--INDVD-----GWE  
  
 GLRG\_09842 AHGT-GTPAG---DPVEA-EAIHSVFTDSSRDGVRT-----DPLYVGSVKT  
 CH030B\_PKS AHGT-GTPAG---DPIEA-AAIREAFFGADS-----HFVPRGPDDT  
 CSP24 TGAF-SIPIG---KPIAN-YGTY--VVDEES-----NPVPVGPWGE  
 GLRG\_09715 EDIM-SDSDG---IRALPN-YNLT--IVDEKL-----NPVPAGFPGE  
 CSP6 DGLG-LVHAG---FALDN-VSIY--IVDDRM-----NLVPDGCAGE  
 CH063\_0617 DDLEEPIPVG---RTLDN-VSVY--VVDEAM-----NLLPDGCAGE  
 CH063\_0392 VSGDGAVPVG---SPLHN-YAAY--VLDSSQ-----RPVPIGMTGE  
 CS.P.NRPS AKQL-PAAAG---RPLHN-YSVY--VLDESL-----RPVPPGVVGE  
 GLRG\_10367 AKQL-PPAAG---RPLQN-YSVY--VLDESL-----RPVPPGVVGE  
 GLRG\_07434 FYQG-TIPVG---VASAN-EWFY--ILDASQ-----NLQPVGOTGE  
 CSP1 YDDG-VMPAG---FSLPN-KSMY--IVDEDM-----HLLPVGLPGE  
 CSP10 ASSKLPLTAG---HVAPN-CSVC--IVDSL-----RPLPIGMPGE  
 CSP20 SSSS-SSSIADLSLMTWPN-YSIS--IVDRDL-----QPVPAGVSGE  
 CH063\_0306 SNSG-GRNIAALPLTTWPN-YSIS--IVDRDL-----RPVPAGVSGE  
 GLRG\_05027 FETH-VCPVG---PPMDP-EHIY--IMDESG-----NRVGTNVSGE  
 CH.NRPS1 FETR-VCPVG---PPMDP-EHIY--IMDENG-----NRVGNNVSGE  
 GLRG\_08225 KPSD-LSRVA---PPFLG-RFW--IVNPED-HG-----KLMPIGALGE  
 CHN24A GCVN-GLPIG---RAIHN-SGAF--VMDSRQ-----QIVPLGVIGE  
 CSN9D RCVN-GLPIG---RAIKH-SGAF--VMDSRQ-----QIVPLGVIGE  
 G0043A RCVN-GLPIG---RSIKN-SGAF--VMDSRQ-----QLVPLGVIGE  
 CSN9G FSNV-GVPIG---RAVSN-SGAY--VMDRRQ-----RLVPIGVMGE  
 CH.NRPS2 HCAN-GVPIG---RAVSN-SGAY--ITDTKQ-----RLVPIGVMGE  
 CHN29A SFVN-GVPIG---GAISN-SGSY--IMDAHQ-----QLTPIGTMGE  
 CSN9I RCVN-GVPLG---RSVTN-SAAY--IMDARQ-----QLTPVGTIGE  
 G0046A RCIN-GVPIG---RAISN-SGAY--VMDALQ-----QLTPIGTIGE  
 CSN9C ----HMRPG-----  
 CHN23A LCIN-GVPVG---RAISN-SGAL--VLDPQQ-----RPVPLGVMGE  
 CHN210A AFEH-GVPIG---QAVSS-SGAY--IMDSRQ-----QLVSIGVMGE  
 G0047A LFEH-GVPIG---RAVSN-SGVY--IMDSRQ-----QLVSIGVMGE  
 CS.NRPS VFEH-GVPIG---QAVSN-SGAY--IMDSRQ-----QLVSIGVMGE  
 CSN9H DFVN-GVPIG---RAIDN-TGAY--IVDARQ-----QVVGAGVMGE  
 CHN28A GFAN-GVPIG---RAINN-TGAY--IVDARQ-----QLVSMGVMGE  
 CHN22A CFAS-GVPVG---RAVSN-SGAY--IMDPIQ-----QLVPVGVVGE  
 CSN9B ASLANGVPIG---RVVSD-SGAF--IMDSQ-----QLVPLGVMGE  
 GLRG\_00469 SLAN-GTPIG---RAISN-SGAF--IMDPDQ-----QFVPVGMGE  
 G0044A SFVN-GVPIG---KSVSH-SGAY--ILDNTQ-----QPVPAGVMGE  
 CSN9E PFVN-GVPIG---KAVSN-SGAY--ILDANQ-----QLVPVGMGE  
 CHN25A PFVN-GVPIG---RAVSN-SGAY--ILDPSQ-----QPVPVGMGE  
 G0045A QYVN-GVPIG---HALSN-SGAY--IMDVRQ-----NPVPIGVMGE  
 CHN26A PYVN-GVPIG---RTATN-SGAY--IMDARQ-----QPVPVGMGE  
 CSN9F PYVN-GVPIG---GAVSN-SGAY--VMDPRQ-----NPVPIGVMGE

CH.NRPS3 QNGR-ASNIG---RQFDN-VGSF--VLQPGT--D-----IPVMKGAVGE  
 CSN1C QNGR-ASNIG---KQFDN-VGSF--VLQPGT--D-----IPVMKGAVGE  
 GLRG\_06542 QNGR-ASNIG---KQFDN-VGSF--VLLPGTD-----IPVMKGAVGE  
 CHN32A PGDF-IDHLG---FSFEN-TSTF--VLGSST-TE-----VVPICVGE  
 CSN1B PGDF-IDHLG---FSFEN-TSTF--VLGSAT-TD-----VVPICVGE  
 G0652A PGDF-IDHLG---FSFEN-TSTF--VLDSAT-TD-----IVPICVGE  
 CSN1A RDCA-SSNIG---FPLDS-VSCF--VASIQEGD-----AYEFRILPLGEPGE  
 G0651A RDSS-PSIIG---FPLDS-VSCF--VASIQEGN-----AYEFRILPRGEPGE  
 CHN31A -----  
 CHN41A QVGR-GHVSF---YGI-N-TRVW--IVRRGD-QA-----LSPVGCGRGE  
 CSN7 SHGP-AGCIG---RPYPT-AVAF--ILDPDGN-----SLCPYGAVGE  
 CHN62A ADTP-PACIG---RSP-G-AVSW--VASQND-HR-----KLVPIGAIGE  
 CSN2A -TDQ-ASSIG---RGL-N-TTTW--IVDPN--RG-----QLAPIGAVGE  
 CH.NRPS9 PDDN-PAVIG---AAVAPHCRVW--LVDAAD-PS-----RLVPVGSSE  
 CHN91A GNSP-PGTIG---RPV-G-CRAW--VLGPVQTNRDEATSKQDPNPRMLRLVPLGCVGE  
 CH.NRPS5 QSPR-ANNVG---RALEGACWGW--VVNPDN-HS-----QLLPVAKGE  
 CH.NRPS6 GGSS-PSNIG---RGN-G-STLW--VLSMTD-PS-----RLAPVGAMGE  
 CHN21A DTTD-LQNI---RALG--ARYW--VTDPPDPG-----RLAPIGAVGE  
 CSN9A DTTD-LQNI---RAL-G-SRYW--ITNPDDPG-----RLAPIGCVGE  
 G0041A DTAD-LQNI---RAL-G-ARYW--ITNPDDPG-----RLAPIGCVGE  
 CSN4B NPSK-PDSIG---RGA-N-SLIW--IVNSNN-HD-----ILVPIGSIGE  
 CHN102A GHFG-TDAIG---RGA-N-SLVW--IVEPDN-HD-----RLVPIGSTGE  
 CH.NRPS7 KGVK-SISLG---RPA-N-CAVW--IVGEDG-----RLAPFNTVGE  
 CSN6A KGVK-SISIG---QPAN--CAVW--IVTENG-----KLAPFNSIGE  
 G0681A KGVK-SISIG---RSA-N-CAVW--IVTENG-----KVAPFNSIGE  
 CSN2B RNTN-ASNIG---RGY-R-CVTW--VVDEND-HE-----RLRPIGSVGE  
 CH.NRPS4 RDSL-PSNIG---RAHRS-----  
 CH.NRPS11 VDTD-ATNIG---WPV-G-CRW--VVHPENHD-----ILMPIGAVGE  
 CSN8B ADSD-PANIG---YPV-G-CHGW--IVHPENHK-----ILAPVGAVGE  
 GLRG\_00920 AHSD-PANIG---HPV-G-CHAW--IVHPEN-HR-----VLAPVGAVGE  
 CSN2D PSTS-PNTIG---RPVV--CRAW--VVNPLK-PS-----ELTPIGGIGE  
 CHN44A QATP-IGTVG---FPLDS-CRLW--VVNPLKQQQQQDDQEYHPGQY--ELAPIGAIGE  
 CSN2C PVDS-SSNIG---TPIA--AATW--LVSPFTR-----TLAPIGAVGE  
 CHN43A LSTS-PRTVG---YPV-G-ASLW--LVSPVSG-----MLAPPVAVGE  
 CHN111A LYSD-PTNIG---SAVPG-CRTW--IVRPDD-PN-----ALQAIGVVGE  
 CSN8A QDGD-PTDIG---TAVPN-CKTW--IVKPGN-PD-----NLQVIGTVGE  
 G0091A PDGD-PTDIG---TALPN-CKTW--IVKPSD-PD-----SLQVVGAVGE  
 CSN4A LSAK-AGVIG---RGV-G-TRCW--IADPEN-PS-----RLSPIGAPGE  
 CH.NRPS10 PSAK-AGVIG---RGV-G-TLCW--IADPED-PN-----RLSPVGAPGE  
 CHN72A AHTD-PRTIG---LPV-G-CSSW--IVNADDNEA-----SLVADGDIGE  
 CSN6B HEMD-PRTIG---LPV-G-CSSL--IVDPDD-ES-----SLVPEGEVGE  
 GLRG\_06838 HETD-PRTIG---ISV-G-CSSL--IVDPDN-EL-----SLAPEGEVGE  
 CHN87A SRPD-ASCIG-----  
 CH063\_1253 -SST-STNIG---KGCGG--LTW--IVDPDDHE-----RLMPVGAVGE  
 GLRG\_08065 -SSS-STNIG---KGCGG--LTW--IVDPDD-HE-----RLMPVGAVGE  
 CSN5 -SST-STNIG---KGCGG--LTW--IVDPDD-HE-----RLMPVGAVGE  
 CHN84A PSAT-ALDTG-----AG-CATW--IVDPNDHN-----VLMPLGATGE  
 CHN85A TPEA-AARIG---KGA-G-AVTW--VANPDD-HE-----RLVPIGAVGE  
 CHN81A GPET-AVRIG---TGA-G-ALTW--VVHADD-HN-----KLVPLGLVGE  
 CH.NRPS8 SPEA-ASLIG---TGS-G-AVTW--VADIRD-PS-----RLAPIGAVGE  
 CHN86A TSIR-ETCIG---KST-G-SASW--IVAPDDSD-----SLTPVGSVGE  
 CHN83A DLRR-PHCIG---TAV-G-SATW--VVAPGD-AS-----QLVPVGAIGE

GLRG\_09842 VIGHTEGTAGLA-GLL-----KTTAALQ--RKTIF----P-NRHFKRL-----  
 CH030B\_PKS LY--VGSi-KTVIGHTEGTAGLA-AVVKa--TEALKAATLPP-NRLFNR-----  
 CSP24 IA--ISGP-GVASGYA-----N-LPLL-----  
 GLRG\_09715 IC--ISSPYSIRGYN-----N-RPEET--ERQFI----D-SKLIRGG-----  
 CSP6 VC--IGGA-GVGHGYL-----D-RPEPT--TKAFV--ED-P-CATVEDQ-----  
 CH063\_0617 VC--IGGA-GVSRGYFGLSSGFG-SGTRN--SSAFV----P-DPFASPE-----  
 CH063\_0392 VY--IAGA-GICKGYL-----G-NPSLT--GQKFV----SDPFVRSDL-----  
 CS.P.NRPS IF--VGGA-GVAAGYVSGETETG--TETESATADKFLPDHLAP-AAFLSQG-----

GLRG\_10367 IF--VGGA-GVAAGYVLGETETG-TETESATADKFLPDHLAP-AAFLSQG-----  
 GLRG\_07434 IA--IGGV-GVAMGYL-----N-MPDR--QASFL----P-DPFAATEE-----  
 CSP1 IV--LGGV-GVAQGYN-----N-NELMT--KASV----P-NTFAPNG-----  
 CSP10 VL--IGGA-KVAIGYL-----G-NDTLT--KERFFDHDHVP-EEFVAEG-----  
 CSP20 VC--IGGA-GVGLGYF-----N-NEELT--SGAFVLDKSAP-PEFAARG-----  
 CH063\_0306 VC--IGGA-GVGLGYL-----N-NDKLT--SEAFVSDKTAP-PEFAARG-----  
 GLRG\_05027 LY--VGGD-LLARGYL-----N-LPETT--TKAFQ----PDPFDKKAG-----  
 CH.NRPS1 LY--VGGD-LLARGYL-----N-LPETT--AKAFH----PDPFVKKEG-----  
 GLRG\_08225 LI--IESP-TLAHHFV-----P-GQSLS--HLPQD----S-DFSLEDG-----  
 CHN24A LV--VTGD-GLARGYT-----DPSLD--LNRV----Q-VTVEGKS-----  
 CSN9D LV--VTGD-GLARGYT-----DPSLD--AARFV----RVTIGDG-----  
 G0043A LV--VTGD-GLARGYT-----DSSLD--QARFV----Q-VTIDGQS-----  
 CSN9G LV--VTGD-GLALGYT-----DAKLD--AHRFV----T-VTIAGRD-----  
 CH.NRPS2 LV--VTGD-GLARGYT-----DPALD--RGRFI----H-ISVAGRK-----  
 CHN29A LV--VTGD-GLARGYT-----DSTLN--ADRFV----E-VDIAGRS-----  
 CSN9I LV--VTGD-GLARGYT-----DPALD--KDRFV----Q-VEIAGRS-----  
 G0046A LV--VTGD-GLARGYT-----DSSLE--KDRFV----Q-VHVGGQS-----  
 CSN9C -----  
 CHN23A LV--LTGD-GVARGYT-----DAALN--KGRFF----DMTTADGET-----  
 CHN210A LV--VTGD-GLARGYT-----DPSLD--VNRFL----H-VTIEGQL-----  
 G0047A LV--VTGD-GLARGYT-----DHALD--KNRFM----Y-VTIEGRL-----  
 CS.NRPS LV--VTGD-GLARGYT-----DQALD--KNRFM----H-VTIEGRL-----  
 CSN9H LV--VTGD-GLARGYT-----DPALD--VDRFV----P-ISIGSNN-----  
 CHN28A LV--VTGD-GLARGYT-----DPSLD--TDRFVHIRI-P-GYDEP-----  
 CHN22A LV--VTGD-GLARGYT-----DPALD--RDRFI----H-ISVAGRE-----  
 CSN9B LV--VTGH-GLARGYT-----DPTLN--RDRFV----E-IDMAGKL-----  
 GLRG\_00469 LV--VTGD-GLARGYT-----DQKLN--QDRFI----Q-IDIAGEL-----  
 G0044A LV--VTGD-GLARGYT-----DASLN--EGRFA----E-VTIHDKV-----  
 CSN9E LV--VTGD-GLARGYT-----DAALN--KDRFV----Q-VAVHGQV-----  
 CHN25A LV--VTGD-GLARGYT-----DAALN--KDRFA----Q-VAVNGQV-----  
 G0045A LV--VSGD-GLARGYT-----DPALD--KGRFV----EVTLDGDG-----  
 CHN26A LV--VLGD-GLARGYT-----DPALE--VGRFV----E-ISVDGQP-----  
 CSN9F VV--VVGA-GLARGYT-----DRTLD--EGRFVEIIS-P-EGGGGGG-----  
 CH.NRPS3 LC--VSGK-LVGKGYL-----N-RPELT--RERF----P-LLER-LG-----  
 CSN1C LC--VSGK-LVGKGYL-----N-RPELT--RERF----P-SLER-LG-----  
 GLRG\_06542 LC--VSGK-LVGKGYL-----N-RPELT--QERF----PFLEGFC-----  
 CHN32A LC--FGGD-QVAAGYL-----G-MPDVT--NAKFL--DH-P-EF-----  
 CSN1B LC--FGGD-QVAAGYL-----G-MPELT--NAKFI--DH-P-RF-----  
 G0652A LC--FGGD-QVAAGYL-----G-MPELT--NAKFI--DH-P-RF-----  
 CSN1A LV--VGGH-QLAKGYL-----N-RPEQT--QSAFL--DT-P-Y-----  
 G0651A LV--VGGH-QLANCYL-----N-RLEQT--QSAFL--DT-P-Y-----  
 CHN31A -----  
 CHN41A IW--LEGP-MVTQGYI-----NVDPLLE--QRSFP--NR-P-DWAE-RL-----  
 CSN7 LC--IGGP-QLTDGYV-----G-RDDLT--SAAFV-----WHDDLQ-----  
 CHN62A LL--VEGP-VVSSGYL-----G-DAEKT--DAAFLSPSDPP-SWLT-QI-----R  
 CSN2A LV--VESL-TLALGYL-----N-DPSRT--AESFI--IN-P-SWAQ-LD-----  
 CH.NRPS9 LL--IEGQ-HVASGYL-----N-DPEKT--ARAFISADEAP-AAIRPLL-----  
 CHN91A LA--IEGP-ILAEGYI-----S-NPRLT--AERFI--FA-S-HVAG-DF-----  
 CH.NRPS5 LL--IQGP-TLSRGYL-----N-EPEKT--AKVFI--ES-P-AWLP-AK-----  
 CH.NRPS6 LV--IEGP-SVARGYL-----N-DPERT--ERSFQ--TE-P-EWKR-AFLQRLVENKDE  
 CHN21A LL--IESA-GIARGYV-----V-PPPPE--QSPFL--DAAP-AWYPAEW-----  
 CSN9A LV--IESP-GIARGYV-----V-PPPPD--QSPFL--DEAP-AWYPAEW-----  
 G0041A LM--IESP-GIARGYV-----V-PPPPD--QSPFL--DDAP-AWYPAEW-----  
 CSN4B IL--IEGP-NVARGYL-----C-DQEKT--DKAFI--TN-P-AWIT-HF-----  
 CHN102A IL--IEGP-NVARGYL-----G-DPEKT--AKAFI--TN-P-AWIA-HF-----  
 CH.NRPS7 IV--IEGP-PVARGYL-----G-DREKT--GAVFL--DD-P-GFLRGVT-----  
 CSN6A II--IEGP-PLARGYL-----G-DEEQT--DAVFL--GDP-SFLRGVV-----  
 G0681A IV--VEGP-PLARGYL-----G-DEGQT--NAVFL--GDP-SFLRGIV-----  
 CSN2B LL--IEGP-ILARGYL-----K-RPEKT--AEVFI--DA-P-SWL--KN-----  
 CH.NRPS4 ----VTS-----  
 CH.NRPS11 LL--LDGP-IVGKGYL-----D-DEAKT--AAAFI--DPP-AWAVEVE-----  
 CSN8B LL--LDGP-IVGKGYW-----R-DEVKT--EAAFV--EPP-EWWARGV-----



GLRG\_00920 LV--LDGP-IVGNGYW-----G-DEAKT--GEAFV--ES-S-DWWGRVV-----  
 CSN2D LC--VEGP-CVARGYL-----G-NEEAT--QAAFL--ES-P-SWLPQSS-----  
 CHN44A LY--IEGV-NVARGYL-----H-DDAKT--AAAF--ER-P-PWLDGRR-----IEDS  
 CSN2C IC--IEGP-TLARGYL-----G-DPERT--AMAF--RNPP-CFPNDVG-----  
 CHN43A LF--LEGP-ALARGYH-----E-DRPRT--QTSFV--DD-P-AWL--V-----  
 CHN111A LL--MEGP-ILAKGYL-----N-SPEQT--NNSFT--QG-L-HWAP-EK-----  
 CSN8A LL--VEGP-ILAKGYL-----G-DAERT--RMAFV--EG-L-RWAP-DK-----  
 G0091A LL--VEGP-ILAKGYL-----G-DAERT--SMAFV--EG-L-RWAP-DK-----  
 CSN4A LL--LESP-QLARGYL-----N-FPENEGPNRAFI--RN-S-FGLT-KH-----  
 CH.NRPS10 LL--LESP-QLARGYL-----N-FAENEGPNQAFI--RN-P-PWLAKCG-----  
 CHN72A ML--LGGA-TLADGYL-----N-DPART--AAAFV--DS-S-SWPL-ES-----  
 CSN6B LL--IGGP-ILADGYL-----N-NPERT--HSAFI--NT-P-SGSV-DT-----  
 GLRG\_06838 LL--IGGP-ILADGYL-----N-DPERT--HSAFT--NT-P-LCSV-GT-----  
 CHN87A -----  
 CH063\_1253 LL--IEGP-VVGLGYL-----N-DKAKT--DEVFI--EDP-AWLLAGG-----  
 GLRG\_08065 LL--IEGP-VV-LGYL-----N-DKTKT--DEVFI--ED-P-AWLLAGG-----  
 CSN5 LL--IEGP-VVGLGYL-----N-DKAKT--DEVFI--ED-P-AWLLAGG-----  
 CHN84A LI--LEGP-IIGTYL-----D-EPEKT--AATYI--RD-P-AFLALGA-----  
 CHN85A LL--LEGP-IVGAGYL-----D-APDKT--AEAFV--ND-P-SWLRRGA-----PGGG  
 CHN81A LL--LEGP-ILSQGYL-----D-DPEKT--AKAFI--ED-P-TWLLQGS-----  
 CH.NRPS8 LL--LEGP-IVGNGYL-----H-DAEKT--KASFI--ES-P-PWLLGGG---SSSSSS  
 CHN86A LL--VEGP-ILARSYL-----DPQNT--AKAFI--EN-P-PWLLQGG---GGGDADG  
 CHN83A LL--VEGP-ILARGYF-----K-DPQLT--AAAFI--EA-P-AWLT-RGGGGG-GGCSP

GLRG\_09842 -----NPRIEPFYKNLKV-----P--TVEIPWPKPPE--NQPLRASVNSFGFGGTNAH  
 CH030B\_PKS -----SPKVEPFYKNLEVV TSA--RP-WPKIPANGMRR-----VSVNSFGFGGAN CHAI  
 CSP24 -----  
 GLRG\_09715 -----ALPPGIPVYRTGDLGRLS--AE-DGTLTVLGRIG-QDSQVKIR-----  
 CSP6 -----RNDWKTMYRTGDSGYLR--P--DGSLVILGRIN-GDSQVKIR-----  
 CH063\_0617 ----DVSRGWTGMYRTGDSGYLK--P--DGSLVVLGRIG-GSTQVKIR-----  
 CH063\_0392 ----AKSRGWTRMYRTGDKARIN--Q--DGTL SILGRID-GDAQLKIR-----  
 CS.P.NRPS -----WTTMHRTGDNGHWR--A--DGQLVIQGRRS-GDTQHKIR-----  
 GLRG\_10367 -----WTTMHRTGDNGRWR--A--DGQLVIQGRRS-GDTQHKIR-----  
 GLRG\_07434 ----DSRCGWATMYRTGDVGFLLQ--H--DGSLVLKGRIGD-----  
 CSP1 --SPFHRKGWTTMYRTRDRGRLL--P--DGSIVVEGRIG-GDTEIKLR-----  
 CSP10 -----WTSVHRTGDVGRLL--L--DGQLILEGRVA-GDSQIKLR-----  
 CSP20 -----WTKKHRTGDRGRLS--P--DGGLILEGRIA-GDTQIKLR-----  
 CH063\_0306 -----WTKKHRTGDRGRLS--P--DGGLVLEGRIA-GDTQIKLR-----  
 GLRG\_05027 -----ARMYRTGDLARIL--P--SGLLEITGRAG--GMIKTRGYTVQPGAVENA  
 CH.NRPS1 -----ARMYRTGDLARVL--P--SGLLEITGRAG--GMIKTRGYTVQPGAVENA  
 GLRG\_08225 -----KPTTRYFKTGHLVRYM--E--DGLLDFVSSGR--DAVESNGRVVPVTEIEQR  
 CHN24A -----LRAYRTGDRARYR--PA-DGEIEFIGRVD--NQIKIRGYRIETAEVETA  
 CSN9D ----RRTATVRA YRTGDRVRYR--PS-DGEIEYIGRTD--NQVKIRGYRIETA EVEQA  
 G0043A -----VRAYRTGDRARYR--PA-DGELEFIGRTD--NQVKIRGYRIETA EVEQA  
 CSN9G -----VRAYRTGDLARFR--PV-DGQIEFHGRMD--QVVKVRGHRIEPAEIERA  
 CH.NRPS2 -----VRAYRTGDRARFR--P--DGQIEFHGRMD--QVVKVRGHRIEPAEIEQA  
 CHN29A -----VRAYRTGDRARIR--PR-DGQIEFFGRMD--QVVKIRGHRIEPVEIEQA  
 CSN9I -----VKAYRTGDRARYR--PG-DGQMEYCGRMD--QVVKIRGHRIEPAEIEHA  
 G0046A -----IKAYRTGDRARYR--PG-DGQIEFFGRMD--QVVKIRGHRIEPAEIEQT  
 CSN9C -----  
 CHN23A -----TRAYRTGDRARYR--PV-DAQMEFFGRMD--QVVKIRGFRVPEAEVEQV  
 CHN210A -----VRAYRTGDRARYR--PS-DGQIEFFGRID--QVVKIRGHRIEPAEVELA  
 G0047A -----VRVYRTGDRARYR--PS-DGQIEFFGRID--QVVKIRGHRIEPAEVELA  
 CS.NRPS -----TRVYRTGDRARYR--PS-DGQIEFFGRID--QVVKIRGHRIEPAEVELA  
 CSN9H --NNNNDQQLVRA YRTGDRARIR--PG-DGQIEFFGRID--HQIKVRGHRIEPAEIEQA  
 CHN28A -----IRAYRTGDRARFR--PG-DGQIEFFGRID--QVVKIRGHRIEPAEIEQA  
 CHN22A -----VRAYRTGDRVRYR--PT-DGHIEFAGRID--QVVKIRGHRIEPAEIEQA  
 CSN9B -----VRAYRTGDRVRYR--PV-DGQIEFSGRMD--QVVKVRGHRVPEAEIEQA  
 GLRG\_00469 -----VRAYRTGDRVRYR--PT-DGQIEFSGRID--QVVKIRGHRIEPAEIEQA  
 G0044A -----LRAYRTGDRVRHR--PT-DGQIEFFGRMD--QVVKIRGHRVPEAEVEQA  
 CSN9E -----VRAYRTGDRVRYR--PT-DGQIEFFGRMD--QVVKIRGHRVPEAEVEQA

CHN25A -----VRAVRTGDRVRYR--PT-DGQIEFFGRMD---QQVKIRGHRVEPAEVEQA  
 G0045A -----QPPIRAYRTGDRARYR--P--DGEMEFFGRID---DQVKVRGHRIEPAEIEHA  
 CHN26A -----PARAYRTGDRVRYR--P--DGQIEFFGRMD---QQVKIRGNRVEPAEVEQA  
 CSN9F ---GGGGSQSPVRAVRTGDRARYR--P--DGQMEFFGRMD---QQVKIRGHRVEPAEVEQA  
 CH.NRPS3 -----ERVYRTGDLVRL--H--DGCDFDLGRAD---DQVKLRGQRLEIGEINHS  
 CSN1C -----ERVYRTGDLVRL--H--DGCDFDLGRAD---DQVKLRGQRLEIGEINHS  
 GLRG\_06542 -----ERVYRTGDLVRL--H--DGCDFDLGRAD---DQVKLRGQRLEIGEINHS  
 CHN32A -----GRIYRSGDMGRML--P--DGSLMVLGRMD---DQLKLRGQRIDTGEIGSI  
 CSN1B -----GRIYRSGDMGRML--P--DGSLMVLGRMD---DQLKLRGQRIDTGEISSI  
 G0652A -----GRIYRSGDMGRML--P--DGSLMVLGRMD---DQLKLRGQRIDTGEISSV  
 CSN1A -----GRVYRTGDKAIMN--P--DGTLECLGRIS--DGQVKLRGQRIELGEIEQA  
 G0651A -----GRVYRTGDKAIMK--S--DGTLECLGRIS--DGQVKLRGQRIELGEIEQA  
 CHN31A -----  
 CHN41A -LGRTHDDGQHRFYRTGDLGRLC--A--DGALEVMGRTD---TQAKLGGQRLELSEVEHY  
 CSN7 -----MRIYRTGDLARSL--P--GGDIECLGRKD---NQVKLHGFRVELGEIEAV  
 CHN62A GEAQGGKV--GRVYKTGDLVRQR--E--DGTLLFLGRKD---DQIKLHGQRLEVEEVEHS  
 CSN2A ---MPETPLSRKFYKTGDLARYL--P--DGSLECLGRLD---TQVKLGGQRVELSDIEHH  
 CH.NRPS9 -----PRAGLYLTGDLARLV--P--HHGLVFLGRKD---LQIKLRGQRVEMGEIEFH  
 CHN91A -CRVTGCRPETRLYRTGDLVRQN--P--DGSLLFLGRID---SQVKVRGVRVELEAIEHC  
 CH.NRPS5 -----TAPQLRRLYKTGDLVRQL--V--DQSYEVYGRID---TQVKLNGQRIELGEIEHK  
 CH.NRPS6 DGKSDERFPWTRAFRTGDLVRYSPRL--DGTLELMGRRD---HQVKVNGQRLELAAVEGH  
 CHN21A --RQTQRANGYRFYRTGDLVCYR--P--DGTVAYLGRRD---SQVKIRGQRVELGDVESH  
 CSN9A --RRHQKANGYRFYRTGDLVCYR--P--DGTVAYLGRRD---SQVKIRGQRVELGDVESH  
 G0041A --RRHQKANGYRFYRTGDLVCYR--P--DGTVAYLGRRD---LQVKIRGQRVELGDVESH  
 CSN4B --ETSSAA--RKMYSKTGDLGMLN--H--DGTISYLGRKD---AQIKLRGQRIEPGEVEHQ  
 CHN102A --EAGSVA--RRMYKTGDLGMLN--S--DGTVSYLGRKD---TQIKLRGQRIEPGEVEHH  
 CH.NRPS7 -----RDSGRCYRTGDLGRMR--V--DGSVDFLGRKD---GQVKINGQRVELGEVEHH  
 CSN6A -----VPMRCYRTGDLGRMN--V--DGSIDFLGRRD---SQVKINGQRVELGEIEHQ  
 G0681A -----ADPMRCYRTGDLGRMN--V--DGSIDFLGRRD---SQVKINGQRVELGEIEHQ  
 CSN2B --GPHPRT--NRLYKTGDLVRYN--S--DGTINFIGRKD---TQLKINGQRVEIGEIEHS  
 CH.NRPS4 -----DGSINYVGRKD---TQLKIHGQRVEVGDIEQH  
 CH.NRPS11 -SAFPEEGLPRKLYKTGDLVSYA--E--DGSLLIHRKD---HSQVKIRGQRVELGEIQFH  
 CSN8B --SSAVSPRSQRKFYKTGDLVRYA--E--DGSLLIQGRKD---DSQVKIRGQRVELREIQHH  
 GLRG\_00920 ---DSPEPCRSRRRVYKTGDLVRYA--E--DGSLLIQGRKD---DSQVKIRGQRVELREIQHH  
 CSN2D -----PGTRVYRTGDFVYYN--A--DGTLSFFGRKD---SQVKIRGQRVELSEIEEA  
 CHN44A DGTTPDDEKHHRVYRTGDLAYYN--P--DGSRLVGRKD---AQVKLRGQRVELGEIEEA  
 CSN2C -----RRIYHTGDLARYN--T--DGS�TFIGRTD---EQVKLYGHRIELGEIEEN  
 CHN43A -----DG-----  
 CHN111A -----RLYKTGDLVKYD--S--SGHLHFVHRRD---GQVKLRGQRLELGEIERQ  
 CSN8A -----RLYRTGDLVRYD--S--AGHLHFVGRRD---TQVKLRGQRLELGEIERQ  
 G0091A -----RLYRTGDLVRYD--S--AGHLHFVGRRD---TQVKLRGQRLELGEIERQ  
 CSN4A ----GRQ--SRLYRTGDCRFD--E--NGVIHFEGRKD---TQLKISGQRLEASEVEYH  
 CH.NRPS10 -----RRSRLYRTGDMCRFD--Q--NGVIHFEGRKD---TQLKISGQRLEASEVEYH  
 CHN72A ---RPRDA--GRLYKTGDLVRKD--S--DGNMVYVGRKD---MQVKVNGQRLEIGDIESH  
 CSN6B ---QPTVN--RRFYKTGDLVKRI--E--DGNMVYVGRKD---RQVKITGQRLEIGDIESN  
 GLRG\_06838 ---RAGVD--RRFYKTGDLVKKT--E--DGNMVYVGRKD---MQVKINGQRLEIGDIESN  
 CHN87A -----  
 CH063\_1253 ---GPARGRHGRLYKTGDLVRYD--PDGTGSIAFVGRKD---QQVKLRGQRLELAEVEHH  
 GLRG\_08065 ---GPARGRHGRLYKTGDLVRYD--PDGTGSIAFVGRKD---QQVKLRGQRLELAEVEHH  
 CSN5 ---GPARGRHGRLYKTGDLVRYD--PDGTGSIAFVGRKD---QQVKLRGQRLELAEVEHH  
 CHN84A --PGHHRGRRGRLYKTGDLVHYS--AG-DGRLHFVSRMD---TQVKIRGQRVELGEVEHG  
 CHN85A GGGGGKAGRSGLTYKTGDLVRYD--R--EGSLEYIGRKD---TQVKVRGQRVELDEVAHV  
 CHN81A -AQTQKQPRRGRLYKTGDLVRYN--D--DGS�TFVGRKD---TQSKIRGQRLELAEVEYR  
 CH.NRPS8 AAAAAAPGRRGRLYKTGDLVRYE--A--DGS�GYLGRKD---TQVKIRGQRVELGEVEFH  
 CHN86A GGGRRRPGRRGRLYKTGDLVRYD--Q--DGNLCYVARKD---NQVKIRGQRMELGEIEAH  
 CHN83A SRRRSGR--GRLYKTGDLVCYD--G--SGKLTfVGRKD---TQVKIRGQRMELEEVELH

GLRG\_09842 VILES-----  
 CH030B\_PKS LES-----  
 CSP24 -----  
 GLRG\_09715 -----

CSP6 -----  
CH063\_0617 -----  
CH063\_0392 -----  
CS.P.NRPS -----  
GLRG\_10367 -----  
GLRG\_07434 -----  
CSP1 -----  
CSP10 -----  
CSP20 -----  
CH063\_0306 -----  
GLRG\_05027 IVKHLAVRDCAV---  
CH.NRPS1 IVKHLAVRDCAV---  
GLRG\_08225 LRRC-----  
CHN24A IAGLSGVRDSAV---  
CSN9D LAGLAGVRDSAV---  
G0043A LAGLTEVRDSAI---  
CSN9G MLGYAGIGDAVI---  
CH.NRPS2 MLSYAGVGDIV---  
CHN29A ML-----  
CSN9I MLGIDSVLDAAV---  
G0046A ILTISTVHDAAV---  
CSN9C -----  
CHN23A LLRHPAVRDAAI---  
CHN210A MLRHPVVRDAAV---  
G0047A MLRHPVVRDAAI---  
CS.NRPS MLRHAIVRDAAI---  
CSN9H MLCQPDVLDAAV---  
CHN28A MLGHPAVLDAAV---  
CHN22A VLAH-----  
CSN9B VLGHGAVLDAAA---  
GLRG\_00469 ILSQGGVLDAAA---  
G0044A LLNHADVLDAAV---  
CSN9E LVQHKEVLDAAV---  
CHN25A LLTHADVLDAAV---  
G0045A MLAQKAVLDAAV---  
CHN26A ILRHTAASDAAV---  
CSN9F ILRH-----  
CH.NRPS3 IRTRVPEISDVA---  
CSN1C IRTRVSEVSDVA---  
GLRG\_06542 IRTRVSEVSDVA---  
CHN32A LTTSGLATSSAV---  
CSN1B L-----  
G0652A L-----  
CSN1A AMR-----  
G0651A AMRTRGCHSAVA---  
CHN31A -----  
CHN41A LREASAFQAASV---  
CSN7 IRKS-----  
CHN62A ITSLCSSVRKVAV---  
CSN2A IRSNTSILES AV---  
CH.NRPS9 VRR-----  
CHN91A ILELTDVRGVVV---  
CH.NRPS5 ISHQKDIS-----  
CH.NRPS6 VVAC-----  
CHN21A LRKVLPGHLTPVAEAV  
CSN9A LRKALPKHLVPVAEAV  
G0041A LRKALPKHLVPVAEVV  
CSN4B LAKL-----  
CHN102A LAKL-----  
CH.NRPS7 LARC-----  
CSN6A LKQ-----  
G0681A LKQSLQVA-----

CSN2B LRNS-----  
 CH.NRPS4 LR-----  
 CH.NRPS11 LDNLSGIIQ-----  
 CSN8B LDNLPDVVRC-----  
 GLRG\_00920 MDNLPD-----  
 CSN2D IR-----  
 CHN44A IRQ-----  
 CSN2C IREI-----  
 CHN43A -----  
 CHN111A MALDPRVQHC-----  
 CSN8A LALNPSVQHC-----  
 G0091A LALNPSVQHC-----  
 CSN4A LRAAFNTTEIVV----  
 CH.NRPS10 LRAAFGVTDIVV----  
 CHN72A LSGC-----  
 CSN6B LAGCLGVNRGVV---  
 GLRG\_06838 LAGCLDVNLAVV---  
 CHN87A -----  
 CH063\_1253 LR-----  
 GLRG\_08065 LR-----  
 CSN5 LRGKMPPGVKIVA---  
 CHN84A LR-----  
 CHN85A VRDGLPGCEQAVV---  
 CHN81A LRESVPRLQQVAV--  
 CH.NRPS8 VRECVPSARQVAA---  
 CHN86A LRDCVVVSDA-----  
 CHN83A LR-----

### PART 3: TS protein sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

CH063\_0494 -----MGVIFY-LLHPSQLRAIIQWKV  
 GLRG\_07002 -----MGVIFY-LLHPNQLRSIIQWKV  
 CSUB\_TS\_8 -----MGIIFY-LLHPNQLRSIIQWKV  
 CH063\_0492 -----MNRTRIAGHGLRRLI  
 GLRG\_10556 -----MNRTRIAGHGLRRLV  
 CSUB\_TS\_12 -----MNRTRIASHGLRRLV  
 CH063\_01\_0 -----MAAKTFH  
 CH063\_0916 -----MISLQSLASF  
 CSUB\_TS\_17 -----MTAVLPFKDDEIH  
 CSUB\_TS\_15 -----MIES-PTAAADARAERRSKY  
 CH063\_0378 -----  
 GLRG\_02475 MGYDYALVHVKYTIPLAALLTVFSYPVFTRLDVVRTLFIIVTIAFVATIPWDSYLIRTNVW  
 CSUB\_TS\_2 MGYDYALVHVKYTIPLAALLTFFSYPVFTRLDVVRTLFIIVTIAFVATIPWDSYLIRTIW  
 CH063\_0666 -----  
 GLRG\_03139 -----  
 CSUB\_TS\_4 -----  
 CH063\_01\_3 -----MQLRSGAAVLRSTSSITSRSL  
 GLRG\_09287 -----MQLRQGAAVLRSTTSITSRSL  
 CSUB\_TS\_11 -----MQLRPGAAVLRSTTTTITSRSL  
 GLRG\_10302 -----MASTIENYQDCGPMRYKSSVPPASLYANTAYPSRFRPRISKHVD  
 CH063\_0097 -----MASTINNYQDCGPMRYKSSIPVPASLYENTAYPSKFRPRISKHVD  
 CH063\_0347 -----MLSEEELYPYSVPVDRETVVRS-GALTTLPVRIYKHDD  
 CSUB\_TS\_16 -----MEVRDGEAKSCGNHSHSVRIKNLISTAVLECIKVDRAGAMCMLEAYGKKWLR

CH063\_0282 -----  
 CH063\_1125 -----MLPATSTSSASSKTTNKSPASAPTPAP  
 GLRG\_03419 -----MLPTTPTLPTSKKAADKSRDL  
 CSUB\_TS\_5 -----MLPTTSTLSTSKKAADKSWNP  
 CH063\_0190 -----MDDIWKYSVDIDREVALSTGCFTSLAIRIHQRND  
 CH063\_1346 -----  
 GLRG\_00455 -----MPYFSLPIPESLIQQSGSHTRFATVRHCNYE  
 CH063\_0519 -----MASFSRPVPGAIVASS-GSRSQFPTYIHQNF  
 CSUB\_TS\_1 -----MPSFSRPVPDGISS-GLRSRFPYVHQNYE  
 GLRG\_07956 -----MASSVAPVQACLHPALSLQPRDNP  
 CSUB\_TS\_9 -----MSSSVVPAPVSLHPALSLQNSEKSSS  
 CH063\_0939 -----MA  
 GLRG\_03977 -----MCVDQQLFHSGVRGRADTYKKNK  
 CSUB\_TS\_6 -----MCVGRQLFHSGARSANTRKQKSK  
 GLRG\_02961 -----MISPSQLTPGAHDAAVAAGSPMFRMLGSLWG  
 CH063\_1495 -----  
 CSUB\_TS\_3 -----MISASQLTPGAHDAAVAASPLFRMLSSLWG  
 GLRG\_11695 -----MKDGLVHPSTSPRREPTLAI  
 CH063\_0640 -----  
 CSUB\_TS\_13 -----  
 CSUB\_TS\_14 -----  
 GLRG\_08962 -----  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----  
 GLRG\_06676 -----  
 CSUB\_TS\_7 -----

CH063\_0494 WHDPVHRRDPSKEDPTLTACFEHLNKTSRSFSA---VIQELNPELLVPVCLFYLVLRGL-  
 GLRG\_07002 WHEPVHRRDPSKEDPTLRACFEHLNKTSRSFSA---VIQELNPELLVPVCLFYLVLRGL-  
 CSUB\_TS\_8 WHEPVHRRDPSKEAPTLRACFEHLNKTSRSFSA---VIQELNPELLVPVCLFYLVLRGL-  
 CH063\_0492 QAPPRGTRGIVTEADVVKARKYCQSQLQHSYD---AHLISRLVPVRTADAYLALRSLN-  
 GLRG\_10556 KTPPRGTRGIVTDADVVKARKYCQSQLQHSYD---AHLISRLVPARTADAYLALRSLN-  
 CSUB\_TS\_12 KTPPRGTRGIVTDADVVKARKYCQSQLQHSYD---AHLISRLVPARTADAYLALRSLN-  
 CH063\_01\_0 NFPLLPDELREIWDKAVRRQGYRGVHYFSVFD---ATSYCTRVPVEEFTNEYLLARVS-  
 CH063\_0916 ILPLSTASPKTAFEAEPKQYNPANGIAGALF---KAKIHPREPEISAEVDSFFLDHWP  
 CSUB\_TS\_17 NGLSPIGQPLKNGGITKQKLLNGCRTIPLSSW---APVCHPLVDPVTAEVDWFLDNWA  
 CSUB\_TS\_15 HEADVVVVGAGVFGTAIAYALAQQGRSVILLER---WMKEPNRIVGELLQPGGIVALRQ-  
 CH063\_0378 -MSSVSLRVVEGTEGAMTEGIVSSVAREAXFFG---SLEIEEASFFLVTNMLIVFGLAA-  
 GLRG\_02475 TYPPDAVLGPTLYDIPAEELFFFIIQTYITAQL---YIILNKPVLHAQYLNSPATLPQW-  
 CSUB\_TS\_26 TYPPDAVLGPTLYDIPAEELFFFIIQTYITAQL---YIILNKPVLHAQYLNSPATLPQW-  
 CH063\_0666 MAKKTTLAEFESVFPKLEEVLEHAQKYKLPKE---ELAWYKKASIQPHQPAEVHRHR-  
 GLRG\_03139 MAKKTTLAEFESVFPKLEEVLEHAQKYKLPKE---ELAWYKKASIQPHRPEKLLHYSHH-  
 CSUB\_TS\_4 MAKKTTLAEFESVFPKLEEVLEHAQKYKLPKE---ELAWQAYSHTNPTSPATTTTTNT-  
 CH063\_01\_3 LSSTASRCAQCRRRIAPLEVRPLPLTSALYHSSSR---RSSAWGAAVSVASNMAANAVNRV-  
 GLRG\_09287 LSSSSSRCAQCRRATLTLRPLPLTSALYHSSSK---RSSAWGAAVSVASNMAANAVNRV-  
 CSUB\_TS\_11 LSSSSSRCPQCRSAPLDLRLPLPLTSALYHSSSK---RSSAWGAAVSVASNMAANAVNRV-  
 GLRG\_10302 VADKACWEACDDFENATGLKCLKADSVGCINPIGGNVNLFPEAIPERLHIISYLSSELL-  
 CH063\_0097 VADKACWEACDDFENATGLKCLKADSVGCINPIGGNVNLFPEAIPERLHIISYLSSELL-  
 CH063\_0347 LADAGAICLTGDWGRIMRDGQDKKSNVSPCVVG---NWG--SFIWPESIPDRMGLLCYL-  
 CSUB\_TS\_16 VMETYKSNEIDNIDDYFEARANNNGMGAYYAML---EFSLGIITDSEYDLMSEAIRHV-  
 CH063\_0282 -----MSPAIIIRPAAKVQLMDSKA-  
 CH063\_1125 TNNSSLPPSPNINPSRKSSIRQSSAVPVSFSA---HTSAPLPATSSKAPLRTVIEADW-  
 GLRG\_03419 TNNPSFPPPPNFIPSRKSSIGYTSTVP---G---SLAAAPTPTTSRVPFRPVLETDW-  
 CSUB\_TS\_5 TNNPSFPPPPNFIPSRKSSI-GHTSAVPVLSL---THAAAPTSSSRAPLRLVPETDW-  
 CH063\_0190 LADEATKLSIQDWGSHVGDGWERKSGSSWSPVG-----NWGAFFPESLPERLGVITYL-  
 CH063\_1346 -----  
 GLRG\_00455 ICTKAAEQVQAEFNQAMDTEIITNTVAPVPGIG---HLHAVAFITIPNCLPERLSVLTRF-  
 CH063\_0519 KCVEAAKETEHEYNQAMSTNIKSNTLAEIPGLG---LVHPMALTIANCLPDRLAAITRF-  
 CSUB\_TS\_1 MCVEAAKMEDEYNQTMSTNIKSRTLANVPGLG---LVHPMALTIANCLPERLAAITRF-  
 GLRG\_07956 GRNSQPQNSQGQTRAQILRQLHGQDVRIPDVGK---MMADWPRVQNMHLEDVNSRILEI-  
 CSUB\_TS\_9 RGNSSAKNSQGLTREQILWQLHGQNVQIPDLGK---LMADWPNDQSMHLEAVNSKILRI-

CH063\_0939 NTTADATNPKTJETQAMITALKGTAIRVFNLYG--ILKGWVPKANINYNKRLPIVIEST-  
 GLRG\_03977 DTNASKMSPKTQEARVISEALKGQTIHVFNLYS---ILKGWVPKANVNYERLVPVVEDA-  
 CSUB\_TS\_6 ERVAEKRSSKSQETRVIIIEALKGQAIHVFNLYS---IFKGWVPKANINYNERLVPVVEDA-  
 GLRG\_02961 MFSPKRTRSEDDLATAPERLASLVKPICTDMLK---GLR-YPGVVPQIKESVEALLQYM-  
 CH063\_1495 -----ML--EELSYPGVPPKLRKRESVEALLKYM-  
 CSUB\_TS\_3 LFAPKRIRLEDDLASAPDRLASLVKPICTDMLK---GMK-YPGAPQLKESVEALLKYM-  
 GLRG\_11695 DVELNDMAKSPVVTPTVGLRQRLSYLISEYLK---CINYTRPPKV---DQEALRDAL-  
 CH063\_0640 -----MSEPAVTPTVEGLRHHLSCLIPDFLK---CINYTQPPKA---DQDALREAL-  
 CSUB\_TS\_13 -----MTGSVFLQNAESPRQRLSILIPKFLK---SINYIPPSNV---NKQVLRQAV-  
 CSUB\_TS\_14 -----MPGPPVSQAAEGVRQRLSILIPKFLQ---SINYTPPLAVNKQELREAV-----  
 GLRG\_08962 -----MQKIVNACDFSIFCSVA-  
 CSUB\_TS\_10 ---MSTTNISTQVFPFDDSRHLHLSGGH---G---AVADWCSVFDSDGYLRSDELTSRQ-  
 CH063\_1011 -----  
 GLRG\_06676 -----MAVTQTESAQLASQLDGRVMVIPDLRR---MLSHWPSGRNIHAPDIEVLINDL-  
 CSUB\_TS\_7 -----MAVMQTESAQLASQLDGRVMIPDLGR---MLSHWPSGKNIHAPDIEVLINGL-

CH063\_0494 DTIEDDMTIDI----KEKEPLLRFQDQYMEQD--GWTFTKNGPNEKDRDLLV-----  
 GLRG\_07002 DTIEDDMTISI----EEKEPLLREFDQYMVKD--GWTFTKNGPNEKDRDLLV-----  
 CSUB\_TS\_8 DTIEDDMTIDI----KEKEPLLREFDQYMEKD--GWTFTKNGPNEKDRRELLV-----  
 CH063\_0492 LELVRLPELVS----NPAIGQMRMQFWRESID--KTFA-----  
 GLRG\_10556 LELVRLPEQVS----NPVIGQMRMQFWRESID--KTFA-----  
 CSUB\_TS\_12 LELVRLPEQVS----NPVIGQMRMQFWRDSID--KTFA-----  
 CH063\_01\_0 EKQLHVFGAPT----SSGNPSSPSWTIGNQST-----  
 CH063\_0916 FVNEEARKRFV----AAGFSKVTCTFYYPHALD-----  
 CSUB\_TS\_17 FVNEEARKRFV----AAGFSKVTCTFYYPHALD-----  
 CSUB\_TS\_15 LGLADALEGID----AVPCYGYKVSYHGEGVD--VPYPSFDENGHVIHPSAN-----  
 CH063\_0378 FDKAVAVCDFAF----PDKFDK-----  
 GLRG\_02475 IKSGKLVGQLA----LSGSVLLGTWLIACKGE--GTYLGLLIVWACTFALFT-----  
 CSUB\_TS\_2 IKSGKLVGQLA----LFGSVLLGTWLIACKGE--GTYLGLLIVWACTFALFT-----  
 CH063\_0666 RDDLPEPPGRPS----CWRSLLEINTLGGKCNRG-----  
 GLRG\_03139 HHHQHCRHHDN----DRCSLEINTLGGKCNRG-----  
 CSUB\_TS\_4 TTTDTPTTRLG----CR-SLEINTLGGKCNRG-----  
 CH063\_01\_3 IPKGDMDHIDPL----RTVAKEMKFLTGNIRKLLGSGHPSLDRVAKYYTQAE-----  
 GLRG\_09287 LPKGDMDHIDPL----RTVAKEMKFLTGNIRKLLGSGHPSLDRVAKYYTQAE-----  
 CSUB\_TS\_11 IPKGDMDHIDPL----RTVAKEMKFLTGNIRKLLGSGHPSLDRVAKYYTQAE-----  
 GLRG\_10302 FRHDDLTDVAITPEQFDEVHGPLARFLGSESKQSDHTTKHNAMNTMQRVAIE-----  
 CH063\_0097 FRHDDLTDVAITPEQFDEVHGPLARFLGSESKQSDHTTKHNAMNTMQRVAIE-----  
 CH063\_0347 LDVGCFFHDDAC----EEMTIA-AAHAHLLDLD--AAMDVEDNRSLSNDSRSA---KTKE  
 CSUB\_TS\_16 ERCMLLTNDYVW----SWPREREQAKHQETGKV--FNIVWFLMHTNKSWSRDD-----  
 CH063\_0282 HGNH-----  
 CH063\_1125 LARANYTSSNS----GNHPYSHKSHSHSHSQ--SRRPSFANQTTTSFTLTM-----  
 GLRG\_03419 LSRPRCASSR----THSRSQSSHRPRQPSLG--NHATSFTVTMQ-----  
 CSUB\_TS\_5 LSRPNCVFSSR----THSRSHSSHRPRRPSLGNQATSFTITMQPH-----  
 CH063\_0190 ANMGNIHDDLC----DDLTYEEALKEHNNLSQ---AMEISAIAPHQGPALDRSMKMKK  
 CH063\_1346 -----  
 GLRG\_00455 AEFTILNDDFY----DIAKNE-DIHKTNDDIQ----SVLNGAIESSRSIESNMTKSKQ  
 CH063\_0519 ADFTELNDYY----DSAKKE-EIQEVNDGIQ--SAIRGFSSPSALPTPSSSAFKAQK  
 CSUB\_TS\_1 ADFTELNDYY----DFAKKE-EIQALNDGIQ--SAIQDVSAAGIKSEASSTSTFKSKQ  
 GLRG\_07956 IETHAINDTVR----MRLTKAMLSSQ-----  
 CSUB\_TS\_9 IETHAVNDTVR----MRLTKAMLSSQ-----  
 CH063\_0939 FDRLVKPSQLR----EKYRKA-----  
 GLRG\_03977 FDRQSPNLREK----YRRAD-----  
 CSUB\_TS\_6 FDRQSPNLREK----YRRAN-----  
 GLRG\_02961 YKRAVEIGYPL----DTPISA-KAFRLGHSGLVNYLPRPSHYLTASHRRIV-----  
 CH063\_1495 YKRAVDIGYPL----DTPISA-KAFRLGYSLG-----  
 CSUB\_TS\_3 YERAVDIGYPL----DTPISA-KAFRLGYSGLVLRCHHLANSCHV-----  
 GLRG\_11695 LERGRQAGVHI----DPDDGSNMRFEAGLAVA-----  
 CH063\_0640 LERGRQAGVHV----EPEDGSNMRFEAGLAVA--AVCKPDGSMREIDK-----  
 CSUB\_TS\_13 LERGRQAGVDV----VQKDGSKMRFEAGIAVA-----  
 CSUB\_TS\_14 LERGRQSGVSV----DSDNGSVMRFETGISVA-----  
 GLRG\_08962 AP-----

CSUB\_TS\_10 V-----  
 CH063\_1011 -----  
 GLRG\_06676 LDKDDAPAQDI----ANIREA-----  
 CSUB\_TS\_7 LNKNGASPQDI----ANITEA-----  
  
 CH063\_0494 -----  
 GLRG\_07002 -----  
 CSUB\_TS\_8 -----  
 CH063\_0492 -----  
 GLRG\_10556 -----  
 CSUB\_TS\_12 -----  
 CH063\_01\_0 -----  
 CH063\_0916 -----  
 CSUB\_TS\_17 -----  
 CSUB\_TS\_15 -----  
 CH063\_0378 -----  
 GLRG\_02475 -----  
 CSUB\_TS\_2 -----  
 CH063\_0666 -----  
 GLRG\_03139 -----  
 CSUB\_TS\_4 -----  
 CH063\_01\_3 -----  
 GLRG\_09287 -----  
 CSUB\_TS\_11 -----  
 GLRG\_10302 -----ALEQDERLGKLVIEKWKGIVSVRGQDAFMEHKTLD SYMHVRHYDAGAYSVW  
 CH063\_0097 -----ALEQDERLGKLVIEKWKGIVSVRGQDAFMEHKTLD SYMHVRHYDAGAYSVW  
 CH063\_0347 LVSMILECVKVDRV GALRMLEAYRKKWLAIMETYNTEEIDN LDDYFFSRANNGGMGAYY  
 CSUB\_TS\_16 -----  
 CH063\_0282 -----  
 CH063\_1125 -----  
 GLRG\_03419 -----  
 CSUB\_TS\_5 -----  
 CH063\_0190 YISKCLLEAMNIDRPRALRMINTYRSKWLDVMERRDVNEIETLDEYLVFRNLNGGMEAFW  
 CH063\_1346 -----  
 GLRG\_00455 FQSSLILDMVNIDADLAMDIMTTYSKGL--DLATFAPDTLKTLD DYLPIRMVNSGLDVFQ  
 CH063\_0519 LQAGFMLELFSLDQEFALHIMSSYSQGL--DIATFAPDDLKTLD DYL PVR SINSGLDVTE  
 CSUB\_TS\_1 LQAALILDLMILDQEFAMDIMSSYSQGL--EIATFAPDDLKTLD DYL PVR SINSGLDVTA  
 GLRG\_07956 -----  
 CSUB\_TS\_9 -----  
 CH063\_0939 -----  
 GLRG\_03977 -----  
 CSUB\_TS\_6 -----  
 GLRG\_02961 -----  
 CH063\_1495 -----  
 CSUB\_TS\_3 -----  
 GLRG\_11695 -----  
 CH063\_0640 -----  
 CSUB\_TS\_13 -----  
 CSUB\_TS\_14 -----  
 GLRG\_08962 -----  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----  
 GLRG\_06676 -----  
 CSUB\_TS\_7 -----  
  
 CH063\_0494 -----  
 GLRG\_07002 -----  
 CSUB\_TS\_8 -----  
 CH063\_0492 -----  
 GLRG\_10556 -----

CSUB\_TS\_12 -----  
 CH063\_01\_0 -----  
 CH063\_0916 -----  
 CSUB\_TS\_17 -----  
 CSUB\_TS\_15 -----  
 CH063\_0378 -----  
 GLRG\_02475 -----  
 CSUB\_TS\_2 -----  
 CH063\_0666 -----  
 GLRG\_03139 -----  
 CSUB\_TS\_4 -----  
 CH063\_01\_3 -----  
 GLRG\_09287 -----  
 CSUB\_TS\_11 -----  
 GLRG\_10302 SQILFCCDISLTDEELTSLEPLTWLAFTQMILWHDYCSWDKEAATYLEREEGGSNMSAVQ  
 CH063\_0097 SQILFCCDMSLTDEELTGLEPLTWLAFTQMILWHDYCSWDKEAATYLEREEGGSNMSAVQ  
 CH063\_0347 AMLEFSLGIVVTDEEYEMMAAPIKHVERCMLLTNDYWSWPREREQAKTQEAGKVFNTVWF  
 CSUB\_TS\_16 -----  
 CH063\_0282 -----  
 CH063\_1125 -----  
 GLRG\_03419 -----  
 CSUB\_TS\_5 -----  
 CH063\_0190 SMVEFGMAMDVSDSDKTRIRPLFAAAESALVLTNDYWSWDREWRQAQQAESRIVNAVHL  
 CH063\_1346 -----  
 GLRG\_00455 TMSCFGMGIKLSADDKEKLSEFVNTAMFSTTLINDLHWPKEVKHHIEHPGSEYPFNAVA  
 CH063\_0519 DMACFGTGVRISRAEKEKLRKATDMAKYAITIVNDLYSWPKEIKCHLETPGSNPPFNAVA  
 CSUB\_TS\_1 DMACFGTGVRVSKSEKEKLRGIVDLTKFAITIVNDLYSWPKEIKCHLETPDSKPPFNAVA  
 GLRG\_07956 -----  
 CSUB\_TS\_9 -----  
 CH063\_0939 -----  
 GLRG\_03977 -----  
 CSUB\_TS\_6 -----  
 GLRG\_02961 -----  
 CH063\_1495 -----  
 CSUB\_TS\_3 -----  
 GLRG\_11695 -----  
 CH063\_0640 -----  
 CSUB\_TS\_13 -----  
 CSUB\_TS\_14 -----  
 GLRG\_08962 -----  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----  
 GLRG\_06676 -----  
 CSUB\_TS\_7 -----

CH063\_0494 -----  
 GLRG\_07002 -----  
 CSUB\_TS\_8 -----  
 CH063\_0492 -----  
 GLRG\_10556 -----  
 CSUB\_TS\_12 -----  
 CH063\_01\_0 -----  
 CH063\_0916 -----  
 CSUB\_TS\_17 -----  
 CSUB\_TS\_15 -----  
 CH063\_0378 -----  
 GLRG\_02475 -----WTITAHFLLAL  
 CSUB\_TS\_2 -----WTITAHFLLAL  
 CH063\_0666 -----  
 GLRG\_03139 -----  
 CSUB\_TS\_4 -----



CH063\_01\_3 -----  
 GLRG\_09287 -----  
 CSUB\_TS\_11 -----  
 GLRG\_10302 VYMAMYGLDQHAADKDFLLSEIGRIEDEY CERKAVYMAECSPAHHITHYIGLIELCMAGNT  
 CH063\_0097 VYMAMYGLDQYAAKDFLLSEIGRIEDEY CERKAIYMAECSPAHHITHYIGLIELCMAGNT  
 CH063\_0347 LMKQERC-SEAEAKLKVADMVAEEEARWVEAKRKIYQEHPD-LRADLVKFLENLHTALAG  
 CSUB\_TS\_16 -----AIVKVRKMVYEEEEQKWVEAKKSLYQKVPNLGRDAIKFLENLHTTLAG  
 CH063\_0282 -----  
 CH063\_1125 -----  
 GLRG\_03419 -----  
 CSUB\_TS\_5 -----  
 CH063\_0190 FMRTHGLAMHEAREAVRDRILAYEAE--YLRLKTEFY SQNPNVGADLKKYIEVCGVITAG  
 CH063\_1346 -----  
 GLRG\_00455 ILMRHGGLSEAEAFRLRLREKQAELEDKHLRLLSAL--EAAGPIPEHMLYLAAQYAAASG  
 CH063\_0519 VLMRHSGYSESEAFQALADKQAELEDKHLRLLVEALREQEGGSLPENQERYIANAQQAVSG  
 CSUB\_TS\_1 VLMRHGGYSESEAFKILCVKQAKLEEEHLRLLLEALRQQEGGRLPENQELYVANAQQAVSG  
 GLRG\_07956 -----  
 CSUB\_TS\_9 -----  
 CH063\_0939 -----  
 GLRG\_03977 -----  
 CSUB\_TS\_6 -----  
 GLRG\_02961 -----  
 CH063\_1495 -----  
 CSUB\_TS\_3 -----  
 GLRG\_11695 -----  
 CH063\_0640 -----  
 CSUB\_TS\_13 -----  
 CSUB\_TS\_14 -----  
 GLRG\_08962 -----  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----  
 GLRG\_06676 -----  
 CSUB\_TS\_7 -----

CH063\_0494 -----  
 GLRG\_07002 -----  
 CSUB\_TS\_8 -----  
 CH063\_0492 -----  
 GLRG\_10556 -----  
 CSUB\_TS\_12 -----  
 CH063\_01\_0 -----  
 CH063\_0916 -----  
 CSUB\_TS\_17 -----  
 CSUB\_TS\_15 -----NETTSSSAKQKEGRSFHHGRFIMNL-----  
 CH063\_0378 -----  
 GLRG\_02475 PLACTALPILLPTVYLWIVDEMALGRGTWAIES-----  
 CSUB\_TS\_2 PLACTALPILLPTVYLWIVDEMALGRGTWAIES-----  
 CH063\_0666 -----  
 GLRG\_03139 -----  
 CSUB\_TS\_4 -----  
 CH063\_01\_3 -----KHMRLIVLLMSRATALCPKGPRLH-----  
 GLRG\_09287 -----KHMRLIVLLMARATALCPKGPRLH-----  
 CSUB\_TS\_11 -----KHMRLIVLLMARATALCPKGPRLP-----  
 GLRG\_10302 LWHLSSRRYDPAAPLPRREDIGKVNQVPLDVSE-----  
 CH063\_0097 LWHLSSRRYDPAAPLPRREDIGKVNVRGPLDVFE-----  
 CH063\_0347 NDYWSSQCYRHNDWAHVPEQPSENHPKVHELAAALGRAVMPAECSSGSLSNAPKSEELGGV  
 CSUB\_TS\_16 NDYWSSQCYRHNDWKHVPELPGKCAPRVNDLVG-----  
 CH063\_0282 -----  
 CH063\_1125 -----QPPPPDPERYATSDLNYTNKTWPAAK-----  
 GLRG\_03419 -----PPPPDPERYATSDLNFTRKTWPEEK-----  
 CSUB\_TS\_5 -----PPPPDSRYATSDLNFTRKTWPEEK-----

CH063\_0190 NHYWCANCPRHHSWRDQDAPLDPIGRRLSVSSE-----  
 CH063\_1346 -----  
 GLRG\_00455 SEFWSVHVPRYPYPSKKDLAQPDVEFVNGEFLYRT-----GPATTDIDSIPRILAKGT  
 CH063\_0519 SELWSVFTTRYPSKADLQPPVEFVDGKLRYS-----  
 CSUB\_TS\_1 SELWSIFTRYPSKADLQQAEEVFMMDGKFGYVS---EGRKVDGVEPGTNTTASTELEDQL  
 GLRG\_07956 -----  
 CSUB\_TS\_9 -----  
 CH063\_0939 -----  
 GLRG\_03977 -----  
 CSUB\_TS\_6 -----  
 GLRG\_02961 -----  
 CH063\_1495 -----  
 CSUB\_TS\_3 -----  
 GLRG\_11695 -----  
 CH063\_0640 -----  
 CSUB\_TS\_13 -----  
 CSUB\_TS\_14 -----  
 GLRG\_08962 -----  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----  
 GLRG\_06676 -----  
 CSUB\_TS\_7 -----  
  
 CH063\_0494 -----  
 GLRG\_07002 -----  
 CSUB\_TS\_8 -----  
 CH063\_0492 -----  
 GLRG\_10556 -----  
 CSUB\_TS\_12 -----  
 CH063\_01\_0 -----  
 CH063\_0916 -----  
 CSUB\_TS\_17 -----  
 CSUB\_TS\_15 -----RKACQK  
 CH063\_0378 -----  
 GLRG\_02475 -----GTKLELQLFGSLEIEEATFFLVNMLIVFGIAA  
 CSUB\_TS\_2 -----GTKLELQLFGSLEIEEATFFLVNMLIVFGIAA  
 CH063\_0666 -----  
 GLRG\_03139 -----  
 CSUB\_TS\_4 -----  
 CH063\_01\_3 -----  
 GLRG\_09287 -----  
 CSUB\_TS\_11 -----  
 GLRG\_10302 VSKPVDASGSEGILTPASSCLGTKRLRPFWNNQRTEYTTMTPAETNSDH--KKKAKASHE  
 CH063\_0097 VSKPVDSSSESDGILTPVSSRLGTKRLRPFWNNQRTEYTTMTPETSSDHKKKKKAKASHE  
 CH063\_0347 IAESDLDFEANAASLLSFLDKASPQDQVVEVVGSSASASEANTQSTAELSATLRSNSVS  
 CSUB\_TS\_16 LGRALLRNESDLDDADNVAATVHSHKSSPGSPAFHDGQSNHHSSTLSTHSQSSCTTPPSP  
 CH063\_0282 -----  
 CH063\_1125 -----  
 GLRG\_03419 -----  
 CSUB\_TS\_5 -----  
 CH063\_0190 -----DTVEDDCATSPGATTSSMSQKSSPTTEMTLSE  
 CH063\_1346 -----  
 GLRG\_00455 VATERAGLTNGHTNGSINGSSNGSSNGFSNGITNSHTNGLTNGLANGHANGHARHHTNGN  
 CH063\_0519 -----SSSSACENKSHSTGDLATIE TTCDSQSITACPSEKEYKRTKAL  
 CSUB\_TS\_1 ASLEVASIDEASFTPSISSEPGCDDKDDIKRDAIHEEIQDSQSITIPRQGHGHGRMKAL  
 GLRG\_07956 -----  
 CSUB\_TS\_9 -----  
 CH063\_0939 -----  
 GLRG\_03977 -----  
 CSUB\_TS\_6 -----  
 GLRG\_02961 -----

CH063\_1495 -----  
 CSUB\_TS\_3 -----  
 GLRG\_11695 -----  
 CH063\_0640 -----  
 CSUB\_TS\_13 -----  
 CSUB\_TS\_14 -----  
 GLRG\_08962 -----  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----  
 GLRG\_06676 -----  
 CSUB\_TS\_7 -----

CH063\_0494 -----HFDDVITEMKKIKKPYDIIKIDITIKMGNGMADYAKNAAFNEN-  
 GLRG\_07002 -----HFDDVITELKKIKKPYFDVIKIDITIKMGNGMADYAKNAAFNEQ-  
 CSUB\_TS\_8 -----HFDDVIAELKKIKKPYDVIKIDITIKMGNGMADYAKNAAFNEQ-  
 CH063\_0492 -----GNPPAEPICLLLHQ--ALQDLRARSTSGTASSIKFWV-----  
 GLRG\_10556 -----GKPPAEPICVLLHQ--ALQDLRARSTASTASSIKFWV-----  
 CSUB\_TS\_12 -----GNPPAEPICVLLHQ--ALQDLRARSTTSTASSIKFWV-----  
 CH063\_01\_0 -----YAIDAGLWTACRESRAAMYRRYTPEKWANWYTEP-----  
 CH063\_0916 -----DRISLACRLLTLLFL-----  
 CSUB\_TS\_17 -----DRIAFACRLLAILF--LIDNAAGLGWAGLGWTGLDWADNQLT  
 CSUB\_TS\_15 QKNITIFETEVTATIRGDDKDTVLGVRSKTTD--PATGEKKDDF--FFGQLTHIADGY--  
 CH063\_0378 -----PADALSMSLLRARVF--PSSKYDMQRILGIRQAVARLAKKSRS  
 GLRG\_02475 FDKAVAVCDAPPEKFDKPADALMSLLRARVF--PSSKYDMQRILGIRQAAARLAKKSRS  
 CSUB\_TS\_2 FDKAVAVCDAPPENFDKPADALMSLLRARVF--PSSKYDMQRILGIRQAVARLAKKSRS  
 CH063\_0666 -----MSVPDSVSLLLDKPLNEEEYFQAATLGWMTELLQAFFLV  
 GLRG\_03139 -----MSVPDSVSYLLGKPLNEEEYFQAATLGWMTELLQAFFLV  
 CSUB\_TS\_4 -----MSVPDSVSLLLDKPLSEEEYFQAATLGWMTELLQAFFLV  
 CH063\_01\_3 -----SGQAIGGVDTAISPPNILVDVN--PSSPLTSAPEPAEVDSDILPSQRR-  
 GLRG\_09287 -----SAQPMGGVDTAISPPNILVDVN--PSSPLTSPAPEPTEVDSILPSQRR-  
 CSUB\_TS\_11 -----SGQPIGGVDTAISPPNILVDVN--PSSPLTSPAIEPTEVDSILPSQRR-  
 GLRG\_10302 TREDLLTVSPCAWPAELDEKNILAPYLYTAAR--PASGARDKLM DALDNWYRVPPGALA-  
 CH063\_0097 TRADLLTVSPCAWPAEPDEKDILAA YLYTAAR--PASGARDKLM DALDNWYRVPPNALA-  
 CH063\_0347 SA-GSASTSDETKARVSDTDIISAPIQYVQSL--PSKGFRTTLIDCLNRWLEVPQQEME-  
 CSUB\_TS\_16 RSPAINPHLPCSSTSGFAKSPVTGPIDYIRSL--PSKKVRTQLIDSLNIWFVPHSALS-  
 CH063\_0282 -----EEIIRAPLNYLLDL--PGKEVRSKLSAFNQWLRIPPEKLE-  
 CH063\_1125 -----ERVVAGPFDYLSAL--PGKDFRAQLIQAFNVWLEVPQESID-  
 GLRG\_03419 -----EKVIAGPFDYLSAL--PGKDFRSQLIQAFNVWLEVPQESID-  
 CSUB\_TS\_5 -----DKVITGPFYLSAL--PGKDFRSQLIQAFNAWLEVPQESID-  
 CH063\_0190 VLSFTTISGXEQPQRSLSDSPLRQPCQYIRSM--PSKGLRHLMAEALDQWLLVDNASLD-  
 CH063\_1346 -----  
 GLRG\_00455 SKEEETLAVKYPEDVKCRSELVLPYKYLTSM--PSKGIRELFIRALNWWLKV PDDKLA-  
 CH063\_0519 LPDGSDLSTYASRVA AAPDHPVMAPFKYIASL--PSKGV RDTFIDALNWWLKV PDDSL-  
 CSUB\_TS\_1 LPDGPEFPTYASRVA AAPDHA AVAPFKYIASL--PSKGIRD TFI DALNWWLAVPEDSLR-  
 GLRG\_07956 -----LAGWYPYASCE-----RIKALTSFQAWMFIIDMLDQ  
 CSUB\_TS\_9 -----LAGWYPYASCD-----RIEALTSFQAWMFIIDMLDQ  
 CH063\_0939 -----NYARFVSLYYPH--PKWD-QVRILALYIIWLF CWDDAIDQ  
 GLRG\_03977 -----YARFVSLYYPH--PEWD-RVQTLALYIIWLF CWDDAIDQ  
 CSUB\_TS\_6 -----YARFVSLYYPH--PEWN-QVRILALYIIWLF CWDDAIDQ  
 GLRG\_02961 -----YPLPRKTSQLLTRQLCH--PNHPTEVQGYVGLFTWL VVQYDDIV-  
 CH063\_1495 -----LARLCH--PGHPTEVQGFVGLFTWL VVQYDDIV-  
 CSUB\_TS\_3 -----VVYPSKTSQLLTSRQLCH--PNHPTEVQGFVGLFTWL VVQYDDIV-  
 GLRG\_11695 -----AMEY--PLHPFNTQLHIGLFTWLGFIIDDLN-  
 CH063\_0640 -----HELFRLLT VQEMY--PLHPFDIQVHIGLFTWLGFIIDDLN-  
 CSUB\_TS\_13 -----AWHCMRLTLVKDMY--PLHPLEIQVHIGLFTWLGFIIDDLN-  
 CSUB\_TS\_14 -----ADMY--PLHPFEVQVHIGLFTWLGFIIDDLN-  
 GLRG\_08962 -----DAPEGRLQTMCDWGNWVFPFDDMFD-  
 CSUB\_TS\_10 -----  
 CH063\_1011 -----MVLWQ GKLD-  
 GLRG\_06676 -----NPTLLAS----SLWPDVSKGKLGTLTFMVLWQGRLD-  
 CSUB\_TS\_7 -----NPTLLASSGANS LWPVNSKGKLGTLTLMVLWQGRLD-

CH063\_0494 -----GVATVKEYELYCHYV-----AGLVGDGLTRLFVEGNMANPKLLERP  
 GLRG\_07002 -----GVATIKEYELYCHYV-----AGLVGDGLTRLFVEGNMANPKLLERP  
 CSUB\_TS\_8 -----GVATIKEYELYCHYV-----AGLVGDGLTRLFVEGNMANPKLLERP  
 CH063\_0492 -----QRLIRTRERHMDNRPYASLAALE  
 GLRG\_10556 -----QRLIKTREKHMDNRPYASLAALE  
 CSUB\_TS\_12 -----QRLIKTREKHMDNRPYASLAALE  
 CH063\_01\_0 -----FTSFLEHMALRPGNRELPAAFKI  
 CH063\_0916 -----VDDILEDMSLQEGSLYNEKLILL  
 CSUB\_TS\_17 -----PQDLEHMSLEEGKAYNKRMLPL  
 CSUB\_TS\_15 -----ASKFRKEYLPQAPVV-----KSKFYALELIDAPMPSP--GFGH  
 CH063\_0378 FHLASSVFPGRRLRIDLTLLYSYCR-----ADDLVDDAESSEEAAWISKLDR  
 GLRG\_02475 FHLASSVFPGRRLRIDLTLLYSYCR-----ADDLVDDAATPEEAAVWISKLDR  
 CSUB\_TS\_2 FYLASSVFPGRRLRIDLTLLYSYCR-----ADDLVDDAATPEEAAWISKLDR  
 CH063\_0666 -----SDDIMDSSITRRGKPCWYRHEGV  
 GLRG\_03139 -----SDDIMDTSITRRGKPCWYRHEGV  
 CSUB\_TS\_4 -----SDDIMDTSITRRGKPCWYRHEGV  
 CH063\_01\_3 -----LAEITELIHTASLL-----HDDVIDHSVSRRGSPSANLEFGN  
 GLRG\_09287 -----LAEITELIHTASLL-----HDDVIDHSVSRRGSPSANLEFGN  
 CSUB\_TS\_11 -----LAEITELIHTASLL-----HDDVIDHSVSRRGSPSANLEFGN  
 GLRG\_10302 -----TIRTIVRIMHNASLM-----LDDVQDNSPVRRGSPSAHVVFGT  
 CH063\_0097 -----TIRTIVRIMHNASLM-----LDDVHDNSPMRRGSPSAHVVFGT  
 CH063\_0347 -----CIKKVINSLHDSSLI-----LDDIEDGAKLRRGFPATHVVYGT  
 CSUB\_TS\_16 -----VIKEVVDCLHDSSLI-----LDDVEDGSDLRRGFPATHVVYGT  
 CH063\_0282 -----VIKRIVMLLHNASLL-----LDDIQDSSTLRRGLPVSHSIFGI  
 CH063\_1125 -----VITNVVGMMLHTASLL-----IDDVEDSSSLRRGLPVAHNIFGV  
 GLRG\_03419 -----VITNVVGMMLHTASLL-----IDDVEDSSSLRRGLPVAHNIFGV  
 CSUB\_TS\_5 -----VITNVVGMMLHTASLL-----IDDVEDSSSLRRGLPVAHNIFGV  
 CH063\_0190 -----RIKNIIDLLHNSSLI-----LDDIEDDSPLRRGLPATHMVFR  
 CH063\_1346 -----MDDFQDDSPRRGKVA AHTIFGP  
 GLRG\_00455 -----CIQDVVSYLHQSSLM-----LDDIEDGSKLRRGQPSTHAVYGI  
 CH063\_0519 -----SIKTIVSMLHDSSLMQ-----VEILDDIEDDSTLRRGSPAHTIYGT  
 CSUB\_TS\_1 -----SIKTIVSMLHDSSLIPEGLKTDKGVTRLDDIEDDSSLRRGSPAAMIYGT  
 GLRG\_07956 Y-----SIVEKFDLHSLHVLADC-----RDFVERSLDVANTMEKAPRYKE  
 CSUB\_TS\_9 Y-----SLVEKFDLHSLHVLADC-----RDFVERSLKVSNTTENASMQYKD  
 CH063\_0939 Q-----GTGDLSDILHAKAH-----RDNTIRVLEHFLGLAPPKTKLSV  
 GLRG\_03977 Q-----GTVDLSDNLLRAKTR-----RDNTIRVLEYVLGLAP--KFGS  
 CSUB\_TS\_6 Q-----GTGDLSDNLLRAKTR-----RDNTIRVLEHVLGLDN--KFDS  
 GLRG\_02961 -----GQNDEMAE-----AQLFQERFFKGEKQPNAMLE--  
 CH063\_1495 -----GQNDEMAE-----AQLFQKRFFNGETQPNAMLE--  
 CSUB\_TS\_3 -----GQNDEMAE-----AQLFQERFFRGETQPNAMLEG--  
 GLRG\_11695 -----ADLQTD-----LDNFQSRFFRGDPQPCAILQ--  
 CH063\_0640 -----AELGSD-----LDNFQSRFFRGDTQPCVILQ--  
 CSUB\_TS\_13 -----TVLQKD-----LEQFQSRFHRGEEQPCALLQ--  
 CSUB\_TS\_14 -----TEFGTD-----LEQFQSRFHCGEKQPGPVLEC--  
 GLRG\_08962 -----SGHLRSLETSRQVLGSLMSNML  
 CSUB\_TS\_10 -----LDSLMSNMLGKRCYTGTCL--  
 CH063\_1011 -----DYIEALEYQS-----QAKSREFRFRVKEYIAQYLELSE  
 GLRG\_06676 -----DYIEALEYES-----QGKAKEFRSNAKGYIAHYLQLSE  
 CSUB\_TS\_7 -----DYIEALEYES-----QAEAKQFRSNAKEYIAQYLQLSE

CH063\_0494 ELTESMGQFLQ--KTNIIRDVHED-----YLDKRRFWPKEIW-----GK  
 GLRG\_07002 ELTESMGQFLQ--KTNIIRDVHED-----YLDKRRFWPKDIW-----SK  
 CSUB\_TS\_8 ELTESMGQFLQ--KTNIIRDVHED-----YLDKRRFWPKDIW-----SK  
 CH063\_0492 EYAENTYSTLM--Y---ATLAAM-----PLRSMHVDHL-----AS  
 GLRG\_10556 EYAENTYSTLM--Y---ATLAAM-----PLRSMHVDHL-----AS  
 CSUB\_TS\_12 EYAENTYSTLM--Y---ATLAAM-----PLRSMHVDHL-----AS  
 CH063\_01\_0 VEGDNSQYFTV--LPFYNLFLVQF-----SSFQPYFKSLGKEMPFSSR-----KF  
 CH063\_0916 SRGDIVPDRSVPVEWITYDLWNL-----RTCDKSLADEILEPVF-----SF  
 CSUB\_TS\_17 ARGDAQPDRTIQVEAMFYNLWASM-----REKDQHLADQVLEPTF-----VF

CSUB\_TS\_15 VVIGKAFPVLM--YQIGTHETRALIDVPANIPASP---AAGGVRGYIKNVVMPTLPPQM  
 CH063\_0378 HLSLLYKDPDAVSAPLAAKYAAEN-----FPA--SALSALDMLPASLLPREPLAEL  
 GLRG\_02475 HLSLLYKDPDATSTPLASKYAAEN-----FPP--SALSALDMLPTSLLPREPLAEL  
 CSUB\_TS\_2 HLSLLYKDPDATSAPLASKYAAEN-----FPP--SALSALDMLPTSLLPREPLAEL  
 CH063\_0666 GMVAINDAFLL--EAAIYTLLRKF-----FREHASYVDLLELFHEVTF-----QT  
 GLRG\_03139 GMVAINDAFML--EAAIYTLLRKF-----FREHASYVDLLELFHEVTF-----QT  
 CSUB\_TS\_4 GMVAINDAFML--EAAIYTLLRKF-----FREHASYVDLLELFHEVTF-----QT  
 CH063\_01\_3 KMAVLAGDFLLGRASVALARLNS-----EVIELLATVIA-----NL  
 GLRG\_09287 KMAVLAGDFLLGRASVALARLRNA-----EVIELLATVIA-----NL  
 CSUB\_TS\_11 KMAVLAGDFLLGRASVALARLRNA-----EVIELLATVIA-----NL  
 GLRG\_10302 AQTNSASYLMIKCVDLARRLGN-----TLSCLLSELG-----QL  
 CH063\_0097 AQTNSASYLMIKCVDLARRLGED-----TLSCLLSELG-----QL  
 CH063\_0347 SQAINSATFLY--VQAV-EAIHEL-----ENK----EMMDVLLGHLK-----QL  
 CSUB\_TS\_16 GQAVNSATFLY--VQAV-EAVHRL-----VKEGGGRLELMDMLLASLK-----EL  
 CH063\_0282 AQTINAANYAF--FLAQQEIPKL-----EDP----RAFEVFTTEELL-----NL  
 CH063\_1125 AQTINSANYIY--FCTLQELQRL-----KNP----KTISIFAEELV-----HL  
 GLRG\_03419 AQTINSANYIY--FRAL-QEVQRL-----KNP----KAITIFAEELV-----HL  
 CSUB\_TS\_5 AQTINSANYIY--FRALQELQRL-----KNP----KAITIFAEELV-----HL  
 CH063\_0190 AQSINTANYMFVQAVQQAQTLNSP-----ACLGTLLEGELE-----CL  
 CH063\_1346 AQAINASTYTIVKAINQVAEFRSQ-----SCVHETTEKIM-----AL  
 GLRG\_00455 GQTINSANFVF-----VQAFARM-----QSLGHGRAEAIDIFIDEVE-----NL  
 CH063\_0519 AQCINAANYMV--VMVLVEIQKL-----RSP----RKLDELSEELE-----NL  
 CSUB\_TS\_1 AQCINAANYMV--VMVLAELQKL-----RSP----AKTSILIEELE-----SL  
 GLRG\_07956 HDAVVSFYEYAQAVCKSYSDNRPY-----RARIAKEAV-----AT  
 CSUB\_TS\_9 YDAVVSFEYEAQAVCKSYSDNRPY-----RARIAKEAI-----VT  
 CH063\_0939 ELQANANPELKMIGDKLQMGYSLEQ-----RQTFMTQMRRYID-----NC  
 GLRG\_03977 LTQFSQADYEL--KVIQDELKKA-----YTQ-EQRQVFMSQMRRYIG-----NC  
 CSUB\_TS\_6 DIEFAQADYEL--KVIQDELKKA-----YTQ-EQRQVFMNQMRRYIG-----NC  
 GLRG\_02961 ----GLASLM-----REAPRW-----FDP----VMANLLQISTL-----KF  
 CH063\_1495 ----GLAGLL-----REAPRL-----FDP----VMANLLQISTL-----KF  
 CSUB\_TS\_3 ----LANLMREAPRW-----FDP----VLANLLQISTL-----KF  
 GLRG\_11695 ----CFASVL-----RSMTDY-----YDP----VVANLIVLSAL-----AF  
 CH063\_0640 ----CFASVL-----RSTTDY-----YDP----VVANLIVLSAL-----AF  
 CSUB\_TS\_13 ----CFANLL-----RSTTDF-----YEP----VVANLIVLSAL-----TF  
 CSUB\_TS\_14 ----FANVLRSTTDH-----YEP----VVANLIVLSAL-----AF  
 GLRG\_08962 GKGCIDIGTKLP-VVQVHDDIFRRL-----SEVGLTYAGVQRRRLAKSME-----LY  
 CSUB\_TS\_10 -PVVQAHNDIFRRLSEVGVTLRCL-----EDYAGVQRRRFARSME-----LY  
 CH063\_1011 GPKEPATSSIISTFQPVARMICQQ-----YNK----DQRRRLKASLF-----EY  
 GLRG\_06676 RPTPEAMTPIISDFQPVAKMICQQ-----YNK----DQRRKLKTSLF-----EY  
 CSUB\_TS\_7 GPTEPAMTSVISDFQPVAKMICQQ-----YDK----DQRRKLKASIF-----EY

CH063\_0494 HVDTWDDLF--KPENQRKALDCSSEMVLNALKH-----AEECL-F  
 GLRG\_07002 HVDTWDDLF--KPENQRKALECSSEMVLNALKH-----TEECL-F  
 CSUB\_TS\_8 HVETWDDLF--KPENQRKALECSSEMVLNALKH-----TEECL-F  
 CH063\_0492 HIGKACGIVAVLR-GI--PVLAAPQQ-QLAKSHA-----GPGGG-R  
 GLRG\_10556 HIGKACGIVAVLR-GI--PVLAAPQQ-QPAKSHA-----GPGGG-R  
 CSUB\_TS\_12 HIGKACGIVAVLR-GI--PVLAAPQQ-QPAKSHA-----GPGGG-R  
 CH063\_01\_0 GLEGIPHIAVEF-----DPSWTLEE-LDQHE-----KDSGWEW  
 CH063\_0916 MRAQTDKSR-----LSIKQLGH-YLKYRE-----KDVGK-A  
 CSUB\_TS\_17 MRAQTEGIR-----TEITELGQ-YLQYRER-----DVGKAWN  
 CSUB\_TS\_15 RPCFEAALA----DG--KIPPSMPNSYLPASRQ-----TAKGM-L  
 CH063\_0378 LKGFEMDLS--FDSST--FPITDPED-LELYAA-----RVASTVG  
 GLRG\_02475 LKGFEMDLS--FSNSA--FPITDPED-LELYAA-----RVASTVG  
 CSUB\_TS\_2 LKGFEMDLS--FSNSA--FPITDPED-LELYAA-----RVASTVG  
 CH063\_0666 ELGQLCDLLTAPEEQVN--LDNFSMEK-YRFIVVY-----KTAYYSF  
 GLRG\_03139 ELGQLCDLLTAPEDKVN--LDNFSMEK-YSFIVY-----KTAYYSF  
 CSUB\_TS\_4 ELGQLCDLLTAPEDKVN--LDNFSMEK-YSFIVY-----KTAYYSF  
 CH063\_01\_3 VEGEFMQLKNTEQDER--RPVWSEET-LTYYLQKTYL-----KTASLIS  
 GLRG\_09287 VEGEFMQLKNTERDER--RPVWSEDA-LAYYLQKTYL-----KTASLIS  
 CSUB\_TS\_11 VEGEFMQLKNTERDER--RPVWSEEA-LTYYLQKTYL-----KTASLIS  
 GLRG\_10302 HLGQSHDLAWTFH-----CRAPSMPEYYSHLEQ-----KTGGL-F

CH063\_0097 HLGQSHDLAWTFH-----CRAPSLPEYYSHLEQ-----KTGGL-F  
 CH063\_0347 FCGQSLDLY--WT-FN--RRCPTETE-YLDMIGQ-----KTGAL-L  
 CSUB\_TS\_16 FQGQSCDLY--WT-HH--RICPTEKE-YLYMVDR-----KTGAM-M  
 CH063\_0282 HRGQGMADIY--WR-DA--SICPTEEE-YFTMVS-----KTGGL-F  
 CH063\_1125 HRGQGMDLH--WR-DT--LTCPTTEED-YLEMVGN-----KTGGL-F  
 GLRG\_03419 HRGQGMDLF--WR-DT--LTCPTTEED-YLEMVGN-----KTGGL-F  
 CSUB\_TS\_5 HRGQGMDLF--WRDT--LTCPTTEED-YLEMVGN-----KTGGL-F  
 CH063\_0190 FIGQSWDLYWKFH----LQIPTEKE-YFAMVDS-----KTGAM-F  
 CH063\_1346 FQGQAMDLFWTYN----SRCPTMNE-YIRMVDN-----KTGQL-F  
 GLRG\_00455 HKGQSYDLF--WK-DQ--VHCPVDE-YFMMIDN-----KTGGL-F  
 CH063\_0519 FLGQSEDLF--WK-YQ--VECPTTEE-YMEMIEN-----KTGGL-F  
 CSUB\_TS\_1 FLGQSEDL--WK-YQ--VECPSTDE-YMEMIDNSKRTDQTVYIQRANGIDSETGGL-F  
 GLRG\_07956 LDGYQAEALNRYA----GRIPTEE-YLGYRQ-----ASSCI-M  
 CSUB\_TS\_9 LDGYRQEALNRYA----GRVPTLEE-YLGYRE-----ASSCI-M  
 CH063\_0939 HEEQKMLRQ-----GTLPDIES-YSELRH-----GTA AV-W  
 GLRG\_03977 HEEQTMRLQ-----GTLPSIES-YSELRH-----GTA AV-W  
 CSUB\_TS\_6 HEEQAMRLQ-----GTLPGIDS-YSELRH-----GTA AV-W  
 GLRG\_02961 LTCNLLERHKGFM-NM--NITRAGVK-FPDFVR-----DLSGINV  
 CH063\_1495 LTCNLLERHDGFR-NM--PVTRSGVK-FPDFLR-----DLSGI-N  
 CSUB\_TS\_3 LTCNLLERHKGFL-NM--PVTRAGVK-FPDFVR-----DLSGINV  
 GLRG\_11695 VNSNAIELRREYQ-TI--ALTKDALS-WPYYFR-----DKEGL-P  
 CH063\_0640 VNSNAIELRREYQ-TI--ALTREALS-WPYYFR-----DKEGL-P  
 CSUB\_TS\_13 VNCNAIELRHDIYQ-TM--IPTKETVN-WPYYFR-----DMEGL-P  
 CSUB\_TS\_14 VNCNAIEPRDYQ-AI--IPTKQTAN-WPYYFR-----DKEGL-P  
 GLRG\_08962 AEGVARHIVETFTS----HRISLQD-MLQTRR-----LSVGV-A  
 CSUB\_TS\_10 AEGVARHIETFTS----HPPSLQY-MLQTRR-----LSVGV-A  
 CH063\_1011 IDSTVQETRYVES----GEVPTDLK-YEMLRK-----KTGGT-G  
 GLRG\_06676 IDSTVQEIR--YI-DS--GEVPTDLK-YDMLRK-----RTAGT-G  
 CSUB\_TS\_7 IDSTVQEMR--YI-DS--GEVPTDLK-YDMLRK-----KTAGT-G

CH063\_0494 YMAGIKDQSVFNFAIPQSMIAITLLEL VFRNPAIFSS-----HIKITKGDACQLMSDS  
 GLRG\_07002 YMAAIKDQSVFNFAIPQSMIAITLLEL VFRNPAIFNS-----HIKITKGDACQLMSSES  
 CSUB\_TS\_8 YMAAIKDQSVFNFAIPQSMIAITLLEL VFRNPAIFNS-----HIKITKGDACQLMTES  
 CH063\_0492 QDPALL-----LPLDVMAEANLRE-----EDVFRY  
 GLRG\_10556 QDPALL-----LPLDVMAEANLRE-----EDVFRY  
 CSUB\_TS\_12 QDPALL-----LPLDIMAEASLRE-----EDVFRY  
 CH063\_01\_0 KYAHVY-----HPRGAKEEGEPMQ-----PLSHERYAEYDSL  
 CH063\_0916 LLSAL-----MRFSMKLHITPAE-----LKSVDNIEN  
 CSUB\_TS\_17 RLLSAL-----MRFAMDLHLSGEE-----LAEMKEVEQN  
 CSUB\_TS\_15 LLGDASN-----MRHPLTGGGMTVA-----FNDCVILSDL  
 CH063\_0378 QACLEL-----VFRHCHHGL-----PDYMQAYLRN  
 GLRG\_02475 QACLEL-----VFCHCQHGL-----PDYMKAYLRN  
 CSUB\_TS\_2 QACLEL-----VFGHCQHAL-----PDYMQAYLRN  
 CH063\_0666 YLPVA-----LALHCLNIATPKN-----LKQAEDILIP  
 GLRG\_03139 YLPVA-----LALHCLNIATPKN-----LKQAEDILIP  
 CSUB\_TS\_4 YLPVA-----LALHCLNIATPKN-----LKQAEDILIP  
 CH063\_01\_3 KSC-----RASAILGGSDAAS-----VEAAYAYGKN  
 GLRG\_09287 KSC-----RASAILGGSDAAS-----VEAAYAYGKN  
 CSUB\_TS\_11 KSC-----RASAILGGSDPAS-----VEAAYAYGKN  
 GLRG\_10302 RMASRM-----MRASATQNKH-----LDACKLMSLL  
 CH063\_0097 RMASRM-----MRASATQNKH-----LDACKLMSLL  
 CH063\_0347 SMVSDL-----M-----VAASPRYRKNSPGQ--QPLALTAFSRFSRL  
 CSUB\_TS\_16 QLLVGL-----MQIAATSSDPRYGSGLDPTHLEDKDGARQLLRFTQL  
 CH063\_0282 RLAVRL-----MQLASES-----DRDYVPLVNV  
 CH063\_1125 RLGIKL-----MQAESRS-----PTDCVELVNL  
 GLRG\_03419 RLGIKL-----MQAESRS-----LTDCVELVNL  
 CSUB\_TS\_5 RLGIKL-----MQAESRS-----LTDCVELVNL  
 CH063\_0190 RLLTRL-----MFHARSVVANDKV-----AQLLDEMCRLL  
 CH063\_1346 SIVTR-----LMLDNHECISTKT-----SAALDKFTTL  
 GLRG\_00455 RLCVRL-----MEFFATGSTRGIS-----SEFFVVKR  
 CH063\_0519 RLCVRL-----LLQAESTRNDVRN-----LDP-RPFVVRQ

CSUB\_TS\_1 RLCVR-----LLQAESSRADVHY-----LDP-RPFVRQ  
 GLRG\_07956 QVAVNLE-----FANGISLPEEVME-----SEEMRELYRA  
 CSUB\_TS\_9 QVAVNLE-----FANGISLPEEIME-----SEEMRELYRA  
 CH063\_0939 TLCALIE-----YGLSDNIPEDIRH-----MGQIQTIWSE  
 GLRG\_03977 TLCALVESVIQEIRYSSLVDQYIRFGLSENISEHIRY-----MEQIQTIWSE  
 CSUB\_TS\_6 TLCALVD-----  
 GLRG\_02961 AFAVF-----CFPKAQYPDVEQY-----LEAIPDM--  
 CH063\_1495 VAYAVF-----CFPKAQYPDVGQY-----LEAIPDM--  
 CSUB\_TS\_3 AFAVF-----CFPKAQYPDVEQY-----LEAIPDM--  
 GLRG\_11695 EVYTYF-----CFYREACPDISRF-----MPAAPEM--  
 CH063\_0640 EVYTYF-----CFYKEVCPDISRF-----MPAAPEM--  
 CSUB\_TS\_13 EAYTYF-----CFYRELCSDISQF-----MPAAPEM--  
 CSUB\_TS\_14 EVYTYF-----CFYRDLCPDISQF-----MPAAPDM--  
 GLRG\_08962 PLYHLVE-----YAHSLQLPDEVFE-----DPAIQALERL  
 CSUB\_TS\_10 PLYHLVE-----YAHSLQIPDEVFE-----DPAIQALERL  
 CH063\_1011 PLCSLAE-----FATGLQWPSFVFD-----SHPYKMMLQA  
 GLRG\_06676 PLCALAE-----FASGLEWPSTVFN-----SHPFKMMLQS  
 CSUB\_TS\_7 PLCALAERR-----FASGLEWPSTVFN-----SHPFKMMLQS  
  
 CH063\_0494 T--QNLVVCVCD--VFRKYTR--RIHKKNDPRDPNFVQISVQC--AKIEQFIDSLFPRQDP  
 GLRG\_07002 T--QNLVVCVCE--VFRRYIR--KIHKKNDPRDPNFVQISVQC--AKIEQFIDTLFPRQDP  
 CSUB\_TS\_8 T--QNLQVVCE--VFRRYIR--KIHKKNDPRDPNFVQISVQC--GKIEQFIDTLFPRQDP  
 CH063\_0492 G--PQAEQFQD--AVFAVAT--RA-----NDHLITAREMLK-SLQAGQGAGHEFEHQG  
 GLRG\_10556 G--PRAEGFQD--AVFKVAT--RA-----NDHLITAREMLK-NLKAGQEAGHEFEHQG  
 CSUB\_TS\_12 G--PRAEGFQD--AVFKVAT--RA-----NDHLITAREMLK-NLKAGQEAGHEFEHQG  
 CH063\_01\_0 ILASRSVGLPQGVLDLWIDR--RL--RRKPSVPDNEKEKPSFGRYGIYGTQLVFESQNC  
 CH063\_0916 C--SRHISVVN--DIYSWEK--EL---KASQTGHKEGAALCSSVSVLTSETNLDFAASK  
 CSUB\_TS\_17 C--AKHISIVN--DIYSWEK--EL---KQSQVAVEEGSILCSGVKVLADSTGLSIEAAK  
 CSUB\_TS\_15 LHPSRVTDLADPVAIKNVLR--EFHWRRKSLTSIINVLAMAL--YALFAANDRQLRALQM  
 CH063\_0378 T--ARQMGLAL--QFVNIAR-----DVAVDKIGRVYL--PTMWLKEEGLTSEDVL  
 GLRG\_02475 T--ARQMGLAL--QFVNISR-----DIAVDKIGRVYL--PTTWLKEEGLTPEDVL  
 CSUB\_TS\_2 T--ARQMGLAL--QFVNVAR-----DIAVDKIGRVYL--PTTWLKEEGLTPEDVL  
 CH063\_0666 L--GEYFQIQD--DYLDNFG--LPEHIGKIGTDIMDNKCSW-----LVNQALQIATP  
 GLRG\_03139 L--GEYFQIQD--DYLDNFG--QP-EHIGKIGTDIMDNKCSW-----LVNQALQIATP  
 CSUB\_TS\_4 L--GEYFQIQD--DYLDNFG--QP-EHIGKIGTDIMDNKCSW-----LVNQALQIATP  
 CH063\_01\_3 L--GLAFQLVD--DMLDYTR--SEKELGKPAGADLELGLATA--PLLFAWK-----TMP  
 GLRG\_09287 L--GLAFQLVD--DMLDYTR--SEKELGKPAGADLELGLATA--PLLFAWK-----TMP  
 CSUB\_TS\_11 L--GLAFQLVD--DMLDYTR--SEKELGKPAGADLELGLATA--PLLFAWK-----TIP  
 GLRG\_10302 --GRLYQLRD--DYQDITS-----ESLSTYDDLDEGSFTL--PLIHALQ-RENEHGDV  
 CH063\_0097 --GRLYQLRD--DYQDITS-----ESLSTYDDLDEGSFTL--PLIHALQ-REDERGDV  
 CH063\_0347 X--GLYYQVRD--DYMNIIVS--ADYAGKKGYAEDLDEQKFSY--MLVHMAR-RAPEMMD-  
 CSUB\_TS\_16 F--GRFFQVRD--DYLNISSESSYMEKKGFAEDLDEQKFSY--ILVHMY--ARNPEARL  
 CH063\_0282 M--GLIFQVRD--DYLNLQS--TAYTKNKGFEDLDEGKFSF--PIIHSIR--SNPSNI  
 CH063\_1125 M--GLIFQIRD--DYMNLSS--KEYSDNKGMCEDLDEGKFSF--PIIHSIR--ADPSNL  
 GLRG\_03419 M--GLIFQIRD--DYMNLSS--KEYSDNKGMCEDLDEGKFSF--PIIHSIR--SDPSNL  
 CSUB\_TS\_5 M--GLIFQIRD--DYMNLSS--KEYSDNKGMCEDLDEGKFSF--PIIHSIR--CEPSNL  
 CH063\_0190 L--GRFFQIRD--DFMNLNS--SEYDLKGFCELDDEGKMSY--PMILVLR--QNPEYQD  
 CH063\_1346 L--GRYFQVRD--DYQNLAS--ADYTKQKGFCELDDEGKYSV--PLIYTLQTPDNIQLI  
 GLRG\_00455 L--SRYFQIRD--DYQNLMS--DQYAKEKGFCELDDEGKISL--PLIYTLQSSPYRDAIS  
 CH063\_0519 L--SLYFQIRD--DYQNLVS--DQYAKQKGFCELDDEGKISL--PMILTQ--RMTRTP  
 CSUB\_TS\_1 L--SLYFQIRD--DYQNLVS--DQYAKQKGFCELDDEGKISL--PMILTQ--RVRTRP  
 GLRG\_07956 A--VAVVWIVN--DIVSLRK--EI--REGFVENL-----VVLLSQGDIQKQID  
 CSUB\_TS\_9 A--VAVVWIIN--DIVSLRK--EI--KEGFVENL-----VVLLSDGKVQKQID  
 CH063\_0939 T--SRAIWITN--DILSLRK--EIPKEGSESIVNAI-----PILMKHKGISPPQAVD  
 GLRG\_03977 T--SRAVWITN--DILSLRK--EIPKEGSESIVNAI-----PIIMKHEGKCPQAVD  
 CSUB\_TS\_6 -----TN--DILSLRK--EIPKEGSESIVNAV-----PIIMKHEGKCPQAVD  
 GLRG\_02961 ---ARFIDISN--DVLSFYK--EE---LSGDTRNYVHNRAMA-----TGR  
 CH063\_1495 ---ARFIDISN--DVMSFYK--EE---LSGDTRNYVHNRAMA-----TGK  
 CSUB\_TS\_3 ---ARFIDISN--DVLSFYK--EE---LSGDTRNYVHNRAMA-----TGR  
 GLRG\_11695 ---GKFINLTN--DILS-----  
 CH063\_0640 ---GKFINLTN--DILSFYK--EE-----

CSUB\_TS\_13 ---GKFINLTN--DIVSFYK--EE---KAGEVRNYIHKKAVS-----LGC  
 CSUB\_TS\_14 ---ARFINLTN--DILSFYK--EE---KAGEIRNYIHKKAVS-----LDC  
 GLRG\_08962 G--ADLVILSN--DILSYRK--EE---SEGCPFNM-----VAASQMNGNSAQEAFD  
 CSUB\_TS\_10 G--ADLVILSN--DILSYRK--EE---SEGCPFNM-----VAACRMNGKSAQEAFD  
 CH063\_1011 V--AIVVGLTN--DLLSLGK--EL---RKGRILNAVPRVRLWN-----SREGGDLASVVR  
 GLRG\_06676 V--AIVVGLTN--DLLSLRK--EL---RKGRILSAVP-----VRLWNNPDGDLEAVVG  
 CSUB\_TS\_7 V--AIVVGLTN--DLLSLKK--EL---RKGRILSAVP-----VRFWNNPDGDLEAVVE

CH063\_0494 KKLGEAAKQKE-----RGQPGMDPGEAFILGGMVLL--TLFFVSALMIGTAWYFGA  
 GLRG\_07002 KMLGGSAAKQKE-----RGQPGMDPGEAFVLLGGLVLL--TLFLLSGLMIGTAWFFGA  
 CSUB\_TS\_8 KKLGESAAKQKE-----RGQPGMDPGEAFILGGMVLL--TLFFISGLMIGTAWFFGA  
 CH063\_0492 EAEHVYE-----HEEDDTQRDIRRGFVLLSV--PAQEYLTNLEGTNFDPA  
 GLRG\_10556 EAEHVYE-----HEEDDTQDIRRGFVLLSV--PAQEYLTNLEGTNFDPA  
 CSUB\_TS\_12 EAEHMYE-----HEENDTQRDIRRGFVLLSV--PAQEYLTNLEGANFDPA  
 CH063\_01\_0 KYYAVPE-----EFIQEFCFYEEPETISTTIWDFLSLSRLA  
 CH063\_0916 RVLWVMC-----REWELVHRDLVAKRLRSPPECSQDLQDYMRGLEFQMS  
 CSUB\_TS\_17 TCLWSLV-----REWELKHELLSSEAHIT--SQFSEAQKLYLKGLEYQM  
 CSUB\_TS\_15 GCFQYFQ-----RGHASEPMALMGGLLHQPSKLAYHFFSVAFLAIWNLAL  
 CH063\_0378 SN-----PTSEGVEKVRRRILAKAFDHYAEARDSMKWIPSEARGPMV  
 GLRG\_02475 KS-----PNSEGVGKVRRRILAKALDHYGEARDSMKWIPSEARGPMI  
 CSUB\_TS\_2 KS-----PNSEGVGKVRRRILAKAFDHYGEARDSMKWIPSEARGPMV  
 CH063\_0666 EQRKILE-----DNYGQKDKAKEAVIKKLFDD--MKLKERYHEFEKCRANEI  
 GLRG\_03139 EQRQILE-----ENYGQKDKAKEAIVKKLFDD--LKLKERFEAFEEKRANEI  
 CSUB\_TS\_4 EQRQILE-----ENYGQKDKAKEAVVKKLFDD--LKLKERFEAFEEKRANEI  
 CH063\_01\_3 ELGALVG-----RKFEQEGDVARARELVLQSD--GIEQTRALAQDYSERAI  
 GLRG\_09287 ELGALVG-----RKFEQEGDVARARQLVLQSD--GIEQTRALAEDYSERAI  
 CSUB\_TS\_11 ELGALVG-----RKFEQEGDVARARQLVLQSD--GIEQTRALAEDYSERAI  
 GLRG\_10302 QLHSILQSARAARSASTSSNNNGRLSVETKLLIREVLEES--GSLEYTRGVIRGLYDETR  
 CH063\_0097 QLHSILQSARAARSASASSNNDGKLSLETKLLVREMLEE--GSLEYTRVVIRGLYNETR  
 CH063\_0347 QVEGMFR-AMR-----RGDADPLESKRYIVSLLHRS--GSLEATRELLLEWQ-RGI  
 CSUB\_TS\_16 KVEGVFRAARQMG-----KEKHDGIQWKRYILGLES--GALNATRQVLVEVHKEMM  
 CH063\_0282 QLSSILK-----QRTTDVDVKLFAVAYIEST--GSFEHCRKTLAELMAQAK  
 CH063\_1125 QLINIL-----QKTNDIEVKRFAVSYMEST--GSFEYTKQVVAVLVDRAR  
 GLRG\_03419 GLINILA-----QKTNNVEVKRFAVARMEST--GSFEYTKQVVGVLDLDRAR  
 CSUB\_TS\_5 QLINILA-----QKTNDVEVKRFAVARMEST--GSFEYTKQVVGVLDLDRAR  
 CH063\_0190 LIMGIFR-QQTANAAGGSAESARLPHETKRYMLGLLKSS--DAMALTLKQLQELEAAME  
 CH063\_1346 SLLSTGK-----KTGTLTREQKECILEILEKS--GGLAYTRVLLRLHDQAS  
 GLRG\_00455 RVFKRRD-----DGGEMGLEMKVFIEMRNT--GALDKTYDLLRSMQSDLM  
 CH063\_0519 EIMGIMK-----HKKPGPMSMEMKQFILTEMRS--GALETTHSLLQTMQEDLI  
 CSUB\_TS\_1 EIMGIMK-----HKKPGPMPFEMKQFIV AEMKKS--GALEATHKLLKEMQENLI  
 GLRG\_07956 GAVARLE-----REVAAVNEASEAAERFADT--PHKHHVSTLSRNCKNMCM  
 CSUB\_TS\_9 GAVARLE-----REIAAVNEAAEAERFAST--PHKDNISLLARNCKNMCM  
 CH063\_0939 DLLAELA-----TSVTVFEEAAGILEQSAGK--GGQELMKTYCDACRCMVT  
 GLRG\_03977 ALLAELA-----TSVAAFEAAAIGLEEAADKGERELLKTYCDACRCMVT  
 CSUB\_TS\_6 ALLAELV-----TSVTAFEAAAIDLEEAVGKEGKELMKTYCDACRCMVT  
 GLRG\_02961 PVLVELE-----VIKNEVVEAANRAAVILE---GRGQYEQSMHDSVRGLLA  
 CH063\_1495 PVLTELE-----EVKNEVVEAANRASTILK---GRGRYEQSLHESVRGLLA  
 CSUB\_TS\_3 PVLTELE-----VLKDEVVESANRAAKILK---GRGKYEQSMHDSVRGLLA  
 GLRG\_11695 -----  
 CH063\_0640 -----KAGERCISILVVT-----  
 CSUB\_TS\_13 SPLAVLE-----STISETALAYNQTLKILKDS-----RSYNELWYAYA  
 CSUB\_TS\_14 TPLGVLE-----STISETTLAYNRTLAILKDK-----HPYSELWYAYA  
 GLRG\_08962 IVGSLLE-----ESYIEWDEVISRMPCWDVDVDSEVERYIKGIQNVVQANI-  
 CSUB\_TS\_10 MVGSLLE-----ESYHEWHEVMSRLPDWDVNVGDSEVERYIKGIQDVVQA---  
 CH063\_1011 SIVDDIE-----KAIRHFDACERRLLITKSRED---ADTTRKIAATLKTICT  
 GLRG\_06676 EVVDDIE-----KAIRQFDICERRLVNQSQAD---SDTTRQIAATLKTIFS  
 CSUB\_TS\_7 EVFDDIE-----KAVRQFDVCERRLINQSQTD---GDTTRQIAATLKTICT

CH063\_0494 RFDLWKTDSVY---WPGTQP--GSA-PIDHKEL-----  
 GLRG\_07002 RFDLWKTDSVY---WPSTEP--GSAQPIAHKEL-----



CSUB\_TS\_8 RFDTLWKTGVDY---WPSTEP--GSAQPIDHKEL-----  
 CH063\_0492 V-KASWKLPW-----RLWRATSKYQI-----  
 GLRG\_10556 V-KASWKLPW-----RLWRATSKNQI-----  
 CSUB\_TS\_12 V-KASWKLPW-----RLWRATSKNQI-----  
 CH063\_01\_0 GKHDAEYHGIDE-----EDANPHTLGVLCEME-----  
 CH063\_0916 G-NEAW-----SEMTPRYHSV-----  
 CSUB\_TS\_17 SGNELW-----SRTTPRYLVVD-----  
 CSUB\_TS\_15 D-LMSSGVFGFL-----KAPLAVIDGIL---ILWRASVVFPLVMWRELN  
 CH063\_0378 V-AVESYMEIGRVLMRNGG---AAADGSGRATVPKSRRIWVAVSTLMAS-----  
 GLRG\_02475 V-AVESYMEIGRVLMRNGGS---AAADGSGRATVPKSRRIWVAVSTLMAA-----  
 CSUB\_TS\_2 V-AVESYMEIGRVLIRNSG---AAADGSGRATVPKSRVWVAVSTLMAA-----  
 CH063\_0666 K-VMIDNIDESE-----GLKKGIFEAFI--AKIYKRSK-----  
 GLRG\_03139 K-EMINNVDESE-----GLKKGIFEAFI--AKIYKRSK-----  
 CSUB\_TS\_4 K-DMINNVDESE-----GLKKGIFEAFI--AKIYKRSK-----  
 CH063\_01\_3 --AAISEFPDS-----EAKDGLVEMAV--KALKRTK-----  
 GLRG\_09287 --AAISGFPDS-----EAKDGLVEMAV--KALKRTK-----  
 CSUB\_TS\_11 --AAISGFPDS-----EAKDGLVEMAV--KALKRTK-----  
 GLRG\_10302 --AVLNALENESGP-----GGKNWMLRLLT--FQLKI-----  
 CH063\_0097 --AMLTEIENEAGS-----GGKNWMLRLLT--FQLKV-----  
 CH063\_0347 N-EIERLEGDF-----GAPNPTLRLLM--ESLRIDV-----  
 CSUB\_TS\_16 --AEIGELEREF-----GAGNGMLRLLV--EMLRI-----  
 CH063\_0282 --AIIEGMEGDS-----SESLSVMNQIL--TMLGLDGNEVPR-----  
 CH063\_1125 --KLADKIDDGR-----GLTKGAHAILD--KMALVDRTTS-----  
 GLRG\_03419 --KAVQDIDEGR-----GLTKGMYKILD--KMALADSATS-----  
 CSUB\_TS\_5 --KAAEDIDEGR-----GLTKGVHKILD--KMALSD-----  
 CH063\_0190 --EKISDLEERF-----GETNPVMRILL--SRLSVRDVTL-----  
 CH063\_1346 --AALKKLELSF-----GSSNPEMKVLL--ELLRI-----  
 GLRG\_00455 --DELKRLEKAF-----GAPNASLELVL--RKLWID-----  
 CH063\_0519 --TELRLERDF-----GSKNPMLEMVL--RKLWIS-----  
 CSUB\_TS\_1 --TELRLEREF-----GSKNPTLELVL--RKLWIN-----  
 GLRG\_07956 A-NWLWSMTRPYCLWDIKPDAAGGYNWTVDLEV-----  
 CSUB\_TS\_9 S-NWLWSMKTTRYCLWDVKREADGGYNWIVDAEP--ENH-----  
 CH063\_0939 G-SIQFTLESSR--YKLEGC--LNEDGSLDILL-----  
 GLRG\_03977 G-SILFSLESSRYKLEDC----LNEDGSLDILL-----  
 CSUB\_TS\_6 G-SIQFTFESSRYKLEGC----LNEDGSLDILL-----  
 GLRG\_02961 MHTANPRYKLDK--LGLAE--KHPLAPFEDQI--GELFERMRE-----  
 CH063\_1495 MHTANPRYRLGD--LGLAE--EHPLGPFEDQI--GELFDRMKA-----  
 CSUB\_TS\_3 MHTANPRYKLDKDLGLAE----EHPLAPFEDQI--GELFERMKAK-----  
 GLRG\_11695 -----  
 CH063\_0640 -----TLQK-----  
 CSUB\_TS\_13 MGYVAMHLR-----TRRYCLSELGI-----  
 CSUB\_TS\_14 MGYVAMHLN-----SRRYYLSELGV-----  
 GLRG\_08962 --GWSFHLKKDL-----GADGPQVRKTW--RIDVLNPPYLSNPGEADY  
 CSUB\_TS\_10 --NLSWRYF-----GADGPKVRKTW--RIDVLNPPYLSDSGGAAY  
 CH063\_1011 G-NLTWSLACKRY-----NVGKPAADGSL--RQELRAGE-----  
 GLRG\_06676 -----H-----PVGTVSANQLL--MEV-----  
 CSUB\_TS\_7 G-NLTWSLASKR--Y-----SVGEPASDGSL--RQELRVGKQSD-----

## PART 4: KS domains of PKS and Hybrids sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

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GLRG_10537 -EPIAIVGFACRLPGG-N-YSPQFWEFLKRGEIAYNKV-PESRFNH----AAHYD----
CS_23      --PIAVVGMACRLPGH-S-NSPTALWEFLQRGGVAKNEP-PPSRFNL----DGHCD----
CH063_1191 -EPIAIIIGVMRLPGR-X-HNAADYWDLLVNGKSGRCRV-PESRYNV----DNWYG----
CS_16      -EPIAIIIGMGMRLPGH-I-QNATDYWDLLVNGKSGRCPV-PKSRYNV----ENWYG----
GLRG_06162 -EPIAIIIGMGMRLPGH-V-HNATDYWDLLVKGNSGRCPV-PKSRYNV----NNWYG----
GLRG_09075 ---VAVCGLGLRAPGG-I-RNATDYWDLLVNGRDARGPI-PDTRYNI----DGFND----
CH063_1187 -----RLPQG-V-RDTNAFWEVLVNGKDLQSHV-PESKYNH----QGFAN----
CH063_1411 ---VAICSIGLRLPQG-V-RDTNAFWEVLVNGKDLQSHV-PESKYNH----QGFAN----
CS_5       -DPVVICGMAMRLPGG-V-SNSEQLWELLQKRCASVPI-PGSRFNL----EGYYS----
GLRG_11893 -DPVVICGMAMRLPGG-V-SNSEQLWELLQKRCASIPI-PGSRFNV----EGYYS----
CS_19      -DPIAIVGMAVRLPGG-V-HTTSDFWMLINKKTGLTKI-PKERWDS----EGFY----
CH063_0663 -DPIAIVGMAVRLPGG-V-RNTEDFWMLMMDKRSGLIPI-PKERWNS----EGFY----
GLRG_00918 -EPIAVCGMAARLPGD-V-QTPAEFWDMLISKRNGLVDWPPSGRFDA----AGFQS----
CH063_0821 -----
CS_13      -PIAICGIGVRLPRD-I-CTPEEMYEFLLSGLDARSPP-DESRYNA----RAFHY----
CH063_0053 -EPIAICGIGLRPQG-I-DTPTSLYNFLVNRKDARSKP-DKPRYSS----QTHHF----
CH063_1006 AEPIAVCGMALRLPGG-I-STPEQFWQFLVDKRDARGPI-PETRFSA----ASYYS----
GLRG_07171 -EPIAVCGMALRLPGG-I-STPEEFWQFLIDKKDARGLI-PQTRFNA----SSYYS----
CS_22      TEPIAVCGMALRLPGG-I-STPEEFWQFLVDKRDARGPI-PQSRFNA----SSYYS----
CS_14      --PIAICGMGLRLPGG-S-STPQEFWNFLVNKGDARGRV-PTSRYNV----AAYHE----
CS_26      --PIAICGMACRLPGG-L-HTPQQWDFLLAKGDARSV-PESRYNV----DAFYD----
GLRG_11435 --PIAICGMACRLPGG-L-HTPQQWDFLLAKGDARSV-PESRYNV----DAFYD----
GLRG_08640 -EPIAINGMGCRLPGQ-V-DSESSFQTLVEKRTGQTPKAPESRFDI----DAHYH----
GLRG_10827 -EPIAVVGMGCRLPGD-V-SSASDFWMLMNRSGQNPKV PANRFNV----EAHYH----
GLRG_11425 -----
CH063_0013 --PIAVIGMGCRLPGD-V-SSPSDFWKLMMEKRSQTPKVPSSRFNI----DAHFH----
GLRG_08482 -EPVAIVGMGCRLPGG-V-HDSSQFWEFLCNKTNGWKEF-DEPRFST----SGFYH----
CH063_1130 -----
GLRG_03511 ---IAIIGMACRLPGG-A-DNPESLWSMLAEGRDGRKEI-PKDRWDW----KSFYH----
CS_18      --PIAIIIGCRLPGG-S-DNPKNLWDLLEGRSGWREI-PADRWNK----DSFYH----
GLRG_05714 --PIAVIGMGCRLPGG-S-DNPDKLWMLSEGRSGWREI-PADRWNK----DSFYH----
CH063_0922 -MPIAIIIGMWRGPGE-A-TNVQNFYELLAAREARVAA-HKGKWNH----EAFYH----
CH063_1282 -----
CS_28      DEDIAIIGMACRFPGD-A-TSPSKLWDLMDGRSAWSEV-PASRWNQ----DAHYH----
GLRG_11563 -----MACRFPGD-A-TSPSKLWDLMEGKSAWSEV-PASRWNQ----DAHYH----
C.P.NRPS_6 ---IAIIGVAHRFPGG-A-NTPAKLWEVLNSRRDLSRKP-DPDRNLN----EKFYH----
CH063_0617 ---IAIIGLAHRFPGG-A-NTPGKLWEVLNSRKDLSREP-GVDRLRL----DKFYS----
C.P.NRPS_1 ---IAVIGICRFPGA-S-SFT-ELGDVLSNPPDLSQTI-PSDRFNI----DGYHH----
GLRG_09842 ---IAVIGICRFPGA-S-SFP-ELADALHEPADLSQTI-PSDRFSI----DGFHH----
GLRG_09715 ---VAIVGTSCRFGA-A-HSPSKLWQILRDPDLRKKI-PSTKFNQ----YSFHH----
C.P.NRPS_8 ---IAIIGSACRFPGA-S-VTPSKLWELLRDPDRVGGPL-PASRFNA----EGFHH----
GLRG_10367 ---IAIIGSACRFPGA-S-VTPSKLWELLRDPDRVGGPL-PVSRFNA----EGFHH----
CS/1       ---IAIIGSACRFAGG-V-DSPATLWELLSNPRDVRTEI-PSSRFSA----DGFYH----
CH063_0489 -----
CH063_0392 ---VAIVGSACRFPGA-A-SSPSKLWDLRQPRDVLRTI-PEDRFNP----EGFYH----
GLRG_07434 ---IAIVGSGCRFPGA-A-SSPSKLWELLREPRDVLCEI-PPSRFDV----NGFHH----
CS/20      ---IAVIGSACRFPGA-C-TTPSRLWDLLENPTDIVSEI-TPDRFNV----DRFFH----
CH063_0306 ---IAVGSACRFPGA-S-TTPSRLWDLLENPTDIVSEI-TPDRFNV----DRYH----
CH030B     ---IAIIGTGRFPGA-S-DSPSKLWELLKDPDRDLLEI-PEDRFNV----EGFYH----
CS_30      -EPIAIVGTGCRFPGA-C-NSPSKLWELLEKPRDLLEI-PDDRFNA----DGFYH----
CS_29      -EPIAIIIGSACRFPGA-V-DSPSKFWECLQQRDLRSEV-PKQRFSA----EKFFN----
CH063_0199 -----GCRFPGA-A-NTPSKLWDLLEKPRDVQSRI-PKERFDV----DTFYH----
C.P.NRPS_2 ---IAIIGRACRFPGENS-TSPSGLWDLLEKPRDLTKPIPPESRFNP----DGFYH----
CH063_0288 -EPIAIVGTGCRFAGD-V-TSPSKLWDLLEKPRDLTQEV-PSSRFHA----QGFYH----
CS_4       -EPIAIVGTGCRFAGQ-V-NSPSKLWDLLEKPRDLTQEV-PKNRFNA----QGFYH----
GLRG_08212 -EPIAIVGTGCRFAGQ-V-NSPSKLWELLINPRDLTQEV-PKNRFNA----QGFYH----
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CS\_15 MEDIAIIGMACRFPGN-A-TSPEKLWETMVQKESAWSEF-PKDRNLNI---DGYH---  
 CH063\_1445 -EDIAIIGMACRFPGN-A-TSPEKLWEMMVQKESAWSEF-PKNRLNI---DGYH---  
 GLRG\_02583 -EDIAIIGMACRFPGN-A-TSPEKLWQMMVQKESAWSEF-PKDRNLNI---DGYH---  
 CS\_32 -MPIAVVGMACRLPGS-V-SSPAEFWELCSRARSGFTPV-PKERFNH---EAFYH---  
 GLRG\_03360 -MPIAVVGMACRLPGS-V-SSPAEFWELCSRARTGFTPV-PKERFNH---EAFYH---  
 CH063\_1222 -MPIAVVGMACRLPGS-V-SSPAEFWELCSRARTGFTPV-PKERFNH---EAFYH---  
 CH063\_0134 ---VAVIGMSCKFSGE-A-TTPEKLWQLVVDGRDGTI-PKSRFNA---DAFYD---  
 CS\_21 -----  
 GLRG\_09267 -EPIAIIIGMSSKFSGD-A-TDNEKLWEMLAGRSGWTPF-PSSRFRL---QGIYH---  
 CH0190 --PVAIVGMGCRFSGD-S-TSPQKLWELLEQGRSTWSKI-PTSRFNV---GGVFH---  
 CH063\_0873 -MPIAVVGMGCRFAGN-V-TGPERLWELLKSGQSGWSEI-PESRFAT---SGILH---  
 CS\_9 -EPIAIVGLDAKLPCD-G-DTVEKFFQFLVDGRSARKPV-PSDRYNA---EAFWH---  
 CH063\_1490 -EPIAIVGLSARLPGD-G-DTPERFFESLLAGRSARTEV-PQDRYNA---DAFWH---  
 GLRG\_01860 -EPIAIVGVSARLPGD-G-DTPERFYDSLLAGRSARTEV-PRERYNA---DAFWH---  
 CS\_25 -EPIAIVGLSARLPGD-G-DTPERFFDSLLAGRSARTEV-PRERYNA---NAFWH---  
 CS\_34 DEPVAIIGMACRFPGE-A-TNPSKFWELLKDGGRDAFSEA---DRFNP---EAFYH---  
 GLRG\_11770 --PIAVVGLACRLPDE-A-SSPEEFWKLLEQRDTLSV--PHNRWNT---DAFHH---  
 CH0399 -----  
 CS\_11 --PIAIVGLSCRFPD-A-TDPSKFWMDLKSGRDGYTP--KTNRYNE---DAFHH---  
 GLRG\_11840 --PIAVVGLSCRFPD-A-TDPSKFWMDLKNRGRDGYTP--QTNRYNE---DAFHH---  
 CH063\_0781 --PIAVVGLACRLPGD-A-TCPTYWNLLKEGRDAYST--DTDRYNT---SAFYH---  
 CH063\_1357 --PIAIIIGLRAPGD-A-SDPEKFWQMLQDARSARSEI-PKDRYNV---DGFYH---  
 GLRG\_10317 --PIAIIIGVRAPGD-A-SDPEKFWQMLLDARSARSEI-PKDRYNV---DGFYH---  
 CH063\_1471 ---IAIVGIAGRFPD-A-ENPQKLWMLAEGRSALSDV-PGDRFNV---DAFYH---  
 CH063\_0506 -EPLAIIIGL SVKFPD-A-TSPEEFWELMREGRSAAASKV-PEDRFNV---DAFYH---  
 GLRG\_11835 --PIAIIIGMSMKLPGD-I-KSSQALWDILKNAQCLTGKI-----  
 CS\_3 -EPIAIIIGLNLKFPGN-A-TSVQAFWDMLENLGNASCEV-PASRFNI---DAFYH---  
 GLRG\_11878 -EPIAIIIGLNLKFPGE-A-TSMQAFWAMLENLGNASCEV-PAGRNI---DAFYH---  
 CH063\_0051 -----  
 CH0135 -EPIAIIIGAACRLPGE-A-TSLGGLWDMISHGRSAHGKV-PPSRWDA---DTWYH---  
 CS\_43 -DDIAVIGMACHVAGA---QDLEQYWKLMLEGKSQHKELVPNDRFAM---ETSYR---  
 GLRG\_09268 -NDIAVIGMACHVAGA---QDLEQYWKIMLEGKSQHRELVPNDRFAM---ETSYR---  
 GLRG\_11857 -NDIAVVGMSAKVAGA---DSLEAFWDVLCAGVSHSEV-SPDRFGGFGMDKSMF---  
 CH063\_0213 --DIAVVGMAKCVAGS---DGLEEFWDLNCGEPQHQL-KDQRFSE---QTPFR---  
 CH063\_0477 ---IAIVGASCRPGA---NSLDELWEVISQGTREKL-RTDRFDL---GGSHR---  
 CH063\_0313 ---VAIVGMAGRFPGA---NSVDELWELMSAGRSTVTS--PPERVGL---NQLAE---  
 CS\_44 --IAVVGMAGRFPGA---NSVDELWELLLDAQSMVDR--APDRVGL-----  
 GLRG\_11575 ---VAIVGIAGRFPGA---NSVDELWELLLNAKSMVVK--APTRVGL---DQL-----  
 CH063\_1347 ---IAVVGMACRFPGR---ENLEAFWRLLADGQTGLGTM-PADRFPD---ATLTR---  
 C.PKS --IAVVGMGCKFPGA---DSVEKFWMLDSGESKLSPP-PSGRFPA-----H---  
 CS\_45 DDSIAIVGMACRLPGA---DNLDEFWQLLLSGSSMLGRL-PGERFAT-----  
 GLRG\_11798 -DSIAIVGMACRLPGA---DNLEEFWQLLLSGSSMLRRL-PGERFAT---TGLRR---  
 GLRG\_11778 --RVAIVGMGGRYPR---GDLEEFWQHICQKALHTEI-PKDRFSL---DDYYS---  
 CH0398 ---IAIVGMGGRFPAC---DDLEGLWDIIEGQIVHSEV-PRDRFNV---DELYD---  
 CS\_37 --RIAIVGVSGRPMC---DNISEFWDIIMSKTDTCTEV-PKDRFDI---DEFYC---  
 GLRG\_11836 --RIAIVGVSGRPMC---GNINEFWDIIMSKTDTCTEV-PKDRFDI---DEFYS---  
 CH063\_1255 --IAVVGMAGRAPEC---DDLEQYWQVIETGRDVAREI-PPGRFDA---DDLFPQPG  
 CS\_40 --RIAIVGMGGRGPDHANDSLDQFVNLIRQQDVARTI-PEDRFDA---DSLFP---P  
 CS\_38 ----VVGMSCRPPG-A-NDTELFWKLMMDKRDVHTTV-PPDRFDL---STHFD---  
 CS\_36 DSKLAIVGMACRMPGD-A-DTPERFWELLAQGRDTHTVV-PPDRFDL---GAHFD---  
 GLRG\_08632 ----AIVGMSCRPPG-A-NDCKLFWDLLEKGRDTHTEV-PADRFDL---QAHFD---  
 CH063\_0351 ----AIVGMAGRFPDA---ASHEKLWELLSKGLDVHRVV-PADRFPV---ATHYD---  
 CS\_35 ----AIVGMAGRFPDA---ASHEKLWELLSKGLDVHRVV-PADRFPV---ATHYD---  
 GLRG\_04203 ----AIVGMAGRFPDA---ASHEKLWELLSKGLDVHRVV-PPDRFPV---ATHYD---  
 GLRG\_11956 ----AIIGMSGRFPG--A-KHNEAFWDVLRQGLDLHKPAPA-LHWDV---KTHVD---  
 CH063\_1223 ----AILSASGRFPGN-A-DTMDQFWDILYNGIDTHEMV-PPSRWDA---STHV---  
 GLRG\_08620 --PIAILAASGRFPGR-A-DTMDAFWDILIRGVDTHEMV-PPSRWSS---SAHV---  
 CH063\_0250 KSPIAILAVSGRFPGN-A-DTMDAFWDILHNGVDTHEMV-PASRWNA---SAHV---  
 CS\_39 --DIAIVGYSRCPGA---ETGEDFWRILAEGRDVHGRV---SFSQ---QVLHN---  
 GLRG\_03507 ---IAIVGMSGRFPGG---DNLQAFWDILVQGKDLHKKV-PKDRFDV---DLHCD---  
 GLRG\_03509 ---IAIVGMSGRFPGG---DNLQAFWDILVQGKDLHKKV-PKDRFDV---DLHCD---

GLRG\_10537 ---GSL--KPRTMRQP--GGMFLKD-ID--LAD-FDAKFFELSATE-----  
CS\_23 ---KSR--RPRTMKSP--GGMFIED-VD--PEL-FDGQFFNISRV-----  
CH063\_1191 ---PG--RALHVPTE--FGYFLEE-LN--LAH-VDASFWSFTKQE-----  
CS\_16 ----PG--RVSHVPTK--FGYFLEE-LN--LAH-VDPSFWSFTKQE-----  
GLRG\_06162 ----PG--RVSHVPTK--FGYFLEE-LN--LAH-VDPSFWSFTKQE-----  
GLRG\_09075 ---SLG--GKNTIKTR--FGYFLTD--D--LTR-IDTSLFMSRKE-----  
CH063\_1187 ---TLG--KKGAIETQ--CGYFVNK--D--LGS-LDTSLFMSQKE-----  
CH063\_1411 ---TLG--KKGAIETQ--CGYFVNK--D--LGS-LDTSLFMSQKE-----  
CS\_5 ----RHP--RPGTMHFE--KAYILDH-VD--LDR-FDSCHFPFSRKE-----  
GLRG\_11893 ---RHP--RPGTMHFE--KAYILDH-VD--LDR-FDTCHFPFSRKE-----  
CS\_19 ---ETP--KWGTIQNR--DCYLLSD-VD--LKK-FDTSFFTCGQAE-----  
CH063\_0663 ---PVA---KWGTVQNK--EAYMFSQ-ADNDLTK-FDASYFTCGEKE-----  
GLRG\_00918 ---RLP--AKNTLQAP--QAYLLNH-VS--LSD-FDTSFFPVGAK-----  
CH063\_0821 -----  
CS\_13 -QASGGN--EAGAEKPLPSQGYWLNW-KE--ISS-FDASLFNLPKE-----  
CH063\_0053 ---TESG--TTRPLPTE--EGYWLTH-DD--VTK-FDPSMFSMNPKE-----  
CH063\_1006 ---EAA--KPGHIKTQ--HGYFLDSDVD--LAA-LDTTFFRMPKSE-----  
GLRG\_07171 ---EAA--KPGHIKTQ--YGYFLDESVD--LAA-LDTTFFRMPKSE-----  
CS\_22 ----EAA--KPGHIKTQ--HGYFLDSDVD--LAA-LDTTFFRMPKSE-----  
CS\_14 ----TTK--RPTTVAAE--YGYFLSE--DVKLGA-MDTTRFSMSRAE-----  
CS\_26 ----TSG--RPGTVKTE--YGYFLDY--D--ISK-TDGGFSSFVRGA-----  
GLRG\_11435 ---TSG--RPGTVKTE--YGYFLDY--D--ISK-SDGGFSSFVRGA-----  
GLRG\_08640 ---ERLD--RPGFFNVP--GGHFLDG--H--PEN-FDPTFFNTTPVK-----  
GLRG\_10827 ---RNKE--RPGSLPVM--GGYFINE--D--LEN-FDPGLFNITPVE-----  
GLRG\_11425 -----SFFGITPIE-----  
CH063\_0013 ---SDND--RPGSFGVL--GGYFLNE----TLQEFDPAFFGITPVE-----  
GLRG\_08482 ---PNPD--RPGSISMK--GAFLAKE--D--ARL-FDHAFFGMTGLE-----  
CH063\_1130 -----  
GLRG\_03511 ---KDTN--AKEAINFS--HGYFLDQ--E--ISA-FDARFFGVPGAE-----  
CS\_18 ---PDPE--AKEAVNFK--GLYFLDQ--D--IAA-FDARFFNIHPHE-----  
GLRG\_05714 ---PDPE--AKEAVNFK--GLYFLDQ--D--IAA-FDARFFNIHPHE-----  
CH063\_0922 ---PESS--RKGTSNVE--AGHYFKD--D--LSL-FDAPFFSMTDSE-----  
CH063\_1282 -----D--IHA-FDAGFFNVRDE-----  
CS\_28 ---PSQE--RAGSNTVK--GGHFLRD--GEQNGKQFDAAFFNITKRE-----  
GLRG\_11563 ---PSQE--RAGSNTVK--GGHFLRD--DEQNGKKFDAAFFNITHKE-----  
C.P.NRPS\_6 ---TNGE--YHGKSNVT--KSYFLEE--D--PRL-FDTAFFNVSPLE-----  
CH063\_0617 ---ENGE--HHGKSNVT--KSYFLDE--D--PRL-FDTTFFNISPLE-----  
C.P.NRPS\_1 ---DDGS--HHGSTNVR--KSYFLRD--N--VRK-FDNNFFNITAVE-----  
GLRG\_09842 ---EDGK--YHGNTNVR--KSYFLRD--G--IRK-FDNNFFNITAVE-----  
GLRG\_09715 ---PESV--RHGTSNVT--HSYVLEE--D--VGE-FDYGFFNLPKE-----  
C.P.NRPS\_8 ---PDPL--HHGHSNVKDMRAYFLSG--EGVEHR-FDAGFFGIRSAE-----  
GLRG\_10367 ---PDPL--HHGHSNVKDMRAYFLSG--EGVEHR-FDAGFFGIRSAE-----  
CS/1 ---VDSQ--YHGHSNVR--HAYVLEQ--D--PGV-FDAQFFGVKPVE-----  
CH063\_0489 -----  
CH063\_0392 ---PDGT--HHGTSNVT--QSYLLEE--D--PRV-FDSGFFNIKPV-----  
GLRG\_07434 ---TDSQ--FPGRNTVK--HSYLLQE--D--VAK-FDAQFFNITASE-----  
CS/20 ---PDHK--HHGTSNVR--HSYMLQE--N--FKL-FDAKFFGVKPQE-----  
CH063\_0306 ---PDHK--HHGTSNVR--HSYLLQE--N--FKL-FDAKFFGVKPQE-----  
CH030B ---PKNS--HHGTTNVR--HSYLLDE--D--LRR-FDASFFGIKATE-----  
CS\_30 ---PKSS--HHGTSNVR--HSYLLDE--D--LRT-FDASFFGIKAI-----  
CS\_29 ---SNGM--HHGSTNSR--YGYFLKE--P--LGA-FDAPFFNIHAGE-----  
CH063\_0199 ---PDGT--HHGRTNAS--YAYFLKE--D--LHA-FDAPFFNIQAGE-----  
C.P.NRPS\_2 ---PNSE--HHGSSNIK--ESYFIEE--D--PRL-FDAGFFNIAPRE-----  
CH063\_0288 ---ADGE--YHGATNAT--QGYFLEQ--D--HRV-WDAGFFNITPKE-----  
CS\_4 ---PDGE--YHGATNAT--QGYFLEQ--D--HRV-WDAGFFNITPKE-----  
GLRG\_08212 ---PDGE--YHGATNTT--KGYFLEQ--D--HRV-WDAGFFSITPKE-----  
CS\_15 ---PSGD--RQGSISFR--GAHFLKD--D--IAA-FDASFFSVMMAED-----  
CH063\_1445 ---PSGD--RQGSISFR--GAHFIKD--D--IAA-FDASFFSVMMAED-----  
GLRG\_02583 ---PSGD--RQGSISFR--GAHFIKD--D--IAA-FDASFFSVMMAED-----  
CS\_32 ---PNPG--KTGAYHAE--GGNFLNV--D--LAS-FDAPFFGLTEKE-----  
GLRG\_03360 ---PNPG--KTGAYHAQ--GGNFLDV--D--LAS-FDAPFFGLTEKE-----

CH063\_1222 --PNPG--KTGAYHAE--GGNFLDV--D--LAA-FDAPFFGLTEKE-----  
 CH063\_0134 -----TDHAKIGMQQIT-----  
 CS\_21 ---EED-----VGL-FDAAFFGYSSEA-----  
 GLRG\_09267 ---PNNE--RLNSTHVK--GAHFLOE--D--IGL-FDAAFFGYSSEA-----  
 CH0190 ---PDGQ--RVGSTHVK--GGHFLDE--D--PAL-FDASFFNMTSEV-----  
 CH063\_0873 ---PQKD--KIGSTNVR--GGYFLGT--D--PGA-FDASFFNLSSTEV-----  
 CS\_9 ---PDNH--RDGVIGGK--EGHFMEA--N--VKA-FDAPFFAMTPAE-----  
 CH063\_1490 ---PDAN--RSGATRVR--HGHFLKG--S--ISA-FDAPFFSITPTE-----  
 GLRG\_01860 ---PNAD--RSGATRVR--HGHFLKG--S--ISA-FDAPFFSITPTE-----  
 CS\_25 ---PDAD--RSGTTRVR--HGHFLKG--S--ISA-FDAPFFSITPTE-----  
 CS\_34 ---ANSSGRQNVLPCK--GAHFLOE--S--PYA-FDAAFFNITPGE-----  
 GLRG\_11770 ---PGRN--KPQTIAAR--SAHFLKR--D--VMA-FDAAFFNINASE-----  
 CH0399 -----FDAAFFNIGHAE-----  
 CS\_11 ---PGGNNKRQNVLPVK--GGYMLKQ--D--PYV-WDAAFFNITAAE-----  
 GLRG\_11840 ---PGGNNKRQNVLPVK--GGYMLKQ--D--PYV-WDAAFFNITAAE-----  
 CH063\_0781 ---ANPKDRQNVLPK--GGHFIKE--D--PYA-FDTSFFKLTAAE-----  
 CH063\_1357 ---PDPE--RLGSIQQR--HAHFLKQ--D--FKV-FDAPFFSVTPKE-----  
 GLRG\_10317 ---PDPE--RLGSIQQR--HAHFLKQ--D--FKV-FDAPFFSVTPKE-----  
 CH063\_1471 ---PHNE--RQGTINVR--KAHFMR--D--ISA-FDAPFFNMPPIAE-----  
 CH063\_0506 ---PDPN--RLDSLRVR--DAHFMKE--D--PRA-FDAPFFNMSPAE-----  
 GLRG\_11835 -----RVQ--NAHFME--D--PRA-FDAPFFSMSPAE-----  
 CS\_3 ---PDPA--RLDSIRVR--KASFMAE--D--PRA-FDAAFFNMSPAE-----  
 GLRG\_11878 ---PDPA--RLDSIRVR--KAHFMAE--D--PRA-FDAAFFNMSPAE-----  
 CH063\_0051 -----  
 CH0135 ---PDPD--RKGSISVK--HGYFLEQ--D--VSL-FDAPFFSMTAKE-----  
 CS\_43 ---PG--QKDDERKW--YGNFIDD----HDA-FDYKFFKKSPPRE-----  
 GLRG\_09268 -----PSEKEGTRKWYGNFLDD----HDS-FDYKFFKKSPPRE-----  
 GLRG\_11857 ---RTA--APNRSRKW--FANFLSG----PDE-FDHRFFGKSPRE-----  
 CH063\_0213 -----PADAKRKW--FANLVDG----ADH-FDHKFFKRSPPRE-----  
 CH063\_0477 ---AAQ--DRDWVTKREFYGNFIDD----VRG-FDNAFFGISPRE-----  
 CH063\_0313 -----DNSQVRWR--GNFIED----HDA-FDHKFFNKSSARE-----  
 CS\_44 ---DQL--VEDVSQVKW--WGNFLAD----HDS-FDHKFFNKSSARE-----  
 GLRG\_11575 -----DEDVSQVKW--WGNFLDD----YDS-FDHKFFNKSSRE-----  
 CH063\_1347 -----EPRLANF--YGNFIDA----PDA-FDHRFFGISGRE-----  
 C.PKS ---EHQ--RDTEKTVY--FGNYLDD----IAS-FDNRFKKSPPRE-----  
 CS\_45 ---TGL--RRSPKGDKPLGNFVRD----AFA-FDHKFFNKSSRE-----  
 GLRG\_11798 -----SPKEDTPF--LGNFVRD----AFA-FDHKFFNKSSRE-----  
 GLRG\_11778 ---ASS--SPFTTNTK--YGCFLDN----PGL-FDARFFSLSPRE-----  
 CH0398 ---PKTK--LHLSTSTK--YGCFLDN----PGL-FDARFFQVSPRE-----  
 CS\_37 TEHRGE--KKCTMTTK--YGCFMNN----PGH-FDSRFFHISPRE-----  
 GLRG\_11836 ---AEHGRGEKKCTTTTR--YGCFMDK----PGH-FDSRFFHISPRE-----  
 CH063\_1255 HGENSSCRATCVSTCK--LGCFLDN----PGH-YDARFFRVSPRE-----  
 CS\_40 HAASSSSSSPCRSSTD--YGCFLRD----PGH-FDARFFRLSPRE-----  
 CS\_38 ---PTGQ--TENATQTP--FMNYMEN----PGH-FDAGFFNISPKEVWQPPHFLPSTYF  
 CS\_36 ---PTGE--TENAIGTQ--FGNFISN----PGH-FDAGFFNMSPRE-----  
 GLRG\_08632 ---PTGQ--VPNSTPTP--WGNFIDH----PGL-FDAAFFNMSPRE-----  
 CH063\_0351 ---ITGK--AVNTSHSQ--YGCWIEN----PGY-FDPRFFNMSPRE-----  
 CS\_35 ---ITGK--AVNTSHSQ--YGCWIEN----PGY-FDPRFFNMSPRE-----  
 GLRG\_04203 ---ITGK--AVNTSHSQ--YGCWIEN----PGY-FDPRFFNMSPRE-----  
 GLRG\_11956 ---PTGK--IKNTSATP--FGCWLDD----PGA-FDAKFFNISPRES-----  
 CH063\_1223 ---DTS--IKNVSGTG--YGCWLHQ----AGQ-FDTTFFNISPRES-----  
 GLRG\_08620 ---DTS--IKNVSGTG--YGCWLHQ----ASQ-FDITFFNISPRES-----  
 CH063\_0250 ---DPS--VKNVSGTG--FGCWLHQ----APL-FDAAFFNMSPRES-----  
 CS\_39 ---MPESRCAGFGGNPP--LGCIFYQG----LGL-FDCRLFNMSPRE-----  
 GLRG\_03507 ---PTGK--TPNSTLSA--FGCFLDK----PGH-FDNLMFNMSPRES-----  
 GLRG\_03509 ---PTGK--TPNSTLSA--FGCFLDK----PGH-FDNLMFNMSPRES-----  
  
 GLRG\_10537 -----ATAMDPAQRMLEVVYEGLEN-----  
 CS\_23 -----CIAMDPQQRQMLEVAYECLN-----  
 CH063\_1191 -----AELMDPRQRLFLEVAYEALES-----  
 CS\_16 -----AELMDPRQRLFLEVSYEALN-----

GLRG\_06162 -----AELMDPRQRLFLEVAYEALEN-----  
 GLRG\_09075 -----VERCDPQQRLLLEVAREALE-----  
 CH063\_1187 -----LERCNPQQRQFLEVVRQALEN-----  
 CH063\_1411 -----LERCNPQQRQFLEVVRQALEN-----  
 CS\_5 -----VEQMDPAQRMLLEITWECLN-----  
 GLRG\_11893 -----VEQMDPAQRLLLEITWECLN-----  
 CS\_19 -----AERMDPMQRQLEITRECLDS-----  
 CH063\_0663 -----IERMDPMQRQLEIARECLDN-----  
 GLRG\_00918 -----ASRMDPMQRQLEEVAYECAEN-----  
 CH063\_0821 -----  
 CS\_13 -----IDRLDPQQRVLLRVVWEALES-----  
 CH063\_0053 -----LSKLDPQQRLLLVVWEALES-----  
 CH063\_1006 -----VERADPQQRLLLELTRECLES-----  
 GLRG\_07171 -----VERADPQQRLLLELTRECLES-----  
 CS\_22 -----VERADPQQRLLLELARECLES-----  
 CS\_14 -----VEFADPQQRLLLEVMEAFED-----  
 CS\_26 -----TEREDPHQRQMTEVARECIED-----  
 GLRG\_11435 -----TEREDPHQRQMTEVARECIED-----  
 GLRG\_08640 -----AQWLDSQQRRTLEMCYESLVS-----  
 GLRG\_10827 -----AMWMDPQQRKLCVVYEFES-----  
 GLRG\_11425 -----AMWMDPQQRKLEVDYDSFES-----  
 CH063\_0013 -----ATWMDPQQRKLEVVYEFES-----  
 GLRG\_08482 -----VETMDPSQRKLEVSYEAIEN-----  
 CH063\_1130 -----MDPQQRKLLQVVYECLQS-----  
 GLRG\_03511 -----APGIDPQQRLLLEVTYEAVEN-----  
 CS\_18 -----AHCLDPQQRILLETTYEALN-----  
 GLRG\_05714 -----AHCLDPQQRILLETTYEALN-----  
 CH063\_0922 -----AACLDPQQRLLLETCYEALN-----  
 CH063\_1282 -----AKAMDPQQRITAECFAFEALS-----  
 CS\_28 -----TETMDLQQRIVMENVYEALES-----  
 GLRG\_11563 -----TETMDLQQRIVMENVYEALES-----  
 C.P.NRPS\_6 -----AEAMDPQQRILLETVYEATES-----  
 CH063\_0617 -----AEAMDPQQRLLLETVYEATES-----  
 C.P.NRPS\_1 -----ASSIDPQQRIVLETVHEAFEN-----  
 GLRG\_09842 -----ASAMDPQQRLLLETVYEAFEN-----  
 GLRG\_09715 -----AESMDPQQRLLLETVYEGIEA-----  
 C.P.NRPS\_8 -----AAVLDPQVRLLMETTYEALEA-----  
 GLRG\_10367 -----AAVLDPQVRLLMETTYEALEA-----  
 CS/1 -----AKALDPQQRLLLEVYEGLEA-----  
 CH063\_0489 -----MDPQHRMLLEVYEAEME-----  
 CH063\_0392 -----AHATDPQHRILLETVYEALS-----  
 GLRG\_07434 -----AVAMDPQQRLLLETVYEGLEG-----  
 CS/20 -----AIAMDPQQRLLLETVYESLEA-----  
 CH063\_0306 -----AIAMDPQQRLLLETVYESLEA-----  
 CH030B -----ANSIDPQQRMLMETVYEGLES-----  
 CS\_30 -----ASSIDPQQRLLLETVYEALS-----  
 CS\_29 -----AKSMDPQQRLLLEATYDALSS-----  
 CH063\_0199 -----AESMDPQQRLLLETVYEAASN-----  
 C.P.NRPS\_2 -----AEAIDPQQRLLLETTYEAMEN-----  
 CH063\_0288 -----AEAIDPQQRILEVVYEALES-----  
 CS\_4 -----AEAIDPQQRILEVVYEALES-----  
 GLRG\_08212 -----AEAIDPQQRILEVVYEALES-----  
 CS\_15 -----AKAIDPQQRFLLEVSYEALN-----GKRTPFLLGKQF  
 CH063\_1445 -----AKAIDPQQRFLLEVSYEALN-----  
 GLRG\_02583 -----AKAIDPQQRFLLEVSYEALN-----  
 CS\_32 -----AISMDPQQRLLLECTFEALN-----  
 GLRG\_03360 -----AISMDPQQRLLLECTFEALN-----  
 CH063\_1222 -----AISMDPQQRLLLECTFEALN-----  
 CH063\_0134 -----ANRTDCFV-----  
 CS\_21 -----AASLDPQYRLQLESVYEALES-----  
 GLRG\_09267 -----AASLDPQYRLQLESVYEALES-----  
 CH0190 -----ARDMDPHHRLMLEVVYEALES-----

CH063\_0873 -----ASTLDPQIRMTLEVVFEEAMES-----  
 CS\_9 -----AANLDPQQRMLLECVYTAMEN-----  
 CH063\_1490 -----ARSIDPQQRGMLESVYKALEN-----  
 GLRG\_01860 -----ARSIDPQQRGMLESVYKALEN-----  
 CS\_25 -----ARSIDPQQRGMLESVYKALENGQGSRIHAPTWGGRTCKTSEKKKK  
 CS\_34 -----ALATDPRQLALEVAYEALEN-----  
 GLRG\_11770 -----AVALDPQQRFFVLEVTYEALLES-----  
 CH0399 -----ATALDPQQRFTLELTFEALLES-----  
 CS\_11 -----AIAFDPKQRIAMEVTYEALEN-----  
 GLRG\_11840 -----AIAFDPKQRIAMEVTYEALEN-----  
 CH063\_0781 -----AAVLDPKQRILLEVTYEALEN-----  
 CH063\_1357 -----AKAMDPTQRMLEAAAYEGFEN-----  
 GLRG\_10317 -----AKAMDPTQRMLEAAAYEGFEN-----  
 CH063\_1471 -----AKAMDPQQRMALECTYEALEN-----  
 CH063\_0506 -----ASVLDPQQRGLLEGAYHCLEN-----  
 GLRG\_11835 -----ACILDPQQRGLLEGAFHTFEN-----  
 CS\_3 -----ASILDPQQRGLLEGAYHTFEN-----  
 GLRG\_11878 -----ASILDPQQRGLLEGAYHTFEN-----  
 CH063\_0051 -----MDPMQRLGLEIAYEAFEN-----  
 CH0135 -----AAGMDPMKRLLEVS YEGFES-----  
 CS\_43 -----ALHM DPQQRMLQVAYQAVAQ-----  
 GLRG\_09268 -----ALHM DPQQRMLQVAYQAVAQ-----  
 GLRG\_11857 -----SAAM DPQQRHMLQVAYQAVEQ-----  
 CH063\_0213 -----SATM DPQQRWMLQIAYQAVEQ-----  
 CH063\_0477 -----AAYM DPQQRILLETAFEAMDS-----  
 CH063\_0313 -----ATAC DPQQRKLEVVYEALLES-----  
 CS\_44 -----ALAC DPQQRKLEVVYEALLES-----  
 GLRG\_11575 -----AMAC DPQQRKLFVVYEALLES-----  
 CH063\_1347 -----AKSM DPQQRLLALQVAYEALLES-----  
 C.PKS -----AVSL DPQQRLLLEVS YQALLES-----  
 CS\_45 -----ARSM DPQQRKLLQVAYEALLES-----  
 GLRG\_11798 -----ARSM DPQQRKLLQVAYEALLES-----  
 GLRG\_11778 -----AMEV DPAHRLFLLA VFEALLES-----  
 CH0398 -----ALQM DPAHRLFLMSTYEALLES-----  
 CS\_37 -----AMLM DPCHRQFLMSTYEALLES-----  
 GLRG\_11836 -----AMLM DPCHRHFLMSTYEALLES-----  
 CH063\_1255 -----ALLM DPGSRLFQMACHEALLES-----  
 CS\_40 -----ATLL DPCSRLFLMAA HAAVEM-----  
 CS\_38 PPLFQYLYKDLPLGGAEQMDPMHRLALVTAYEALLES-----  
 CS\_36 -----AAQT DPMQRLALVTAYEALLES-----  
 GLRG\_08632 -----AEQT DPMHRLALVTAYEALLES-----  
 CH063\_0351 -----AFQT DPM-RMALTTAYEALLES-----  
 CS\_35 -----AFQT DPMQRMALTTAYEALLES-----  
 GLRG\_04203 -----AFQT DPMQRMALTTAYEALLES-----  
 GLRG\_11956 -----AAQIDPAQRLALMTAYEAIEQ-----  
 CH063\_1223 -----ASRIDPAQRLALMTAAEALEK-----  
 GLRG\_08620 -----ASQVDPAQRLALLTTAAEALEK-----  
 CH063\_0250 -----AAQVDPAQRLALLTTAAEALEK-----  
 CS\_39 -----AAQTDPGQRLLLMATYEALQL-----  
 GLRG\_03507 -----AAQTDPQQRLLLMAYEALET-----  
 GLRG\_03509 -----AAQTDPQQRLLLMAYEALET-----

GLRG\_10537 ----AGI--PLDKV----NGQSVGCYVGN YASD--Y---GDMG-----ARDPED  
 CS\_23 ----AGV--PLELL----SGTKGVIVGTNFID--Y---AAIQ-----NRDPED  
 CH063\_1191 ----SGS----TSW----RGS DVG VYVGTMGED--W---S LLE-----CHDQQC  
 CS\_16 ----SGS----KSW----KGNDVGVYVGTMGED--W---ATLE-----SRDERN  
 GLRG\_06162 ----SGS----KSW----KGNDVGVYVGTMGED--W---TTLE-----SRDERN  
 GLRG\_09075 ----AGE----TKY----RGERIGCYVGTFGDD--W---AQIA-----GKETQH  
 CH063\_1187 ----AGE----VNY----RGKAVGCYVGTFS ED--W---LQMA-----AREQQH  
 CH063\_1411 ----AGE----VNY----RGKAVGCYVGTFS ED--W---LQMA-----AREQQH  
 CS\_5 ----AGE----MNW----Q GKAVGCYVGSFAED--W---TTEM-----ERDTQY

GLRG\_11893 ---AGE---VNW---QKAVGCVVGSFAED--W---TTEM-----ERDTQY  
CS\_19 ---AGE---VGTST---MDKRVGCYVGTFFSSD--W---QDDQ-----SMDPQA  
CH063\_0663 ---AGE---TNW---RGEEIGCVVGNFSSD--W---QDDL-----SMDPHA  
GLRG\_00918 -AGAGGL--AHDSA---TRKDVGVFVGVFGED--W---LQEN-----VMDSQL  
CH063\_0821 -----  
CS\_13 ---AGE---ADW---RGKNIGCVVGSFGDD--W---RELH-----ARDELD  
CH063\_0053 ---AAE---TDW---QGQRIGCVVGSFGDD--W---REMH-----AVDTQD  
CH063\_1006 ---AGE---TEY---RGKTIGTFVGCFCGED--W---LETL-----TRDSEV  
GLRG\_07171 ---AGE---TDY---RGKTIGTFVGCFCGED--W---LETL-----TKDSEV  
CS\_22 ---AGE---TAY---RGKTVGTFVGCFCGED--W---LETL-----TRDSEV  
CS\_14 ---AGE---AQF---RGKKIGCVYFGNMNED--W---GEMM-----NRDPLW  
CS\_26 ---AGE---TNY---RGGLIGCVVGSFGED--W---CEMF-----AKDVQQ  
GLRG\_11435 ---AGE---TNY---RGGLIGCVVGSFGED--W---CEMF-----AKDVQQ  
GLRG\_08640 ---AGI---TLERI---SGSNTAVFVGSIMAD--YHTNVEQMS-----TWNTDF  
GLRG\_10827 ---GGI---SLDAI---AGSRTGVFAASFTAD--W---QQMS-----MKEPSF  
GLRG\_11425 ---AGV-----TLELYSRLPTDD-----VQEPSF  
CH063\_0013 ---AGL---TLDQL---SGSDTACFMATFTAD--F---QQMS-----FKEPSF  
GLRG\_08482 ---AGE---TWDSV---SGSRTGVFVGNFCLD--H---WMIQ-----SRDWDN  
CH063\_1130 ---SGT---TMESL---SGSSTGVFVANFSVD--F---QPMQ-----ARPDY  
GLRG\_03511 ---AGI---PIEDL---RGSDTSVHMAMFARD--Y---DRMG-----YRDTSQ  
CS\_18 ---AGQ---TIAGI---KGSDTSVHVGAYATDCSF---ERMG-----YKDTAR  
GLRG\_05714 ---AGQ---TIAGI---KGSDTSVHVGAYATD--F---ERMG-----YKDTAR  
CH063\_0922 -----GNA---AGSDTSVVFSTFGAD--Y---TDLL-----QKDPDT  
CH063\_1282 ---AGW---TLRDV---AGRNVAVFAAHQGST--Y---AGHA-----AEDLLT  
CS\_28 ---AGL---QLDDV---KGSKTSVFAAVFTDD--V---RSIL-----QEDPDL  
GLRG\_11563 ---AGL---RLEDV---KGSKTSVFAAQFTDD--V---RSIL-----QEDPDL  
C.P.NRPS\_6 ---SGT---PIDKF---QGSRCVVFVGVMTGD--Y---ETIQ-----YRDTE  
CH063\_0617 ---AGV---SVDRL---RGSRCVVFVGVMTGD--Y---ETIQ-----YRDTE  
C.P.NRPS\_1 ---AHV---SMSQL---KGSDTAVYVGLMCGD--Y---EYIL-----NNDLDT  
GLRG\_09842 ---ADV---STPQL---KGSDTAVYVGLMCGD--Y---ENIL-----LRDIDS  
GLRG\_09715 ---AGY---AMSDL---RGSPTGVYVGMQMTDD--Y---YDIL-----NKDVQC  
C.P.NRPS\_8 ---AGQ---TMEAL---RGSDTGCVYVGM MISE--Y---EQSM-----MRDPES  
GLRG\_10367 ---AGQ---TMEAL---RGSDTGCVYVGM MISE--Y---EQSM-----MRDPES  
CS/1 ---AGI---PAEDL---RGSNTGVYVGLMSND--Y---ESLM-----LRDVT  
CH063\_0489 ---GGF---TLSSL---QNSDTAVYVGM MCTD--Y---NITL-----GLDSTF  
CH063\_0392 ---AGL---PMEGM---AGSQTAVYVGM M WCD--Y---NDHL-----QRDMDS  
GLRG\_07434 ---AGL---TIEGL---KGSNTGVYVGM MYVD--Y---ESLQ-----FRDLQH  
CS/20 ---AGI---PIEDI---KGSKTGVFVGNMGGD--Y---SELL-----GQDLDA  
CH063\_0306 ---AGI---TIQHL---KGSKAGVFGVGNMGGD--Y---SELL-----GQDLDA  
CH030B ---AGL---SIKQL---QGSDTAVYVGM MSAD--Y---TEML-----ARDVEK  
CS\_30 ---AGL---SVKQL---QGSDTAVYVGM MSAD--F---TDML-----ARDIEK  
CS\_29 ---AGL---VQDL---QGSDTAVYVGLMTHD--F---ETIK-----TQDMHH  
CH063\_0199 ---AGM---RMQDL---QGSSTAVYVGM MTHD--Y---ETTS-----TRDLES  
C.P.NRPS\_2 ---AGL---SLQGM---KGSSTSVYVGM IMSAD--Y---TETQ-----LRDPES  
CH063\_0288 ---AGY---NIEKY---AGRKVAVFAGLMTAD--Y---DTLC-----QRDDIT  
CS\_4 ---AGY---NLERY---AGRKVAVFAGLMTAD--Y---DTLC-----QRDDIT  
GLRG\_08212 ---AGY---NLERY---AGRKVAVFAGLMTAD--Y---DTLC-----QRDDIT  
CS\_15 VLTLAGL--RMEDL---RGSPTAVYVGSFVKG--Q---SAPCCILITPAETRQTT  
CH063\_1445 ---AGL---RMEDL---RGSPTAVYVGSFVKD--Y---EQVC-----LRDMDW  
GLRG\_02583 ---AGL---RMEDL---RGSPTAVYVGSFVKD--Y---EQIC-----LRDMDW  
CS\_32 ---AGI---PKHTI---VGKDVGVFVGG SFAE--Y---ESHL-----FRSDT  
GLRG\_03360 ---AGI---PKHTI---VGKDVGVFVGG SFAE--Y---ESHL-----FRSDT  
CH063\_1222 ---AGI---PKHTI---IGKDVGVFVGG SFAE--Y---ESHL-----FRSDT  
CH063\_0134 ---AGV---PLQKL---AGSDTSVVFVGSFSRD--Y---HDSL-----LRDPDT  
CS\_21 ---AGI---PMSAI---AGSGTSVFAGV FVHD--Y---RDGL-----LRDADN  
GLRG\_09267 ---AGL---PISTI---AGSNASVFTGV FVHD--Y---RDGL-----LRDADN  
CH0190 ---AGI---PLESA---AGTNTAVFGGIMYKD--Y---HDSL-----NRDPKS  
CH063\_0873 ---AGI---KLPDI---QGSDTSVFAGCMVRD--Y---HDTL-----ARDPHT  
CS\_9 ---AGY---TMADM---DGSALTGVYAGAFMWD--F---RDLL-----IKDQDV  
CH063\_1490 ---AGI---PLDTV---AGSRTGVYVGCFTAD--Y---NDHI-----TKDLDI  
GLRG\_01860 ---AGI---PLDTV---AGSRTGVYVGCFTAD--Y---NDHI-----AKDLDI  
CS\_25 KKKRAGI--PLDTV---AGSRTGVYVGCFTAD--Y---NDHI-----SKDLDI



CS\_34 ---SGL---PPHRV----AGTRTACFMGAAANMAEY---KDGI-----VRDFGN  
 GLRG\_11770 ---AGL---TMAGV----SGSRTGCVYVGFSSCD--Y---RDSI-----LRDTET  
 CH0399 ---AGL---PIDVV----AGTKTGCFMGSAAAD--Y---RDTI-----NRDPDS  
 CS\_11 ---AGM---SLQKV----AGTQTACYMGTSMSD--Y---RDSI-----VRDFGN  
 GLRG\_11840 ---AGM---SLQKV----AGTQTACYMGTSMSD--Y---RDSI-----VRDFGN  
 CH063\_0781 ---AGL---PLPRV----AGTRTACIIGTAWSD--Y---RDAL-----VRDFEQ  
 CH063\_1357 ---AGL---RLEDV----SGTQTSCYIGTFTND--F---VNLQ-----AQSNEA  
 GLRG\_10317 ---AGL---RLEDV----SGTQTSCYIGTFTND--F---VNLQ-----AQSNEA  
 CH063\_1471 ---AGI---AMENV----DGSNTSCFVGCFTRD--Y---SDML-----ACDRED  
 CH063\_0506 ---AGI---SIPNV----TGSNTSVFVACFGRD--Y---DASI-----ARDVES  
 GLRG\_11835 ---AGY---PIDKI----AGTNTSVFCASFGRD--N---EAIV-----SRDPEF  
 CS\_3 ---AGI---AMECA----AGSKTSVFCASFGRD--S---DAIV-----ARDPAF  
 GLRG\_11878 ---AGI---AMECA----AGSKTSVFCASFGRD--S---DAIV-----ARDPAF  
 CH063\_0051 ---AGI---PMQKL----AKSTTAVYSGAMTND--Y---QMLA-----EADPYR  
 CH0135 ---AGV---LMDDL----MNSQTGVYVGCMTND--Y---EQLS-----THDIYD  
 CS\_43 ---SGY---YHTSQ----PDSRVGCVYGVVGS--Y-----ENNISH  
 GLRG\_09268 ---SGY---YHKPQ----PDSRVGCVYGVVGS--Y-----EN-----NISHTS  
 GLRG\_11857 ---AGY---FNSKQ---DETNGRVGCFVGCSTCD--Y-----EQ-----NIACHD  
 CH063\_0213 ---SGY---FNAVS---PERSVGCYIGLRGND--Y-----YDNVAC  
 CH063\_0477 ---SGY---LRHHR---RERGDVPGCFIGASYTE--Y-----LE-----NTSAYT  
 CH063\_0313 ---SGY---HSADSRV---DPTDYGCYIGAVMNN--Y-----ASNVSC  
 CS\_44 ---AGY---LGADA---RSDCTDYGCYIGAVTNN--Y-----VT-----NVSCHP  
 GLRG\_11575 ---SGQ---LGADA---LSDCSYDGCYIGAVMNN--Y-----VTNVSC  
 CH063\_1347 ---AGYAGGAGTDGDGIKSTGPNVGCYLGVSVD--Y-----DA-----NVAARD  
 C.PKS ---AGF---FGPRN----PDVDVGCFIGVCASD--Y-----ND-----NVASHP  
 CS\_45 ---AGY---FSRGG----QPRDVGCVYIGVAASD--Y---EDNV-----ASHAPT  
 GLRG\_11798 ---AGY---FSRGG----QPRDVGCVYIGVAASD--Y---ED-----NVASHA  
 GLRG\_11778 ---AGY---SRRPH---TKTAACRFATFVGIADD--W-----AEITRL  
 CH0398 ---AGY---GRGRF---ASPHDTRIATFFGQASED--W---RDIM-----HISG  
 CS\_37 ---AGYSNGKTKST---DPNRIATFYGQVTDD--W---HDQ-----SHPTLG  
 GLRG\_11836 ---AGYSDGKTKST---DPNRIATFYGQVTDD--W---HDQ-----SHPTLG  
 CH063\_1255 ---AGYSCGTTRAV----DPSRISVLFQSNVD--GY---ET-----AHHEKG  
 CS\_40 ---SGYSDGPTRTV----DPARIAVVYQGSNED-----GYMT-----SHHERG  
 CS\_38 ---SGY---VPNRT--RSTAGPRIGTYGQASDD--W---RE-----LNASQN  
 CS\_36 ---AGF---VPNRT--PSSHTTRVGTYYGQASDD--Y---RE-----VNASQK  
 GLRG\_08632 ---SGY---VPNRT--PSTILERVGTIFYGQASDD--W---RE-----LNAAQN  
 CH063\_0351 ---CGY---VPNRT--PSTRLDRIPTYGQTSDD--W---RE-----INAAQE  
 CS\_35 ---CGY---VPNRT--PSTKLDRIGTFYGQTSDD--W---RE-----INAAQE  
 GLRG\_04203 ---CGY---VPNRT--PSTKLDRIGTFYGQTSDD--W---RE-----INAAQE  
 GLRG\_11956 ---AGI---IPDAT--PSTRRDRVGVVYGVTSND--W---MET-----NS-AQN  
 CH063\_1223 ---AGI---VPDRT--PSTMHRVGVVWYGSCSMD--W---LE-----TNSAQD  
 GLRG\_08620 ---AGI---VPDRT--PSTQKHRVGVWFGTSND--W---ME-----TNSAQS  
 CH063\_0250 ---AGI---VPNRT--ASTQKHRVGVWFGATSND--W---ME-----TNSAQD  
 CS\_39 ---AGY---SPGTT--SSTDGRRVGTFFGQTTDD--W---RE-----YNVRDD  
 GLRG\_03507 ---AGY---RYDAK---PDRGNVGSFVGLTTDD--W---RE-----YNISQE  
 GLRG\_03509 ---AGY---RYDAK---PDRGNVGSFVGLTTDD--W---RE-----YNISQE

GLRG\_10537 RS--PGH---GLGIARTILANRISHFLNIHGP-----S---  
 CS\_23 RA--DSI---TIGLASSILSNRVSHFLNVTGP-----S---  
 CH063\_1191 LN--QVR---PDVYGDYILANRASIEFDLTGP-----S---  
 CS\_16 LN--PVR---PDVYGDYILANRASIEFDLTGP-----S---  
 GLRG\_06162 LN--AIR---PDVYGDYILANRASIEFDLTGP-----S---  
 GLRG\_09075 QGGLGYV---ATGNGDLMLSNRVSYEYDLRGP-----S---  
 CH063\_1187 AG--GYL---LTGHGDLMIANRASIEYDLKGP-----S---  
 CH063\_1411 AG--GYL---LTGHGDLMIANRASIEYDLKGP-----S---  
 CS\_5 FD--HY----PAGKWDFMLSNRISYVYDFRGP-----S---  
 GLRG\_11893 FD--HY----PAGKWDFMLSNRISYVYDFRGP-----S---  
 CS\_19 SG--VYR---GSGYLDFFQANRISYEWNTGP-----S---  
 CH063\_0663 SG--LYR---GSGYLDLQPNRVSYEYGWGTGP-----S---  
 GLRG\_00918 AG--LYR---GTGFLDFLQANRVSFAMGWQGP-----S---  
 CH063\_0821 -----S---

CS\_13 PG--GYR---LTGYMDYALSNRISVLDLRGP-----S---  
 CH063\_0053 DG--MYR---LTGYMDFVQANRISHAFDLRGP-----S---  
 CH063\_1006 VG--QYK---ITGYGDFMLSNRLAYEYDLKGP-----S---  
 GLRG\_07171 VG--QYK---ITGYGDFMLSNRLAYEYDLKGP-----S---  
 CS\_22 VG--QYK---ITGYGDFMLSNRLAYEYDLKGP-----S---  
 CS\_14 HG--PNK---IDGYQDWMLANRISYEFGLTGP-----S---  
 CS\_26 HG--LYR---VAGYGDFMLSNNVAYEMDLKGPVVAHPRSFLLVHISQTNQDLSS----  
 GLRG\_11435 HG--LYR---VAGYGDFMLSNNVAYEMDLKGP-----S---  
 GLRG\_08640 CH--NCA---ATGVDIRIISNRIGHMFNLRGP-----S---  
 GLRG\_10827 RH--TMA---ATGVDPGILSDRISYVFNLRGP-----S---  
 GLRG\_11425 RH--ALA---ATGVDPGIISNRISHAFNLKGT-----S---  
 CH063\_0013 RH--SLA---ATGVDPGLLSNRVSHIFNLRGP-----S---  
 GLRG\_08482 PR--PYA---FTGAGTSILANRISYIFNLQGP-----S---  
 CH063\_1130 IH--RYQ---ASGSGATVMSNRISHVNLQGP-----R---  
 GLRG\_03511 MH--KQH---STGSGDAILANRISYLLDLKGT-----S---  
 CS\_18 TP--KAH---MIGTGIAILSNRISYVFDLHGP-----S---  
 GLRG\_05714 TP--KAH---MIGTGIAILSNRISYVFDLHGP-----S---  
 CH063\_0922 MP--VYQ---ATNSGYRAIISNRLSHFFDFRGS-----S---  
 CH063\_1282 TS--AYS---ASGTAGCMLANRISYLFDLRGP-----S---  
 CS\_28 SV--KYK---PIGTSAAILAAARVSWFYDLRGS-----S---  
 GLRG\_11563 SV--KYK---PIGTSAAILAAARVSWFYDLRGA-----S---  
 C.P.NRPS\_6 LS--QYA---ASGTSRAILANRISYFFDLSGS-----S---  
 CH063\_0617 LS--QYT---ASGTSRAILANRISYFFDLAGS-----S---  
 C.P.NRPS\_1 AP--RYQ---ATGIARSIISNRISYIWDLHGP-----S---  
 GLRG\_09842 AP--RYQ---ATGVGRSIMANRISYIWDLHGP-----S---  
 GLRG\_09715 AP--QYT---ATGSSRAIIANRVSYFFDWRGP-----S---  
 C.P.NRPS\_8 TG--TYH---ILGTARSLMSNRLSYFFDWHGP-----S---  
 GLRG\_10367 TG--MYH---ILGTARSLMSNRLSYFFDWHGP-----S---  
 CS/1 MA--NYH---AVGTQRSILANRISYFYDWHGP-----S---  
 CH063\_0489 IP--QYT---ATGVSPSNASSRISYFYNWHGP-----S---  
 CH063\_0392 LP--TYA---GVGTARSILSNRISHFFDWHGP-----S---  
 GLRG\_07434 VP--TYL---AIGTARSIVSNRISYFFDWHGP-----S---  
 CS/20 FP--TYF---APGTARSILSNRISYFFDLHGP-----S---  
 CH063\_0306 FP--TYF---APGTARSILSNRISYFFDLHGP-----S---  
 CH030B YP--TYF---ATGTARSILSNRLSYAFDWRGP-----S---  
 CS\_30 FP--TYF---ATGTARSILSNRLSYFFDWHGP-----S---  
 CS\_29 MP--TYF---ATGVAASIASNRLSYVFDWHGP-----SRLIL  
 CH063\_0199 IP--TYS---ATGVAVSIASNRISYFFDWHGP-----S---  
 C.P.NRPS\_2 VS--QYW---VTGSSRALTSNRLSYFFDWRGP-----S---  
 CH063\_0288 SN--QYF---ATGNSRAILSNRISYFFNFRGP-----S---  
 CS\_4 AN--QYF---ATGNSRAILSNRISYFFNFRGP-----S---  
 GLRG\_08212 SN--QYF---ATGNSRAILSNRISYFFNFRGP-----S---  
 CS\_15 RF--AYAIWTGSPSTGNAIMSNRVSYTYDFKGP-----S---  
 CH063\_1445 QP--QYA---ATGTGNAIMSNRVSYTYDFKGP-----S---  
 GLRG\_02583 QP--QYA---ATGTGNAIMSNRVSYTYDFKGP-----S---  
 CS\_32 IP--MHQ---ATGCAHAMQSNRLSHFFDLRGP-----S---  
 GLRG\_03360 IP--MHQ---ATGCAHAMQSNRLSHFFDLRGP-----S---  
 CH063\_1222 IP--MHQ---ATGCAHAMQSNRLSHFFDLRGP-----S---  
 CH063\_0134 LP--RTT---LTGNGVAMMSNRISHFFDLRGP-----S---  
 CS\_21 LP--RLM---ATGTGVPMMSNRVSHFFDLRGA-----S---  
 GLRG\_09267 LP--RLM---ATGTGVPMMSNRVSHFFDLRGA-----S---  
 CH0190 LP--RYF---ITGNAGAMVANRISHFFDLRGP-----S---  
 CH063\_0873 LP--RYF---MTGNAATMAANRVSHFYDLRGP-----S---  
 CS\_9 PM--TYT---ACGTIASTLAGRVSWFYGLRGP-----A---  
 CH063\_1490 PN--KYS---ALGTVASMLSNRVSWFFDFRGP-----S---  
 GLRG\_01860 PN--KYS---ALGTVASMLSNRVSWFFDFRGP-----S---  
 CS\_25 PN--KYS---ALGTVASMLSNRVSWFFDFRGP-----S---  
 CS\_34 QP--QHL---IMGISEELMSNRLSHFFDLHGP-----S---  
 GLRG\_11770 SP--RYT---DLGTHAEMLSNRTSWFYNLKGP-----S---  
 CH0399 NP--RYS---LIGVTTEMISNRTSWFYDLKGP-----S---  
 CS\_11 YP--KYH---LLGTSDEMISNRISHFLDIHGP-----S---  
 GLRG\_11840 YP--KYH---LLGTSDEMISNRISHFLDIHGP-----S---

CH063\_0781 WP--RLY---LMGVSDDEMVSNRLSHYFDLRGP-----S---  
 CH063\_1357 PS--IYH---ATGLSSSLASNRLSWFYNLKGP-----S---  
 GLRG\_10317 PS--IYH---ATGLSSSLASNRLSWFYNLKGP-----S---  
 CH063\_1471 LP--LYH---GTGTGSAIMS NRISWFFNMKGP-----S---  
 CH063\_0506 MS--RYH---ATGSGSSMLANRISHAFDLRGP-----S---  
 GLRG\_11835 QS--RYQ---GTSSGSTMLSNRVSYFYDLHGP-----S---  
 CS\_3 QS--RYQ---ATASGSSMLSNRISHFFDLRGP-----S---  
 GLRG\_11878 QS--RYQ---ATASGSSMLSNRISHFFDLRGP-----S---  
 CH063\_0051 LG--VNS---AAGTGKSMLSNRISWFFDLQGP-----S---  
 CH0135 IG--DVA---ASGMSEAMTANRVSWFFGLRGP-----S---  
 CS\_43 TSPNAFS---AMGALRCYIAGKVSHNFGWTGP-----S---  
 GLRG\_09268 PN--AFS---AMGALRCYIAGKVSHYFGWTGP-----S---  
 GLRG\_11857 AS--AFS---ATGQLRAFIAGKVSHYFGWTGP-----G---  
 CH063\_0213 HPPNAFT---ATGNLQAFVAGKISHQFDWTGP-----A---  
 CH063\_0477 PS--AFT---ATGTIRAFLSGKISYHFGWTGP-----S---  
 CH063\_0313 HPPTAYA---TMGTSRAYFSGAISHYFGWTGP-----A---  
 CS\_44 PT--AYA---TTGTGRSFLSGAVSHHFGWTGP-----A---  
 GLRG\_11575 HPPTAYA---TTGTGRSFLSGAVSHHFGWTGP-----A---  
 CH063\_1347 AN--AFA---ATGTLRAFISGR-----  
 C.PKS AN--AFS---ALGTLRAFVPGRISHYFGLTGP-----S---  
 CS\_45 ---AFS---VTGAVRAAFVSGKISHFFGLSGP-----S---  
 GLRG\_11798 PT--AFS---VTGAVRAAFVSGKISHFFGLNGP-----S---  
 GLRG\_11778 RGGDAFS---LTGIQRSFAAGRVNYHFKWAGP-----A---  
 CH0398 GD--AYS---LTGLQRSFGPGRVNYSMRWSP-----T---  
 CS\_37 CD--AYT---LPGVQRAFGPGRVAWQFNWEGP-----T---  
 GLRG\_11836 CD--PYT---LPGVQRAFGPGRVAWQFNWEGP-----T---  
 CH063\_1255 CD--AYT---LQALARPFAGGRVAFHYGWEGA-----T---  
 CS\_40 CD--AYT---LQTVQRAFAPGRVAHHFGWEGQ-----T---  
 CS\_38 IG--TYA---VPSGERGFANGRINYFFKFSGP-----S---  
 CS\_36 IG--TYG---IPGTERAFGNRINYFFNFQGP-----S---  
 GLRG\_08632 IG--THA---VPGGERAFANGRINYFFKFGGP-----S---  
 CH063\_0351 VD--TYY---ITGGVRAFGPGRINYHFGFSGP-----S---  
 CS\_35 VD--TYY---ITGGVRAFGPGRINYHFGFSGP-----S---  
 GLRG\_04203 VD--TYY---ITGGVRAFGPGRINYHFGFSGP-----S---  
 GLRG\_11956 ID--TYF---IPGGNRAFIPGRINYCFKFSGP-----S---  
 CH063\_1223 ID--AYF---IPGGARAFIAGRVNYFFKFTGP-----S---  
 GLRG\_08620 ID--TYF---IPGGNRAFIPGRINYHFKFRGP-----S---  
 CH063\_0250 ID--TYF---IPGGNRAFIPGRINYHFKFSGP-----S---  
 CS\_39 ID--MFY---VPGTIRAFGPGHLNYFKWDGP-----S---  
 GLRG\_03507 ID--MYF---VTGGLRSFGSGRNLNYFFKLEGP-----S---  
 GLRG\_03509 ID--MYF---VTGGLRSFGSGRNLNYFFKLEGP-----S---  
  
 GLRG\_10537 ----VTLDTA-----SSSLVGLDLACQALRARSIDTGIVAAANVY----  
 CS\_23 ----MTIDTAC-----SASLVSDVACRYLDSFQADGMIVGGANMW----  
 CH063\_1191 ----LVVRTAC-----SASMV ALHQACRDLHSGDCSSALVGGANLI----  
 CS\_16 ----VVVRTAC-----SASLV ALHQACQDLHSGDCSSALVGGVNLI----  
 GLRG\_06162 ----VVVRTAC-----SASLV ALHQACQDLHSGDCSSALVGGVNLI----  
 GLRG\_09075 ----MTIKTGC-----SASLAALHEAFRSIQNGDATGAIIGGTSLI----  
 CH063\_1187 ----MVIKTGC-----SASLVGLHEACRALQNGDCVGAIVGGVNLI----  
 CH063\_1411 ----MVIKTGC-----SASLVGLHEACRALQNGDCVGAIVGGVNLI----  
 CS\_5 ----MTIRTGC-----SGTLVALNLAEQAVKSGECSAIIAGCSMF----  
 GLRG\_11893 ----MTIRTGC-----SGTLVALNLAEQAVKSGECSAIIAGCSMF----  
 CS\_19 ----MLIKTGC-----SSSMVALHLAAEAVQSGSCTSAMALGCNLI----  
 CH063\_0663 ----MLVKTGC-----SSSMVALHLAAEAVQNGACKSAMALGCNLI----  
 GLRG\_00918 ----IVVKTGC-----SASLV ALDMACHSLRAGECSAAVVLGVNLI----  
 CH063\_0821 -----  
 CS\_13 ----MTVRAAC-----AASGLALHLACQAIRAGECDSAIVAGSNII----  
 CH063\_0053 ----MTVRTAC-----SAAGLAVHLACQAIRAGECDSAVVAGSNLM----  
 CH063\_1006 ----MTIRTGC-----SSALIGLHEACMSISHGQCDAAVVAGSNLI----  
 GLRG\_07171 ----MTIRTGC-----SSALIGLHEACMSIHHGQCNAALVAGSNLI----  
 CS\_22 ----MTIRTGC-----SSALIGLHEACMSIHHGQCNAALVAGSNLI----

CS\_14 ----MTIRTAC-----SSALTCVNEACAAIQRGVCDAAVVAGGNLM----  
 CS\_26 ----MTIRTGC-----SAALVGLHEACTAIQRGDCTSAIVGGVNLI----  
 GLRG\_11435 ----MTLRTGC-----SAALVGLHEACTAIQRGDCTSAIVGGVNLI----  
 GLRG\_08640 ----FTINTAC-----SSSIYTINDACRALRMRDCDAIITAGFNLI----  
 GLRG\_10827 ----IVCNTAC-----SSSIYALHNACIALRNDECDGAVVGGTNLV----  
 GLRG\_11425 ----IVVNIAC-----SSSVYAVHNACNALRNKECQAAVFCGVNLI----  
 CH063\_0013 ----IVVNTAC-----SSSVYALHNACNALRTHECSAAVVGGSNLI----  
 GLRG\_08482 ----LTVDTAC-----SSSMYALHLAVNSIRAGDCDAAIVASANWI----  
 CH063\_1130 -----YAGDCESAIVASANLI----  
 GLRG\_03511 ----NTLDTGC-----SGGLVAVHQACRTLKANEASIALAGASQLL----  
 CS\_18 ----STVDTGC-----SGSMVALHQACHGLRARESKMAIVAGTQLV----  
 GLRG\_05714 ----STVDTGC-----SGSMVALHQACHGLRARESKMAIVAGTQLV----  
 CH063\_0922 ----VTIDTAC-----SGGLTALHLACQSIRAGEVVRQAVCGGSSVI----  
 CH063\_1282 -----AASVRLGECEAALVSAANLL----  
 CS\_28 ----FTLDTAC-----SGSIVALHTGAQDLRAGLSEMSIITGVNII----  
 GLRG\_11563 ----FTLDTAC-----SGSIVALHTGAQDLRAGLSDMSIITGVNII----  
 C.P.NRPS\_6 ----ICLDTAC-----SSSLVAMHLAVQDLRNGTAETAIVAGANLI----  
 CH063\_0617 ----VCLDTAC-----SSSLVAMHLAVQ-LRGGGAADTAVVAGTNLI----  
 C.P.NRPS\_1 ----MTIDTAC-----SSSLVALHQAVQALRLGETNLAVVAGSNLL----  
 GLRG\_09842 ----MTIDTAC-----SSSLVALHQAVQALRLGETQLAVVAGSNLL----  
 GLRG\_09715 ----VNIDTAC-----SSSLVALHQAVQALRGGEIDLAVAAGVNLI----  
 C.P.NRPS\_8 ----MTIDTAC-----SSSLVAVHQAVQLLRSGESRVAIAAGSNMI----  
 GLRG\_10367 ----MTIDTAC-----SSSLVAVHQAVQLLRSGESRVAIAAGSNMI----  
 CS/1 ----MVVDTAC-----SSSLVALHLAVQALRTGEARTALACGSNLL----  
 CH063\_0489 ----MTIDTAC-----SSSLVAVHQAVDQLRTGRSRVAVACGTNLM----  
 CH063\_0392 ----ATIDTAC-----SSSLVAVYNAVMTLRNGDAPVAVVAGANII----  
 GLRG\_07434 ----LTVDTAC-----SSSLVAVHQAVQALRSGEVRVALAAGSNLL----  
 CS/20 ----VTIDTAC-----SSSLVAVHQAVQSLRLGETPVAIACGTNLL----  
 CH063\_0306 ----VTVDTAC-----SSSLVAVHQAVQSLRVGETPVAIACGTNLL----  
 CH030B ----MTIDTAC-----SSSLIAMHQAVQALRSGESKVAIAAGTNLL----  
 CS\_30 ----MTIDTAC-----SSSLIALHQAVQVLRSHQSKVAVAAGSNLI----  
 CS\_29 TSHPQMTIDTAC-----SSSLVAVHHAQQQLRSGQSRVAVAAGVNMI----  
 CH063\_0199 ----MTIDTAC-----SSSLAAVHLAVQQLRSGQSTMAVAAGANLI----  
 C.P.NRPS\_2 ----MTIDTAC-----SSSMAALHLAVQSLRNGECQVSCVAGANLL----  
 CH063\_0288 ----MTIDTAC-----SSSLVALHQAVLSLRSGECEMACVAGANLM----  
 CS\_4 ----MTIDTAC-----SSSLVALHQAVLSLRSGECEMACVAGANMM----  
 GLRG\_08212 ----MTVDTAC-----SSSLVALHQAVLSLRSGECEMACVAGANMM----  
 CS\_15 ----MTIDTGC-----SGSLVAVHLAAQALRAGDCSLALAAGAGLI----  
 CH063\_1445 ----MTIDTGC-----SGSLVAVHLAAQALRAGDCSLALAAGAGLI----  
 GLRG\_02583 ----MTIDTGC-----SGSLVAVHLAAQALRAGDCSLALAAGAGLI----  
 CS\_32 ----FTTDTAC-----SASLVALHLACQSLRAGESTTAIVGGCHLN----  
 GLRG\_03360 ----FTADTAC-----SASLVALHLACQSLRAGESTTAIVGGCHLN----  
 CH063\_1222 ----FTADTAC-----SASLVALHLACQSLRAGESSTAIVGGCHLN----  
 CH063\_0134 ----LTTDTGC-----SASLAALHLAVNSIRNGESRMAIVGASNLL----  
 CS\_21 ----MTIETAC-----SSGMVALHQGIQSLRTGEADMSIIGGANLT----  
 GLRG\_09267 ----MTIETAC-----SSGMVALHQGIQSLRTGEADMSIVGGANLT----  
 CH0190 ----VTIDTAC-----STTLTALHLACQSLRAGESDMAVVVGANLM----  
 CH063\_0873 ----MTIDTGC-----STTLTALHLACRSLQAGESGSAIVTGANLM----  
 CS\_9 ----LSVDTAC-----SSSMVALHQAVVGLKSRDCNIAVACGTNVL----  
 CH063\_1490 ----VTVDTAC-----SSSLVAVHEACMSLKLREISMV-----  
 GLRG\_01860 ----VTVDTAC-----SSSLVAVHEACMSLKLREISMAVVGGCNLI----  
 CS\_25 ----VTVDTAC-----SSSLVALHEACMSLKLREIPMAVVGGCNLI----  
 CS\_34 ----ATVETAC-----SSSLVAVHLACQSLRAGESNMAIAGGVNLL----  
 GLRG\_11770 ----MTVSTAC-----SSSLVAVHMACQSLLAGETDLAVAGGVNLM----  
 CH0399 ----ITLTAC-----SSSLVAVHLACQSVLSGESTMAVAGGVNLM----  
 CS\_11 ----ATIETAC-----SSSHVATHLACQSLQSGESEMAIAGGVGMT----  
 GLRG\_11840 ----ATIETAC-----SSSHVATHLACQSLQSGESEMAIAGGVGMT----  
 CH063\_0781 ----LTVETAC-----SSSLVAIHQACESLRSGQAEMAVAGGINMI----  
 CH063\_1357 ----ITIDTAC-----SSSLTAFHLACQSIRSGESEMSIVAGANLM----  
 GLRG\_10317 ----VTVDTAC-----SSSLTAFHLGCQSIRTGEAEMSIVAGANLM----  
 CH063\_1471 ----ISLDTAC-----SSSMAALHLGCQSLRTGETTMSVVGGTNLM----  
 CH063\_0506 ----ITVDTAC-----SAGLSAFHLACQSVRSGESDLSLVCGGNTY----

GLRG\_11835 ----ITVDTAC-----SSGLYALHLGCQSVLSGESDMSLICGANVY----  
 CS\_3 ----LTVDTAC-----SSGLYAVHLAYQSILAGESDMSLVCGSNTY----  
 GLRG\_11878 ----LTVDTAC-----SSGLYAVHLACQSIMAGESNMSLVCGSNTY----  
 CH063\_0051 ----LTVDTAC-----SSSLYAVHLACQSIRTGESDQALVTGHNLI----  
 CH0135 ----LTLDTAC-----SSSLYALHLACQSLKSGETKMGLVAGVNLI----  
 CS\_43 ----MTVDTAC-----SASTIAMNLACQAIKSGDCSAALVGGTNFC----  
 GLRG\_09268 ----MTLDTAC-----SASTIAMNLACQAITSGDCSAALVGGTNFC----  
 GLRG\_11857 ----VTIDTAC-----SSSAVAVHQACQAILAGDCDAALAGGAFVM----  
 CH063\_0213 ----MTIDTAC-----SSSLVAIHQACRAIIAGDCNAALAGGAHIM----  
 CH063\_0477 ----EVIDTAC-----SASLVAVHRACRAIAAGECPVALAGGVNII----  
 CH063\_0313 ----TTIDTAC-----SSSLVAIHTACRAIAAGECSRAVAGGTNVI----  
 CS\_44 ----LTIDTAC-----SSSLVAIHMACRAIATGECSRAVAGGANII----  
 GLRG\_11575 ----LTVDTAC-----SSSLVAIHMACRAIATGECSRAVAGGTNII----  
 CH063\_1347 -----  
 C.PKS ----VALDTAC-----SSSAVAIDAACKAILQGDCCKSALAGGVSI----  
 CS\_45 ----LVFDTAC-----SSSAVAIHTACQAIRSGDCSMALAGGVNVI----  
 GLRG\_11798 ----LVFDTAC-----SSSAVAIHTACQAIRSGDCSMALAGGVNVI----  
 GLRG\_11778 ----VTLDTAC-----SSSMTALDQACTFLLSGQCDMAVAGGTNII----  
 CH0398 ----YSVDSAC-----ASSLSSVNLACSSLLARDCDMAVAGAANLI----  
 CS\_37 ----YALDSAC-----ASTTSCVHLACMSLQTGDIDMAVAGAGNII----  
 GLRG\_11836 ----YALDSAC-----ASTTSCVHLACMSLQTGDIDMAVAGAGNII----  
 CH063\_1255 ----YSVDQAC-----STSLSLIHLACRDLLAGDHDMVVAGAANVL----  
 CS\_40 ----WSVDSAC-----STSSSILHLATRLLASGEVDMVVAGASNVI----  
 CS\_38 ----FNMDTACLSLHSYLPFRSRGSSGLAAVNAACSALWAGEVDTALAGGLNVI----  
 CS\_36 ----FNVDTAC-----SSGLAAVHMACSALWAGEADTVVAGGLNIIGRDII----  
 GLRG\_08632 ----FNIDTAC-----SSGLAAVNAACSALWAGEADTVIAGGLSVI----  
 CH063\_0351 ----LNIDTAC-----SSSAAALNVACNSLWVKDCDTAIVGGLSCM----  
 CS\_35 ----LNIDTAC-----SSSAAALNVACNALWVKDCDTAIVGGLSCM----  
 GLRG\_04203 ----LNIDTAC-----SSSAAALNVACNALWVKDCDTAIVGGLSCM----  
 GLRG\_11956 ----YAVDTAC-----SSSLAGIHLACNALWRGEIDTAIAGGTNVL----  
 CH063\_1223 ----FTVDTAC-----SSSLAALHLACNALWRGEVDTAV-----  
 GLRG\_08620 ----YTIDTAC-----SSSLAALHLACNALWGGEVDTAIVGGTNII----  
 CH063\_0250 ----YTIDTAC-----SSSLAALHLACNALWRGEVDTAIVGGTNVL----  
 CS\_39 ----YSFDTAC-----SSSSVAVQMACASLLARDCDLAVAGGLNII----  
 GLRG\_03507 ----YVLDTAC-----SSSAAASIELACASLLGRDCDMALAGGANVM----  
 GLRG\_03509 ----YVLDTAC-----SSSAAASIELACASLLGRDCDMALAGGANVM----  
  
 GLRG\_10537 ----MSPEHLI-DAGGVGGAHSLTALCHTFDAAAD--GYVKA EAVNTV IIKRLSDA---  
 CS\_23 ----LTPHEHNE-EVGMNITQSGSGRCHSFDADKAD--GHVKA EGINAVYLKRLDDA---  
 CH063\_1191 ----LTPKDTT-VMHQNG-VLSPSGSCKSFADADAD--GFARGE GVS AIYIKKLSDAVRD  
 CS\_16 ----LTPKETV-AMHQNG-VLSSGGSCKSFADADAD--GFVRGEGVSAIYIKKLSDACTA  
 GLRG\_06162 ----LTPKETA-SMHQNG-VLSPSGSCKSFADADAD--GFARGE GVS AIYIKKLSDAVRD  
 GLRG\_09075 ----MTPPTTA-AFTSEG-ILSPDGSKSFADADAD--GFARAEAINAIYIKPLSSAIRD  
 CH063\_1187 ----MGPTTTA-AMTEEG-MLSPEGSCKTFDAAAD--GYGRGEAINAVYIKLLDDAIRD  
 CH063\_1411 ----MGPTTTA-AMTEEG-MLSPEGSCKTFDAAAD--GYGRGEAINAVYIKLLDDAIRD  
 CS\_5 ----LSGPIATKGSFGTVGATVSPQGICKTFDADCD--GLGRGEAVNAIYVKRLSHA---  
 GLRG\_11893 ----LSGPIATKGSFGTVGATVSPQGICKTFDADCD--GLGRGEAVNAIYVKRLSHAVRD  
 CS\_19 ----TSVLTSTV-IFTETG-VLSPSGKCKTFDILLAD--GYGRGEAVNAVYVKKLSKA---  
 CH063\_0663 ----TSVITSI-VFTETG-VLSPSGKCKTFDILLAD--GYGRGEAVNAVYVKKLSDALRD  
 GLRG\_00918 ----TCPLMTL-LYTAQG-LLSPSGICKTFDAAAD--GYGRGEAVNAVFLRRISDAKRT  
 CH063\_0821 -----  
 CS\_13 ----LSPDFGC-FMAEHG-ILSPDASCRTFDAQAN--GYARAEAVNCVFLKSLDSA---  
 CH063\_0053 ----LSPGFTK-LMAEQS-VLSPDASCKTFDAGAN--GYARAEAVNCLFVKRLHCAIRD  
 CH063\_1006 ----MAPGLYV-SMSEQG-VLSPSGSCKTFDAGAD--GYARGEAVNVVYVKRLSDA---  
 GLRG\_07171 ----MAPGLYA-SMSEQG-VLSPNGSCKTFDAGAD--GYARGEAVNVVYVKRLSDALRD  
 CS\_22 ----MAPGLYV-SMYEQG-VLSPNGSCKTFDARAD--GYARGEAVNVVYVKRLSDA---  
 CS\_14 ----LAPGQTQ-QMTEKG-ILSPEGSCKTFSDADAD--GYARGEAVTAVFLKPLDAA---  
 CS\_26 ----LAPGMTQ-AMTEQG-VLSPDGSKTFSDADAN--GYGRGEAVCAVYVKPLTAA---  
 GLRG\_11435 ----LAPGMTQ-AMTEQG-VLSPDGSKTFSDADAN--GYGRGEAVCAVYVKPLEAAIRD  
 GLRG\_08640 ----KKDDQHM-NTAKLG-ILSPKFHMPHIRFCGWLRYGKVEGAGTLFLKRLLLDA---  
 GLRG\_10827 ----FTIDQQM-NTAKIG-VLSPRGTCFTFDESAD--GYGRAEGVGAVYVKRLSDAVRD

GLRG\_11425 ----LAVDQHM-----NTGCCAVYLKRLSDAIRD  
 CH063\_0013 ----LTVDQHM-NTAKLG-VLSPTSTCHTFNSYAN--GYGRAEGVGAIYKRLSDAIRD  
 GLRG\_08482 ----ADPGVQI-ALDKLG-ALSASSRCHTFDARAE--GYARGEGYGAIYKRPSSLAVAD  
 CH063\_1130 ----MSPEPHI-GAAKSG-VLSPTGTCHTFDSSAD--GYGRAEGVNAIYVKRLSAAALRD  
 GLRG\_03511 ----LTPDQ---SMTMFN-LTNKDGRCYTFDDRGA--GYARGEGLGVVLKRLLEQAIAD  
 CS\_18 ----LTPDQII-PMSSVG-MTNPDKGKCYVFSRGS--GYARGEGVVTLILKRLDDA---  
 GLRG\_05714 ----LTPDQII-PMSSVG-MTNPDKGKCYVFSRGS--GYARGEGVVTLILKRLDDAVKD  
 CH063\_0922 ----LHPDQFI-SLSAM-----  
 CH063\_1282 ----NGPELWS-MLDTVG-VLSPEGKCFYSYDHRAS--GFGRGEGSACLVVKPLAAALAD  
 CS\_28 ----ESPEFMF-RASGLG-MVSPDGKCYSLDARAN--GYGRGEGVGTLLILKPVSA---  
 GLRG\_11563 ----ESPEFMF-RASGLG-MVSPDGKCYSLDARAN--GYGRGEGVGTLLILKPVSAAIRD  
 C.P.NRPS\_6 ----FGPDYMI-SESKLR-MLSPGTGKQMWADAMAD--GYARGEGVAALLKPLSKALRD  
 CH063\_0617 ----FGPDYMI-SESKLR-MLSPGTGKQMWADAMAD--GYARGEGVAALLKPLSKAVRD  
 C.P.NRPS\_1 ----LGPECYI-TESNLN-MLSPNGTSRMWDADAEAN--GYARGEGVAAVVLKRLSDAVAD  
 GLRG\_09842 ----LGPEWYV-TESNLN-MLSPGTGSRMWDADAN--GYARGEGVAAVVLKRLSDAVTD  
 GLRG\_09715 ----LGPEKYI-YESQLS-MLSPGTGSRMWDSEAD--GYARGEGFAAVIHKRLSQAIAID  
 C.P.NRPS\_8 ----MDPVTYV-GESKLQ-MLSPDGRGRMWDADVN--GYARGEGVATIVMKRLCDAVAD  
 GLRG\_10367 ----MDPVTYV-GESKLQ-MLSPDGRGRMWDADVN--GYARGEGVATIVMKRLCDAVAD  
 CS/1 ----LGPENFV-GYSKMK-MLSPDGKSMTWDRDAN--GYARGEGVATVVLKTLSSAALAD  
 CH063\_0489 ----INVSPYL-TESKLN-MLSPGTGSRMWDADAD--GYARGDGIATVVLKRLSDAIAID  
 CH063\_0392 ----LTPEPYI-GESKLR-MLSPDSRSMWDEDAD--GYARGDGVAAALVLKPLSAAVAD  
 GLRG\_07434 ----LGPEPYI-HESKLR-MLSPDGRSRMWDQGAN--GYARGDGVAAAVVLKTLSSAALAD  
 CS/20 ----LGPSQYI-AESKLQ-MLSPNGRSMWADASAD--GYARGEGFASVVLKTLSSAALAD  
 CH063\_0306 ----LGPSQYI-AESKLQ-MLSPNGRSMWADASAD--GYARGEGFASVVLKTLSSAALAD  
 CH030B ----LGPEQYI-AESKLQ-MLSPGSRSMWADADAD--GYARGEGVAIVMKPLSQALAD  
 CS\_30 ----LGPEQYI-AESKLQ-MLSPAGSRSMWADAEAD--GYARGEGVAAVVLKTLTQA---  
 CS\_29 ----MSPLSYI-SESKLS-MLSPGTGSRMWDAAAD--GYARGEGVAAVVLKTLQQA---  
 CH063\_0199 ----LGPMTFV-LESKLN-MLSPGSRSMWADAGAD--GYARGEAVCSVVLKTLSSQALKD  
 C.P.NRPS\_2 ----LAPDSFI-GASNLH-LLSPDGKSKMWDITAD--GYARGEGICAMFLKPLSQALRD  
 CH063\_0288 ----ITPEQFI-SEASLH-MLSPGTGSRMWDVSAD--GYARGEGFAALFVKPLSQALRD  
 CS\_4 ----ITPEQFI-SEASLH-MLSPGTGSRMWDVSAD--GYARGEGFAALFVKPLSQA---  
 GLRG\_08212 ----ITPEQFI-SEASLH-MLSPGTGSRMWDVSAD--GYARGEGFAALFVKPLSQALKD  
 CS\_15 ----FTPNTMM-PMTALN-FLSPDGKCFADFARAN--GYGRGEGIGFVVLKLSDA---  
 CH063\_1445 ----FTPNTMM-PMTALN-FLSPDGKCFADFARAN--GYGRGEGIGFVVLKLSDAVVRD  
 GLRG\_02583 ----LTPNTMM-PMTALN-FLSPDGKCFADFARAN--GYGRGEGIGFVVLKLSDAVVRD  
 CS\_32 ----MLPEFWI-SFSSCR-LLADSGRSIAFDQRGT--GFGRGEGCGMILKPLDQA---  
 GLRG\_03360 ----MLPEFWI-SFSSCR-LLADSGRSIAFDQRGT--GFGRGEGCGMILKPLDQAIRD  
 CH063\_1222 ----MLPEFWI-SFSSCR-LLADSGRSIAFDQRGT--GFGRGEGCGMILKPLDQA---  
 CH063\_0134 ----LNPDPFI-IMSSLG-VLGADGRCFAWDSRAN--GYGRGEGMATLLKPLRDAMAD  
 CS\_21 ----LNPDMFK-ALGSAG-FLSGDGKCYAFDSRAS--GYGRGEGVATIVIKRLGDALAA  
 GLRG\_09267 ----LNPDMFK-ALGSAG-FLSGDGKCYAFDSRAS--GYGRGEGVATIVIKRLGDALAA  
 CH0190 ----LNSDVV-FV-TMSNLG-FLSPDGISYAFDSRAN--GYGRGEGVAIVLKPALD  
 CH063\_0873 ----LNPDVV-FV-SMSTIG-FLSPDGISYAFDHRAN--GYGRGDGIAAVILKRLDHAVAA  
 CS\_9 ----LTPMGL-ELNALG-VLDPEGASKSFDKCRAN--GYGRGEGIAAVVLKRLSDA---  
 CH063\_1490 -----  
 GLRG\_01860 ----LTPMGL-ELNALG-VLDPEGASKSFDKCRAN--GYGRGEGIAAVVLKRLSDA---  
 CS\_25 ----LTPMGL-ELNALG-VLDPEGASKSFDKCRAN--GYGRGEGIAAVVLKRLSDA---  
 CS\_34 ----LSPDTFM-QLQNLG-VLSPEGRSRSFDENGR--GYGRGEGCGIVVLKR-----  
 GLRG\_11770 ----LNPECGI-YLSSLT-MISPEGHCKSFDASGD--GYGRGEGCGMIVLKRLLDADALRD  
 CH0399 ----LNPDYSL-YLSNMT-MLSKEGCKSFDASGD--GYGRGEGCGMIVLKRLLDADALRD  
 CS\_11 ----LVPESTM-QLNLLG-FLSPLGQSRAFDKAG--GYARGEGCGVIVMKRLDKA---  
 GLRG\_11840 ----LVPESTM-QLNLLG-FLSPLGQSRAFDKAG--GYARGEGCGVIVMKRLDKAIAID  
 CH063\_0781 ----LGPEATM-ELNSLG-VLNPDGRSLSFDEAAN--GYGRAEGCGIVVLKPLDQA---  
 CH063\_1357 ----FGPDMSI-LLGAAK-ILSPEGKSKMWDANAN--GFARGEGFGVTILKPLDAALRD  
 GLRG\_10317 ----FGPDMSI-LLGAAK-ILSPEGKSKMWDANAN--GFARGEGFGVTILKPLDAALRD  
 CH063\_1471 ----LLPDIMG-AMTRLH-FLSPDGKCSFDHKGKGN--GYARGEGAGFVILKPLHLALDK  
 CH063\_0506 ----LTPESLTIPLDDAG-FLSPDGRCYSFDHKGKGN--GYARGEGAGFVILKPLHLALDK  
 GLRG\_11835 ----MTPECMSIPLSNAG-FLGPDGRSISFDHKGKGN--GYARGEGAGFVILKPLHLALDK  
 CS\_3 ----ITPECMSIPLSNAG-FLGPDGRSISFDHKGKGN--GYARGEGAGFVILKPLHLALDK  
 GLRG\_11878 ----ITPECMSIPLSNAG-FLGPDGRSISFDHKGKGN--GYARGEGAGFVILKPLHLALDK  
 CH063\_0051 ----VNPTLFS-QLSAMH-MVSPDGISHSFDAAAAN--GYGRGEGIAGLVKRLSSALAD  
 CH0135 ----LHPNFMH-QLSSMH-MLSPEGISHSFDHKGKGN--GYGRGEGIAGLVKRLSSALAD

CS\_43 ----SAPLFFQ-NLAAGS-FLSTTGQCKPFDKAD--GYCRGEAIGAVYLKLSKAL--  
 GLRG\_09268 ----SAPLFFQ-NLAAGS-FLSTTGQCRPFDKAD--GYCRGDAIGAVYLKLSKAIAD  
 GLRG\_11857 ----CGPQWFQ-DLAGAS-FLSPTGQCKPFDAGAD--GYCRGDGVAAVFLKRMDKALAD  
 CH063\_0213 ----TSPLWFQ-NLAGAS-FLSTTGQCKPFDAAAD--GYCRGDGVGALFLKRHSQAIAD  
 CH063\_0477 ----TGVNNYF-DLAKAG-FLSKTGQCKPFDSDAD--GYCRADGVGLVVLKSLRQAVSD  
 CH063\_0313 ----TSPHDYR-DLKAAG-FLSPTGQCKPFDADAD--GYCRGEAVGVVVLKSLDAAIEE  
 CS\_44 ----TSPHDYR-DLKAAG-FLSPTGQCKPFDAGAD--GYCRGEAVCVVVLKSLAAAL--  
 GLRG\_11575 ----TSPHDYR-DLKAAG-FLSPTGQCKPFDAGAD--GYCRGEAVCVVVLKSLASIAIE  
 CH063\_1347 -----NLAAGS-FLNTRGSSGAFDDGAS--GYCRGEGAGILVLKPLSRVAVAD  
 C.PKS ----TSPFFYQ-NLAAAS-FLSPTGASKSFDASAD--GYCRGEGVGLVVLKRLSDAM--  
 CS\_45 ----TSPTLHQ-NLAAAN-FLSPTGESKSFDARAD--GYCRGEGAGVLMMLKRYSA--  
 GLRG\_11798 ----TSPTLHQ-NLAAAN-FLSPTGESKSFDARAD--GYCRGEGAGVLMMLKRYSAVAVAD  
 GLRG\_11778 ----TSPSNFN-ILGKAG-FTSTTGGSKSYRADAD--GYCRGEFVGAFVLKRYEDAVAA  
 CH0398 ----TSPVAYH-MLDKAG-FLSKTGCKVYRSDAD--GYCRGEFVGAFVLKRLDAIAD  
 CS\_37 ----NYPHSFA-SLSKSG-VLSDTGNCCTFRDDAD--GYCRGDFVGSVVLKRLLEDAL--  
 GLRG\_11836 ----NYPHSFA-SLSKSG-VLSDTGNCCTFRDDAD--GYCRGDFVGSVVLKRLLEDADAEAD  
 CH063\_1255 ----ASPHGWC-LLSKAG-VLSDTGNCRTFRDDAQ--GYCRGEPHAV-----  
 CS\_40 ----SSPHGWC-ALSKSG-VLSATGNCKPFRDDAD--GYCRGEFVGAVVLKRLLEDAL--  
 CS\_38 ----TDPDNFC-QLGKGH-FLSLTGQCKVWDEAAD--GYCRADGVGSVVIKRLDDAFAD  
 CS\_36 VSPTKSPDIYC-MLGKGH-FLSQGCKVWADAGAD--GYCRSDGVGSVVIKRLLEDAL--  
 GLRG\_08632 ----TNPDNYA-MLGNHG-FLSRTGQCKVWDEGAD--GYCRADGIGSVVIKRLDAVAD  
 CH063\_0351 ----TNPDIFA-GLSRGQ-FLSKTGPCATFDNGAD--GYCRADGCASVIVKRLDDAIAD  
 CS\_35 ----TNPDIFA-GLSRGQ-FLSKTGPCATFDNGAD--GYCRADGCASVIVKRLDDAIAD  
 GLRG\_04203 ----TNPDIFA-GLSRGQ-FLSKTGPCATFDNGAD--GYCRADGCASVIVKRLDDAIAD  
 GLRG\_11956 ----TNPDTA-GLDRGH-FLSRTGNCKTFDDGAD--GYCRGEGIGTILKRLDDAIAD  
 CH063\_1223 -----TFDDAAD--GYCRGEAAVTLVLKRLGDARRD  
 GLRG\_08620 ----TNPDMTA-GLDRGH-FLSHTGNCKTFDSDAD--GYCRGEAVVTIILKRLDDAMVD  
 CH063\_0250 ----TNPDMTA-GLDRGH-FLSRTGNCKTFDDEAD--GYCRGEAVVTIILKRLEDA---  
 CS\_39 ----TGPSMFA-GLGEGR-FLSTTGSKTFDSGAD--GYCRADGVGVIALKRLDSAY--  
 GLRG\_03507 ----TGNLWA-GLSRAG-FVSPTGSKTFDETAD--GYCRGEGVGIIVLKRLDAIQA  
 GLRG\_03509 ----TGNLWA-GLSRAG-FVSPTGSKTFDETAD--GYCRGEGVGIIVLKRLDAIQA

GLRG\_10537 -----  
 CS\_23 -----AVIRGTAANASGRTA-----GLANPSPDAQAAVTRM  
 CH063\_1191 G-----DPVRAVIRSTCVAGNGRTP-----GLTTPNPVIHERLMRR  
 CS\_16 G-----NGRTL-----GLTTPNPEVHERLMRR  
 GLRG\_06162 G-----DPIRSVIRSTCTAGNGRTL-----GLTTPNPEIHERLMRR  
 GLRG\_09075 G-----NAIRAVIRATGANS DGHSQ-----GLFTPNQVAQEALMRK  
 CH063\_1187 N-----NPIRAIIRASGTNSDGNSS-----TLLTPNSAMHEALMRK  
 CH063\_1411 N-----NPIRAIIRASGTNSDGNSS-----TLLTPNSAMHEALMRK  
 CS\_5 -----AVIRSTATQHDSGS-----GMLIPNGHAQESLIRH  
 GLRG\_11893 G-----NPIRAIIRSTATQHDSGS-----GMLIPNGHAQESLIRH  
 CS\_19 -----AIIRSGTNDGRSR-----GIMTPNDVLQEKLIRQ  
 CH063\_0663 G-----NPVRAIIRASATNNDGRSN-----GIMSPNTYLQEALIRK  
 GLRG\_00918 G-----DPIRALVRASATGHDGRKS-----GQLKPDIMGQEVLIRK  
 CH063\_0821 -----ALVRASATGHDGRKV-----GQLKPDAAAQELLIRK  
 CS\_13 -----AIIRGSATNADGRTV-----GMSTPNAGAHETLIRS  
 CH063\_0053 G-----NPIRAVIRGSATNSDGKTL-----GLTTPSAQAQQQLIRD  
 CH063\_1006 -----AVIRGTSSNADGRTP-----SLTIPSFESHEAMIRQ  
 GLRG\_07171 G-----NPIRAVIRGTSSNADGRTP-----SLTIPSFESHEAMIRQ  
 CS\_22 -----AVIRGTSSNADGKTP-----SLTIPSFESHEAMIRQ  
 CS\_14 -----AVIRAAVANS DGKTP-----GITQPNGAAHEAMIRL  
 CS\_26 -----AVIKGTATNFDGRTN-----GISLPSTEAEALIRQ  
 GLRG\_11435 R-----NPIRAVIKGTATNFDGRTN-----GISLPSTEAEALIRQ  
 GLRG\_08640 -----  
 GLRG\_10827 G-----DPIRAVLRGSSVNHNGRVS-----SASISYPGVNGQAYVMAD  
 GLRG\_11425 GDPIRDRDPIRAVMRSSATNNNGKVP-----VVGITHPNRDAQVEVIRH  
 CH063\_0013 G-----DPIRGVIRSSATNNNGKAP-----AVGITYPGFDGQRNVMRH  
 GLRG\_08482 H-----SPIRAMIRGTAINSNRGTG-----GITRPSARGQEMVIRE  
 CH063\_1130 G-----NPIRAIKGS AVNASGRTP-----GISLPSGNMQEVVIRK  
 GLRG\_03511 G-----DAIHAI VMESGSNHDKGTG-----GIFLPSDAQESLARR

CS\_18 -----HAIIRNSGLNQDGKTV-----GLTLPNPIAQANLMRL  
 GLRG\_05714 G----DKVHAIIRNSGLNQDGKTV-----GLTLPNPIAQANLMRL  
 CH063\_0922 -----  
 CH063\_1282 G----DTVRAVVRNTAASHSGRIA-----GGITMPSQDAQEALARR  
 CS\_28 -----AVLRGTGVNSDGRGT-----GGITLPNKAAQESLIRD  
 GLRG\_11563 G----NVIRAVLRGTGVNSDGRGT-----GGITLPNKAAQECLIRD  
 C.P.NRPS\_6 N----DPIQAVIRNSGVNSDGRTP-----GITMPSAMAQAKLTTE  
 CH063\_0617 G----DPIHAVVRNSGVNSDGRTP-----GITMPSAAAQARLTAE  
 C.P.NRPS\_1 G----DFIECVVRETGVGQDGHTN-----GITTPGAKSQASLIRR  
 GLRG\_09842 G----DFIECVVRETGVGQDGRTN-----GITTPSSESQASLIRH  
 GLRG\_09715 N----DHIECIIRETGVNQDGRSE-----GLTVPSSSEAQAALIRS  
 C.P.NRPS\_8 G----DDIECVIRETGVNQDGKAR-----GITMPSASAQSALIRD  
 GLRG\_10367 G----DDIECVIRETGVNQDGKTR-----GITMPSASAQSALIRD  
 CS/1 G----DHIECIIRETGTNQDGATP-----GITMPSAESQALIRD  
 CH063\_0489 G----DAIEGIIRNTGFTHDGRTL-----GITMPSGQAQADLIRR  
 CH063\_0392 G----DHIESVIRECLVNQDGRTA-----EGITVPSSSEAQAELIRR  
 GLRG\_07434 G----DHIEAIIRETGVNQDGRSR-----GITMPSAAAQASLIRA  
 CS/20 G----DHIECIIRETGVNQDGRTK-----GITMPSPLAQANLIRD  
 CH063\_0306 G----DHIECIIRETGVNQDGRTK-----GITMPSPLAQANLIRD  
 CH030B G----DSIECVIRETGLNQDGKTP-----GITMPSSSAQALIRS  
 CS\_30 -----CIIRETGINQDGKTP-----GITMPSAKAQASLIRS  
 CS\_29 -----SIIRETGVNQDGKTT-----GITMPSHLAQEVLIRD  
 CH063\_0199 G----DTIECVIRETGINQDGRTT-----GITMPNHDAQEALIRA  
 C.P.NRPS\_2 G----NRVDALIRETGINS DGRTQ-----GITQPSATAQTALIRK  
 CH063\_0288 G----DSVISIIRETGVNSDGRTQ-----GITMPSPLAQAE LIRD  
 CS\_4 -----SIIRETGVNSDGRTQ-----GITMPSPIAQAE LIRD  
 GLRG\_08212 G----DSVISIIRETGVNSDGRTQ-----GITMPSPIAQAE LIRD  
 CS\_15 -----AVIRGTHVNQDGLTTGELSASKAHNPSKLTVQQGITLPSKEAQVANIRA  
 CH063\_1445 N----DTIRAVIRGTHVNQDGLTT-----GITLPSKEAQVANIRS  
 GLRG\_02583 N----DTIRAVIRGTHVNQDGLTT-----GITLPSKEAQVANIRA  
 CS\_32 -----AVIRGTGINQDGKTP-----GITMPSGAAQEKL MRQ  
 GLRG\_03360 N----DSIRAVIRGTGINQDGKTP-----GITMPSGAAQEKL MRQ  
 CH063\_1222 -----AVIRGTGINQDGKTP-----GITMPSGAAQEKL MRQ  
 CH063\_0134 G----DPIHAVIRETAINQDGKTP-----TITSPSSEAQEELIRA  
 CS\_21 G----DPIRGVIRGSALNQDGKTE-----TITTPSLEAQEALIRA  
 GLRG\_09267 G----DPIRGVIRGSALNQDGKTE-----TITTPSLEAQEALIRA  
 CH0190 H----DPVRTIIRETALNQDGKTP-----AITAPSDMAQEQLIRE  
 CH063\_0873 G----DPIRAIIRGSALNQDGKTP-----TITTPSQEAQERLMGA  
 CS\_9 -----AVIRNTGCNHDGHSP-----GLTAPAKEAQAE LMR  
 CH063\_1490 -----  
 GLRG\_01860 G----DVIRAVIRNSSTNQDGRSP-----GITQPTKAGQVALIKH  
 CS\_25 -----AVIRNSSTNQDGRSP-----GITQPTKAGQVALIKH  
 CS\_34 -----AIIRATGCNSDGWTK-----GMALPSGESQMQLIKD  
 GLRG\_11770 G----DPIRAVIPGTGVNSDGFTQ-----GFTMPSSSESQAALIRD  
 CH0399 G----DPIRAVIRGSGVNADGFTQ-----GFTMPSATAQADLICD  
 CS\_11 -----AIIRGSGVNQDGWTQ-----GVTMPSGEEQAALIKY  
 GLRG\_11840 G----DTIRAIIRGSGVNQDGWTQ-----GVTMPSGEEQAALIKY  
 CH063\_0781 -----AVIRGSGVNSDGWTK-----TISTPSGQAQADLIRQ  
 CH063\_1357 G----DTVRAVVLASAANEDGRTP-----GISLPNSEAQALIRT  
 GLRG\_10317 G----DTIRAVVLASAANEDGRTP-----GISLPNSEAQALIRT  
 CH063\_1471 G----DVIRGVIRNTA-----  
 CH063\_0506 G----NVIRAVVRATGVNQDGKTP-----SITQPSLEAQIALIRK  
 GLRG\_11835 G----DVIRAVIRATGVNQDGRTP-----SITQPNSKAQVDLIRR  
 CS\_3 -----AVIRATGVNQDGRTP-----SITQPSSQAQADLIRS  
 GLRG\_11878 G----DPIRAVIRATGVNQDGRTP-----SITQPSSQAQADLIRS  
 CH063\_0051 G----DVIRAVVRATGVNSDGKTP-----GITVPSEDAQAELIRR  
 CH0135 G----DTIRAVIRGTGTNADGKTP-----GITQPSSEAQAELIRT  
 CS\_43 -----GVISATAVNQNQNST-----PIFVPNPLSLTDVFRT  
 GLRG\_09268 G----DQVLGVVSATAVNQNQNST-----PIFVPNPLSLTDVFRT  
 GLRG\_11857 G----DEILGVIGATVVQQNQNST-----PIVVPNEASLSDLFKT  
 CH063\_0213 G----DQVLGVIPATAVQQLNNT-----PIFVPNAPSLSDLFSS  
 CH063\_0477 G----DHIMGVIPS VATNQGGIGA-----PGITVPDGICQKALYST



CH063\_0313 N----DHILGVVVGSATNQNPKEG-----PIVVPNSKAQASLLRK  
 CS\_44 -----GVVVGSATSQNDQNG-----PIIVPNAKSQASLLRK  
 GLRG\_11575 G----DEIIGVVVGSATSQNDNEG-----PIVVPNAKSQASLLTK  
 CH063\_1347 G----DRVLGVLGSAVNQNSNRS-----PITVPDSSSQSGLYRR  
 C.PKS -----GTILATAVRQSSNKV-----PITVPYSPSQTQLYRK  
 CS\_45 -----GVIAGSAVNQNANTA-----PITVPVSSSQTSLYMR  
 GLRG\_11798 G----DNIFGVIAGSAVNQNANTA-----PITVPVSSSQTSLYMR  
 GLRG\_11778 N----DNILAVILSSARNHSGDAV-----SITHSDPEAQQELMAE  
 CH0398 N----DNILSVIASSARNHSGTAT-----SITRSDHRAQELLMAE  
 CS\_37 -----AVIAGSGRNHSGNSP-----SITSDAGAQRERLFRK  
 GLRG\_11836 N----DKILAVIAASGRNHSGNSP-----SITSDAGAQRERLFRK  
 CH063\_1255 -----G-----  
 CS\_40 -----AVIAASGKNQSGNAA-----SITTPDAGAQRERLFRR  
 CS\_38 N----DTILATILAANTNLSAEAV-----SITHPHAPTQADNYKK  
 CS\_36 -----GTIIAGATNHSAESI-----SITQPHAGAQRDNYRQ  
 GLRG\_08632 N----DNIIATVLAGATNHSAYAA-----SITQPHAGAQKSNYAQ  
 CH063\_0351 K----DNVLAVILGTA-NHSADAI-----SITHPHGPTQSILSRH  
 CS\_35 K----DNVLAVILGTATNHSADAI-----SITHPHGPTQSILSRY  
 GLRG\_04203 K----DNVLAVILGTATNHSADAI-----SITHPHGPTQSILSRH  
 GLRG\_11956 H----DPILGVVLGAYTNHSAESE-----SITRPHVGAQRAIFSK  
 CH063\_1223 N----DPIEACLLSVATNHNAEAE-----SITRPNTAAQRELFGRG  
 GLRG\_08620 K----DPIQACIRGIATNHNAEAE-----SITRPHSEAQVELFEH  
 CH063\_0250 -----ACILGVATNHNAEAE-----SITRPHAAAQKDLFEY  
 CS\_39 -----GVIKSWATNHSSRAI-----SITQPHADTQARLFLK  
 GLRG\_03507 G----DNIQGVIRGIATNHSANAL-----SITQPHGPTQKKLYKQ  
 GLRG\_03509 G----DNIQGVIRGIATNHSANAL-----SITQPHGPTQKKLYKQ  
  
 GLRG\_10537 -----  
 CS\_23 AYKNAGI-SDF---WATQFLE-----CHGTGTLAGDPVEVKGAA  
 CH063\_1191 GHEIAGI-ADL---SKTAMVE-----CHGTGTPVGDPLEVSAVA  
 CS\_16 GHKLAGI-TDL---SKTAMVE-----CHGTGTPVGDPLEVSAVA  
 GLRG\_06162 GHRLAGV-TDL---SKTAMVE-----CHGTGTPVGDPLEVSAVA  
 GLRG\_09075 YLRDAGL--DP---SQTAYVE-----CHGTGTATGDPIETSAVG  
 CH063\_1187 VYLDAGL--DP---SKTAFVE-----CHGTGTPGDPLETNAVG  
 CH063\_1411 VYLDAGL--DP---SKTAFVEVSMCLYVLGGRGATQLERQCFCEGTPGDPLETNAVG  
 CS\_5 TYDIAGI-RDF---SKTAFVE-----CHGTGTVVGDPIEVA AVE  
 GLRG\_11893 TYDIAGI-RDF---SKTAFVE-----CHGTGTIVGDPIEVA AVE  
 CS\_19 VYSQAGI-DDL---SKTAFVE-----CHGTGTSTGDPLEASAVA  
 CH063\_0663 TYEKAGVPF----NKTAFFE-----CHGTGTPTGDPLEVA AVA  
 GLRG\_00918 AYALAGI--PDSQFGETAWIE-----CHGTGTAVGDPIELRSVA  
 CH063\_0821 AYALAGI--PDSQFGDTAWIE-----CHGTGTAVGDPIEMRSVA  
 CS\_13 AYRMAGI--W--DFGETAMVE-----CHGTGTTVGDIAETCAVA  
 CH063\_0053 AYRQAGI--PESDMWQTAMVE-----CHGTGTAVGDTIEACTVG  
 CH063\_1006 AYKMAAI--GGRDIADTGFVE-----CHGTGTAAGDPIETTAIA  
 GLRG\_07171 AYKMAAI--SQQHIAATGFVE-----CHGTGTAAGDPIETTAIS  
 CS\_22 AYKMAAI--GGQHVADTGFVE-----CHGTGTAAGDPIETTAIS  
 CS\_14 AYQQAGI-TDF---SKTAYFE-----CHGTGTAVGDPIETGAVA  
 CS\_26 AYAHAGI-TDF---SETTFVE-----CHGTGTPIGDPIEANAVA  
 GLRG\_11435 AYAHAGI-TDF---SDTTFVE-----CHGTGTPIGDPIEANAVA  
 GLRG\_08640 -----  
 GLRG\_10827 AYKRSGN-LDP---MLTGYFE-----CHGTGTAVGDPIEVEAIG  
 GLRG\_11425 AYHSAGN-LDP---RLTGFFE-----CHGTGAAIGDPLEVHAVL  
 CH063\_0013 AYQRGGL--DP---MLTGYFE-----CHGTGTAIGDPLEVHAVS  
 GLRG\_08482 AYRSAG--DL--PFRDTSYFE-----CHGTGTYYVGDPIEVAALG  
 CH063\_1130 AYRDAGL--DF---ADTDYVE-----CHGTGTPVGDPIEVDIAG  
 GLRG\_03511 VYANAGL--DP---RETTYVE-----AHGTGTAAGDNAEVSSIS  
 CS\_18 VYKNAGL--DP---ADTVYVE-----AHGTGTQAGDNAEISSIA  
 GLRG\_05714 VYKNAGL--DP---ADTVYVE-----AHGTGTQAGDNAEISSIA  
 CH063\_0922 -----  
 CH063\_1282 VHGEVGL--DP---KDTGFVE-----GHGTGTAVGDPIDAAAIA  
 CS\_28 VYARNNL--DL---DHTGFIE-----GHFTGTPAGDPIEASIA

GLRG\_11563 VYARHNL--NP---DETGFLE-----GHFTGTPAGDPIEASAIA  
C.P.NRPS\_6 TYRAAGL--DPSKAEDRCQYFE-----AHGTGTQAGDPTEAKGIF  
CH063\_0617 TYRAAGL--DPLNPDERCQYFE-----AHGTGTQAGDPTEARGIY  
C.P.NRPS\_1 VYAKAGL--DVIK--DGCQYFE-----AHGTGTPAGDPVEAEAIH  
GLRG\_09842 VYAKAGL--DVVK--DGCQYFE-----AHGTGTPAGDPVEAEAIH  
GLRG\_09715 TYSKCGL--DWRRTEDRCQYFE-----AHGTGTQAGDPKEARAIH  
C.P.NRPS\_8 TYRRAGL--DPHRPADRPQYFE-----AHGTGTPAGDPVEAEAIH  
GLRG\_10367 TYRRAGL--DPHRPADRPQYFE-----AHGTGTPAGDPIEAEAIH  
CS/1 TYRRASL--DPRDPADRCQYFE-----AHGTGTQAGDPIEAEAIY  
CH063\_0489 TYEEAGL--DLANRANWPQFFE-----AHGTGTPVGDQPVEASALA  
CH063\_0392 TYAKSGL--DATNVDDRCQYFE-----AHGTGTGAGDPREARAIA  
GLRG\_07434 TYAKAGL--DPESKTDRCQYFE-----AHGTGTPAGDPVEAEAVH  
CS/20 TYKRAGL--DLNKPSDRPQYFE-----AHGTGTPAGDPVEAEAIS  
CH063\_0306 TYKRAGL--DLSKPSDRPQYFE-----AHGTGTPAGDPVEAEAIS  
CH030B TYARAGL--DLSKPSDRPQFFE-----AHGTGTPAGDPIEAAAIR  
CS\_30 TYARAGL--DLCKASDRPQFFE-----AHGTGTPAGDPIEAAAIS  
CS\_29 TYARAGL--DLSRIGDRPQFFE-----AHGTGTPAGDPQEAIAIT  
CH063\_0199 TYARAGL--DINNPOERCQFFE-----AHGTGTPAGDPQEAIAIA  
C.P.NRPS\_2 TYRNAGL--DLGRAEDRPQYIE-----AHGTGTQAGDPQEAIAIS  
CH063\_0288 TYRRSGL--DALSPDRRCQYFE-----AHGTGTQAGDPREAAQIS  
CS\_4 TYRRSGL--DPQSPADRCQYFE-----AHGTGTQAGDPREAAQIS  
GLRG\_08212 TYRRSGL--DPHSPADRCQYFE-----AHGTGTQAGDPREAAQIS  
CS\_15 LYTKHNL--DM---KQTSFVE-----CHGTGTQAGDFRELKAIS  
CH063\_1445 LYSKHNL--DM---KQTAQVE-----CHGTGTQAGDFRELKAIS  
GLRG\_02583 LYAKHDL--DM---KQTAQVE-----CHGTGTQAGDFRELKAIS  
CS\_32 IYRNAGL--DP---SDCGYVE-----AHGTGTVGDPIEATAIH  
GLRG\_03360 IYRNAGL--DP---NDCGYVE-----AHGTGTVGDPIEATAIH  
CH063\_1222 IYRNAGL--DP---NDCGYVE-----AHGTGTVGDPIEATAIH  
CH063\_0134 CYRRAGL--DP---AKTPYVE-----AHMTGTPTGDPIEAKAIS  
CS\_21 CYQNSGL--NP---NDTQYFE-----AHGTGTQAGDTVEARAIA  
GLRG\_09267 CYENAGL--SP---KDTQYFE-----AHGTGTQAGDTIEARAIA  
CH0190 CYNRAGI--DT---SQTSYIE-----AHGTGTPTGDPLEISAIS  
CH063\_0873 CYRSCGL--DP---GETGFVE-----AHGTGTPTGDPIEVTAIA  
CS\_9 TYAQAQL--DP---SETRFFE-----AHGTGTNVGDPTTEASAIS  
CH063\_1490 -----  
GLRG\_01860 VYDRANL--DP---SLTRFAE-----AHGTGTPVGDPIEASALA  
CS\_25 VYDRANL--DP---SLTRFAE-----AHGTGTPVGDPIEASALA  
CS\_34 VYDTFGL--DY---SLTQYVE-----AHGTGTVKAGDPTEAHAIY  
GLRG\_11770 VYEGAEL--DM---SDTQFVE-----CHGTGTVKAGDPPIETRAIY  
CH0399 VYKTAGL--DM---GLTTYVE-----AHGTGTVKAGDPPIEARAIY  
CS\_11 VYESNKL--DY---GGTQYVE-----AHGPGTQAGDPPTETGAIY  
GLRG\_11840 VYESNKL--SY---GDTQYVE-----AHGPGTQAGDPPTETGAIY  
CH063\_0781 VYESNGL--DY---DSTQYVE-----AHGTGTVKAGDPPIELGAIH  
CH063\_1357 AYENAGV--DP---AETGYVE-----AHGTGTQAGDPLEARAIL  
GLRG\_10317 AYRDAGV--DP---AETGYVE-----AHGTGTQAGDPLEARAIL  
CH063\_1471 -----AHGTGTPAGDPVEAAALA  
CH063\_0506 TYEQAGL--GF---STTEYVE-----AHGTGTAVGDPIEARAIG  
GLRG\_11835 TYQAGGL--DL---AHTEYVE-----AHGTGTPVGDPIEASGIG  
CS\_3 TYEAAGL--SL---DDTGYVE-----AHGTGTPVGDPLEAAAIS  
GLRG\_11878 TYRTAGL--SL---DDTGYVE-----AHGTGTPVGDPLEAAAIS  
CH063\_0051 VYEGADL--DM---ARTAYFE-----AHGTGTPKGDPIEVRAIR  
CH0135 TYEAAGL--SL---SDTQYFE-----AHGTGTALGDPIELSAIG  
CS\_43 VISKSGL--GI---KDISVVE-----AHGTGTAVGDPAEYDSIR  
GLRG\_09268 VISKSGL--GV---KDISVVE-----AHGTGTAVGDPAEYDSIR  
GLRG\_11857 VLDKAGV--QP---QQVSVIE-----AHGTGTAVGDPAEYGAIR  
CH063\_0213 VTTRAAI--AP---SQISVVE-----AHGTGTAVGDPAEYNGIR  
CH063\_0477 LLRKSGI--KA---DQVSYVE-----AHGTGTQVGDPIEISSIR  
CH063\_0313 VMDMSGV--LP---EDVTYVE-----AHGTGTGVGDPIEVQSLR  
CS\_44 VMEVSNV--VP---GDVTYVE-----AHGTGTDVGDPIEVSSIR  
GLRG\_11575 VMNMSNV--VP---EDITYVE-----AHGTGTEVGDPIEVSSIR  
CH063\_1347 VLDEAGI--QP---HEVTYVE-----AHGTGTVKAGDPPIEYESVR  
C.PKS ILGAAGI--EA---EEVTYVE-----AHGTGTPVGDPLEFGAIK

CS\_45 VLQRAHM--DP---KSVSYVE-----AHGTGTPKGDPIECASIR  
 GLRG\_11798 VLQRAHM--DP---KSVSYVE-----AHGTGTPKGDPIECASIR  
 GLRG\_11778 VLRKARL--EP---SDVSYVE-----MHGTGTQVGDYAEMMAVS  
 CH0398 TLRKACL--EP---RDISYVE-----MHGTGTQVGDVAEMTAVA  
 CS\_37 VLRNAHV--SA---SDISYVE-----MHGTGTQVGDPAEMGAVG  
 GLRG\_11836 VLRNAHV--SA---DDISYVE-----MHGTGTQVGDPAEIGAVG  
 CH063\_1255 -----EDVTIED-----  
 CS\_40 VLRTAGV--AP---ADVSYVE-----AHGTGTPVGDPCEMTAVS  
 CS\_38 VTAKAGI--SP---LDVSYVE-----LHGTGTQARDREARSMS  
 CS\_36 VMDKAGV--RP---LDVSYVE-----LHGTGTQVGDAVESESVL  
 GLRG\_08632 VTQAAGI--NP---LDVSFVE-----LHGTGTQVGDIAESESVC  
 CH063\_0351 ILDEAGV--DP---LDVDYVE-----MHGTGTQAGDGTMEMVSVT  
 CS\_35 ILDEAGV--DP---LDVDYVE-----MHGTGTQAGDGTMEMVSVT  
 GLRG\_04203 ILDEAGV--DP---LDVDYVE-----MHGTGTQAGDGTMEMVSVT  
 GLRG\_11956 ILNEASV--DP---FSISYVE-----MHGTGTQAGDAGEMSSVL  
 CH063\_1223 LLADAVV--RP---NDISYVE-----MHGTGTQAGDAGETSSIV  
 GLRG\_08620 LLAEANI--SP---IDISYVE-----MHGTATQVGDAAETTSVV  
 CH063\_0250 ILTEANV--SS---NDISYVE-----MHGTGTQAGDAGETTSVV  
 CS\_39 VLSEANL--GP---ADITYVE-----MHGTGTPVGDTAELTSVL  
 GLRG\_03507 VLRKANL--TP---DQIQYVE-----MHGTGTQAGDVTMEMNSVV  
 GLRG\_03509 VLRKANL--TP---DQIQYVE-----MHGTGTQAGDVTMEMNSVV

GLRG\_10537 -----  
 CS\_23 SVFAAG-----RRPGQELVIGS-----IKSNIGHSE  
 CH063\_1191 NIWG-----QDGIYIGS-----V-----  
 CS\_16 NIWG-----ESGIYIGSVRTTATMCNFLTNTNIKCVKPNIGHGE  
 GLRG\_06162 NIWG-----ENGIYIGS-----VKPNIGHGE  
 GLRG\_09075 EVFG-----EKGVYIGS-----VKPNVGHSE  
 CH063\_1187 NVFGEH-----GVYIGS-----VKPNVGHSE  
 CH063\_1411 NVFGEH-----GVYIGSTDDVAVKKSFS-----VKPNVGHSE  
 CS\_5 RIFGEY-----GILIGS-----AKPNFGHSE  
 GLRG\_11893 RIFGEH-----GILIGS-----AKPNFGHSE  
 CS\_19 RIWGP-----GGILMGA-----VKPNVGHSE  
 CH063\_0663 RIWKDN-----EGVMIGA-----VKPNVGHSE  
 GLRG\_00918 NIFG-----QQGIIVGS-----VKPNVGHSE  
 CH063\_0821 NVFG-----RDGIVVGS-----VKPNVGHSE  
 CS\_13 RVFG-----EKGVVIGS-----TKPALGHSE  
 CH063\_0053 TVFG-----EKGMLIGS-----VKPNLGHSE  
 CH063\_1006 KVFGSA-----GVYIGS-----CKPNIGHSE  
 GLRG\_07171 KVFGEH-----GVYIGS-----CKPNIGHSE  
 CS\_22 KVFG-----DVGVIYIGS-----CKPNIGHSE  
 CS\_14 RVFG-----EHGIHITS-----VKPNVGHTE  
 CS\_26 RVFVGGP-----ERGPYIGS-----VKPNLGHSE  
 GLRG\_11435 RVFVGGP-----ERGPYIGS-----VKPNLGHSE  
 GLRG\_08640 -----  
 GLRG\_10827 RAMNHS-----RRPGVDDKLMIGA-----VKTNVGHGE  
 GLRG\_11425 LAMNDQ-----RSDEEPLLIGA-----VKTNIGHSG  
 CH063\_0013 DIMNAT-----RTEADGPLHIGA-----VKTNIGHSE  
 GLRG\_08482 RVFAPE-----RSADDPLLIGS-----VKSINVGHGE  
 CH063\_1130 RCFSPR-----QGPPLIIGS-----VKTNVGHSE  
 GLRG\_03511 KVFGRE-----AGRKSDLPIGS-----IKANIGHLE  
 CS\_18 EVFCPE-----GQREDGLYVGS-----IKSNIGHLE  
 GLRG\_05714 EVFCPE-----GQREDGLYVGS-----IKSNIGHLE  
 CH063\_0922 -----  
 CH063\_1282 SVYASP-----RSTSDPVYLG-----VKSNIHLE  
 CS\_28 AVFQRG-----GPRAAAD-----KPSLYVGA-----VKANIGHVE  
 GLRG\_11563 AVFQRG-----GPRAAAD-----RPSLYVGA-----VKANIGHVE  
 C.P.NRPS\_6 ESFFST-----EGAPG-----AEVSPLYVGS-----IKTVVGHLE  
 CH063\_0617 DSFFAS-----SADSKDTNVDMTARRPLLIGS-----IKTVVGHLE  
 C.P.NRPS\_1 SVFIDP-----SRDKRPT-----GPLYVGS-----IKTVIGHTE  
 GLRG\_09842 SVFTDS-----SRDGVRT-----DPLYVGS-----VKTVIGHTE

GLRG\_09715 DAFFPE----NVQOELE-----KDTLYVGS-----VKTIVIGHLE  
 C.P.NRPS\_8 SAFFGG-----RPTKSNEERGRDILLVGS-----IKTVCGHAE  
 GLRG\_10367 SAFFGG-----RPTKTNEERGRDILLVGS-----IKTVCGHAE  
 CS/1 RSFFDD----DSGDADS-----LYVGS-----LKSIIIGHTE  
 CH063\_0489 SAFYPD----GDNAYEQ-----DDRLLVGS-----IKTLVGHTE  
 CH063\_0392 TAFFGP----NHRSDDP-----SDKLYVGS-----IKTVIGHTE  
 GLRG\_07434 TAFFGN----GNLDADR-----LLVGS-----VKTIIIGHTE  
 CS/20 SAFFGP----ESGFQRKS--DADPKLYVGS-----IKTVIGHTE  
 CH063\_0306 SAFFGA----ESGFQRRP--DADPKLYVGS-----VKTIVIGHTE  
 CH030B EAFFGA----DSHFVPR---GPDDTLVGS-----IKTVIGHTE  
 CS\_30 DAFFGE----DVKSDDK---DKHSTLFVGS-----IKTVIGHTE  
 CS\_29 KAFFGD----EVLDTLQ----PQDLLIGS-----VKTIVIGHSE  
 CH063\_0199 SAFFGQ----GDNSNAG-----DDPLFVGS-----IKTIVIGHTE  
 C.P.NRPS\_2 QTFF-----PPN---EEHDKLFVGS-----VKTIVIGHTE  
 CH063\_0288 TAFFGD----EASAAAD---DDEQKLVVGS-----VKTIVIGHTE  
 CS\_4 TAFFGD----KASTVAE---SDDQKLVVGS-----IKTVIGHTE  
 GLRG\_08212 TAFFGD----KASTVAE---SEDQKLVVGS-----IKTVIGHTE  
 CS\_15 ETLA-----EGRKT----ENPVFVGS-----VKTNIIGHTE  
 CH063\_1445 ETFGDA-----RTTENPVFVGS-----VKTNIIGHTE  
 GLRG\_02583 ETLAEG-----RTTENPVFVGS-----VKTNIIGHTE  
 CS\_32 NVIGQN-----RSAKDPLFIGS-----VKSNIIGHTE  
 GLRG\_03360 NVIGQN-----RSTKDPLFIGS-----VKSNIIGHTE  
 CH063\_1222 NVIGQN-----RSSKDPLFIGS-----VKSNIIGHTE  
 CH063\_0134 CVFGKG-----RGVSNPVLVGS-----IKTNLGHLE  
 CS\_21 SVFKCT-----SEPLLIGS-----VKTNLGHTE  
 GLRG\_09267 SVFSSS-----SEPLLIGS-----VKTNLGHTE  
 CH0190 RAFQOQ-----PLHVGS-----VKANIGHTE  
 CH063\_0873 NIIASA-----SPPG----SDPLLLGS-----VKSAIGHTE  
 CS\_9 EMFTPY-----RSAEPLHIGA-----LKSNIIGHTE  
 CH063\_1490 -----  
 GLRG\_01860 EMFAPH-----RSQDEPLFIGA-----LKSINVGHLE  
 CS\_25 EVFAPH-----RSP-----DEPLFVGA-----LKSINVGHLE  
 CS\_34 NTIGRA-----TRNQKLIMGS-----VKPNIGHLE  
 GLRG\_11770 ETLGKK-----ASSSQPLIIGS-----VKPSIGHLE  
 CH0399 ETLGRN-----VKRGQKLMIGS-----VKPNIGHLE  
 CS\_11 RTIGLK----GLETNPS----RKKLWVGS-----VKPNIGHLE  
 GLRG\_11840 RTIGLE----GLKTNPS----RKKLWVGS-----VKPNIGHLE  
 CH063\_0781 DTIGRN-----ASESRKLYVGS-----VKANIGHLE  
 CH063\_1357 KTIGDL-----PGRTSELYVGS-----VKS-----  
 GLRG\_10317 NTIGDL-----PNRKSPLYVGS-----VKTNIIGHTE  
 CH063\_1471 ATFGSA-----RRAGDPVYMGS-----VKSINVGHLE  
 CH063\_0506 EVFRTS-----RKTPLHVGS-----VKSNIIGHTE  
 GLRG\_11835 AVFRQH-----RTRPVHIGS-----IKSNIIGHTE  
 CS\_3 QVFKTA-----MRNEPLVVGGS-----VKSNIIGHTE  
 GLRG\_11878 QVFQTA-----SRSEPLVVGGS-----VKSNIIGHTE  
 CH063\_0051 ATIAAA-----RKLYHAGPLYVGS-----VKPNIGHTE  
 CH0135 AILGAA-----RSPE----TEPLYVGS-----IKANVGHTE  
 CS\_43 QVFGGS-----ARSGLKPLQIGS-----VKGLIGHTE  
 GLRG\_09268 QVLGGP-----ARSGLKPLQIGS-----VKGLIGHAE  
 GLRG\_11857 QVFGAS-----SKRQTPLSLGS-----VKGSIGHTE  
 CH063\_0213 KVLGGP-----GRDSPLVLGS-----VKSIVGHTE  
 CH063\_0477 QVFGGS-----QRTKPLYIGS-----LKANIGHSE  
 CH063\_0313 DAFGGP-----LRTSTLHFSS-----IKGNIGHAE  
 CS\_44 EAFGGP-----SRKSKLRFAS-----IKGNIGHVE  
 GLRG\_11575 EAFSGP-----SRKSKLRFAS-----IKGNIGHVE  
 CH063\_1347 STFGSP-----LRTGPVYVLS-----VKDNIGHAE  
 C.PKS EVFASG-----SRRVPLHFAS-----VKGNIIGHTE  
 CS\_45 EVFGNQ-----PSRKLYFSS-----VKGSIGHTE  
 GLRG\_11798 EVFGNQ-----PSRKLYFSS-----VKGSIGHTE  
 GLRG\_11778 KALGRR-----RRPLPLPLGS-----IKANAGHGE  
 CH0398 NVFGNA-----SRSVPLSVGT-----IKANIGHSE  
 CS\_37 NLLKHT-----PRADGPVVGGS-----VKANFGHGE

GLRG\_11836 NLLKHK-----SRADGPVPVGG-----VKANFGHGE  
 CH063\_1255 -----AEPLTVGS-----VKANIGHSE  
 CS\_40 NVLARP-----GVEDDTHRLAVGA-----VKANIGHSE  
 CS\_38 DVFAPA----GPGPRRK----ENRLRLGA-----VKSNIHGHE  
 CS\_36 NFFAPL----GQRQRP-----EQRLHMGA-----VKSNIHGHE  
 GLRG\_08632 DFFAPL----SPRRA-----DQPLHLGA-----VKSNIHGHE  
 CH063\_0351 NVFAPA----DRKRPA----DRPLYLGA-----VKSNIHGHE  
 CS\_35 NVFAPA----DRKRPA----DRPLYLGA-----VKSNIHGHE  
 GLRG\_04203 NVFAPA----DRKRPA----DRPLYLGA-----VKSNIHGHE  
 GLRG\_11956 EVFAPPSQVVKVRKSDE-----PVYIGS-----VKPNVGHGE  
 CH063\_1223 NTLSP-----TARGSCLRPASSPLYLGA-----AKANVGHSE  
 GLRG\_08620 TTLSPF-----TARGSSVRPATHPLHIGA-----VKSNIHGHE  
 CH063\_0250 TTLSPL-----TARGTSVRPATSPLHIGA-----VKSNIHGHE  
 CS\_39 SVFGRH-----RSSGNPLYIGA-----AKANVGHGE  
 GLRG\_03507 STFASG-----REPTNPLYVGG-----IKANVGHGE  
 GLRG\_03509 STFASG-----REPTNPLYVGG-----IKANVGHGE

GLRG\_10537 -----  
 CS\_23 AAAGLSGLLKAIMAVERAVIPGNPTFLIPNPID-----  
 CH063\_1191 -----  
 CS\_16 GASGLSSVIKMVLALENSTIPPINFK-----  
 GLRG\_06162 GAAGLSSVIKMVLALENSTIPPINFK-----  
 GLRG\_09075 GASGLNSLIKAVLALEHKTIPPNI-----  
 CH063\_1187 GASGLTSLIKAVLALENNSTIPPNIKFPSTPNPKIPFEDKKLVVPTKPVAWPADRDRRISVN  
 CH063\_1411 GASGLTSLIKAVLALENNSTIPPNIKFPSTPNPKIPFEDKKLVVPTKPVAWPADRDRRISVN  
 CS\_5 GAAALTSIIKATLMLERETIVPNANFE-----  
 GLRG\_11893 GAAALTSIIKATLMLERETIVPNANFE-----  
 CS\_19 GAAGLTSIIKAVLALENRVIPPNI-----  
 CH063\_0663 GAAGLTSIIKAVLALENRVIPPNI-----  
 GLRG\_00918 GAAGLTGLIKAVLSLEHGIIPPNIFD-----  
 CH063\_0821 GAAGLTGLIKAVLSLEHNV-----  
 CS\_13 GASALTSIIKAVLSLEHRTILPNI-----  
 CH063\_0053 AASAITSVKAVLSLETRTIIPNIKFE-----  
 CH063\_1006 GASGITSLIKSVLALEHRTIPPNIKFD-----  
 GLRG\_07171 GASGITSLIKSVLALEHRTIPPNIKFD-----  
 CS\_22 GASGITSLIKSVLALEHRTIPPNIKFD-----  
 CS\_14 GSSGLVSLIKAVMSLEHRTIPPNI-----  
 CS\_26 GASGLTSLIKTVMALEHRTIPPNIKFT-----  
 GLRG\_11435 GASGLTSLIKTVMALEHRTIPPNIKFT-----  
 GLRG\_08640 -----  
 GLRG\_10827 AASGLTALIKAVLIVERGIIPPTIGIK-----  
 GLRG\_11425 AASGLSAVIKAVLVAERGVIPPTRGI-----  
 CH063\_0013 AASGLSAVIKAVLVAERGVIPPTRGI-----  
 GLRG\_08482 GASALASIMKVLSLENGAIAP-----  
 CH063\_1130 GASGLTSLIKVVKSMEEGRIPAS-----  
 GLRG\_03511 SSSGIAGMLKAIMVLKKNQIPPQLNL-----  
 CS\_18 ASSGVAGLLKAILILKHGAIPPNI-----  
 GLRG\_05714 ASSGVAGLLKAILILKHGAIPPNI-----  
 CH063\_0922 -----  
 CH063\_1282 GASGLLSLIKATMMLERGVMLPNANFEKLNPAILEASGADRLRVLKEAMPWPESPSPRR  
 CS\_28 AASGMAQVIKTVLVLENGLIPPNTNFE-----  
 GLRG\_11563 AASGMAQVIKTVMVLENGVIPPNTNFE-----  
 C.P.NRPS\_6 GCAGLAGVIKVLAMKHDSTIPPNLHLRSLSAKIQPYQHALKVPT-KATWPTRVSGQHKR  
 CH063\_0617 GCAGLAGVIKVLAMKHDSTIPPNLHLRSLSAKIQPYQHALKVPT-EPTAWPAPAAGQPKR  
 C.P.NRPS\_1 GTAGLAGVIKTTAALQRKTIFPNKHFNLNPNRIAPFYGNLKVPT-VNVPWPKPPNGQPLR  
 GLRG\_09842 GTAGLAGLLKTTAALQRKTIFPNRHFKNLNPRIEFPYKNLKVPT-VEIPWPKPPENQPLR  
 GLRG\_09715 GAAGLAGLLKASVAIQHGQIPPNLHFNRLNPKIKPFYDHLVPT-SLQSWPELPGVPRR  
 C.P.NRPS\_8 GAAGIAGVLKASLALKHAAVPPNLLFERPNPRIEPLMANLRVPT-SLEPWPTEAGEPRR  
 GLRG\_10367 GAAGIAGVLKASLALKHAAVPPNLLFERPNPRIEPLMANLRVPT-SLEPWPTEAGEPRR  
 CS/1 GTAGIASILKASLALQNKQIPPNLWFNNVNPILPFCKNLRVPTINPKWEAEPLNSQPRR  
 CH063\_0489 GTAGLAGLLKGCALKHAEIPPNI-----

CH063\_0392 GTAGIAGILKASLAVQHGEIPPNLLFDKLSPAVAPFYTNLEIAT-EVK-----  
 GLRG\_07434 STAGLAGILKVVQAMQHGFPNMLLENLNPNVLPFYRHLQIPQ-ELTPWPQPPGQSRR  
 CS/20 GTAGLAALIKASLAMKARRIPPNNLHLDRLNPAVEPFYKDLEVPT-KLMDWPQPEPGQPLR  
 CH063\_0306 GTAGLAALIKASLAMKARRIPPNNLHNRVNPAVEPFYKDLEVPT-RLREWPQLEPGQPLR  
 CH030B GTAGLAADVKAATEALKAATLPPNRLFNRLSPKVEPFYKNLEVVT-SARPWPKIPANGMRR  
 CS\_30 GTAGLAAVIKASRAVQAGILPPNR-----  
 CS\_29 GTAGLAGVLKASLAVQHGAIPPNOHFE-----  
 CH063\_0199 GTAGIAGLLKACLAVKHGVIPPNL-----  
 C.P.NRPS\_2 GCAGMAGLLKITLAMQHKIIPPNNLHFHKLNPSVAPWYRNLIQCT-RPQEWPVAPGHPLR  
 CH063\_0288 GAAGLAGLLKVVHAMINRSIPPNLHLE-----  
 CS\_4 GAAGLAGLLKVIHSMINKSIPPNLHLE-----  
 GLRG\_08212 GAAGLAGLLKVVHSMINNSIPPNLHLE-----  
 CS\_15 GAAGVAGLIKGVLTTEKGRIPPNIINFE-----  
 CH063\_1445 GAAGVAGLIKGVLTTEKGRIPPNIINFE-----  
 GLRG\_02583 GAAGVSGLIKGVLTTEKGRIPPNIINFE-----  
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 GLRG\_03360 AASGIAGVIKAAAMMLERGFLLPN-----  
 CH063\_1222 AASGIVGVIKAAAMMLERGFLLPN-----  
 CH063\_0134 ASSGIVGVIKAIMMLKHGVIPPSLNQANPNIDMNSLGVQVPTSTREWPKDMPPRISVN  
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 GLRG\_09267 AASGLASIVKTVLALERGVIPPSINFE-----  
 CH0190 AASGLAGIHKVALSLEKGLMPPS-----  
 CH063\_0873 AASGLASVIKVVCALEAGLVPPNANFE-----  
 CS\_9 GNSGLASFIKGVLCVERGIIPANAWFE-----  
 CH063\_1490 -----  
 GLRG\_01860 GAAGVAAVIKGVLTLESGVIPGNIWFE-----  
 CS\_25 GAAGVAAVIKGVLTLESGVIPGNIWFE-----  
 CS\_34 PAAGIAGLIKGVLAALERGLIPPNIHLTLSTA-----  
 GLRG\_11770 GASGVAALIKSILALEKGYIPPQ-----  
 CH0399 CAAGIAALIKGVLALEKGVIPPNIHFT-----  
 CS\_11 SAAGVAGLIKGILAMEHGFIPPNIHFD-----  
 GLRG\_11840 SAAGVAGLIKGILAMEHGFIPPNIHFD-----  
 CH063\_0781 PAAGVAGFIKGVLSLEHGMIPPNVHFH-----  
 CH063\_1357 -----  
 GLRG\_10317 GAAGVAGIKAFAVERGLIPQNLWFERGEGVAAIVMKPLSQALADGDSIECVIRETGLN  
 CH063\_1471 GGSGLVQVIKAVLMLEQKIPPSLYYEKPNPRIPMDDWNLRVPTTELLPWPAAGLRRISIN  
 CH063\_0506 GASGLAGIHKTVLVLEHGIIPPICNFE-----  
 GLRG\_11835 GASGIAAILKTVLILENGVIPPANANFE-----  
 CS\_3 GASGLAGLLKTVLTLERGVIPPIADFE-----  
 GLRG\_11878 GASGLAGLLKTVLALERGVIPPIADFE-----  
 CH063\_0051 GCAGLAGLIKTVLCLEAGVIPPVAGLR-----  
 CH0135 GCSGLAGVLKSLCCLKGVLIPTAGIE-----  
 CS\_43 GASGVVSLIKVLLMMHENCIPPQAS-----  
 GLRG\_09268 GASGVISLIKVLLMMQESRIPPQASF-----  
 GLRG\_11857 CTAGVLSLIKVILMMCNGRIPPQASF-----  
 CH063\_0213 CASGVVSAVKILLMLQKGMIPPQVNLK-----  
 CH063\_0477 TAAGVASLLKVLAMLRHRAIPP-----  
 CH063\_0313 AASGAAGLIKTIMLRHEQIPPQASF-----  
 CS\_44 AASGAAGLIKAILMMRHGKIPPQASYR-----  
 GLRG\_11575 AASGAAGLIKAILMMRHGKIPPQASYRSLNPRIPALEPDGMEIPQVLTDWGPAERIACIN  
 CH063\_1347 AASGVAGVIKTLMMQHQAIKQANFFRLNRTIKVLPGDADDVVPESTLAWTTTTTTER  
 C.PKS GASGVAGLIKTIILMMQNRAIPRQVHF-----  
 CS\_45 GASGVTGIIRVLLMMQHRITIPQASFR-----  
 GLRG\_11798 GASGVAGIIRVLLMMQHRITIPQASFR-----  
 GLRG\_11778 AGAGAAAIVIKAIMMFERNIM-----  
 CH0398 AGSGAAALVKSIMMLQANIIPPQAGL-----  
 CS\_37 GAAGMASLMKCIKMFETNIVPPQAG-----  
 GLRG\_11836 GAAGMASLMKCIKMFETNTIPPQAG-----  
 CH063\_1255 ASSGVASVIKAILMFQKQILPPQVGMPHALNKQFPPLDDKKIRILSKAAPFRSASGKPRR  
 CS\_40 AASGASLIKGVFMFRHQIMPPQVG-----  
 CS\_38 AAAGIASFIKVLIMYQKGAVPPQIGVAKLNPTLPPDLEERNIGNLWEYSEWSRSNRAAGR  
 CS\_36 AAAGIASLIKVLLMYRHGTIPRHIGI-----

GLRG\_08632 AAAGITSLKVLVLSFQNEIPPHVGIK-----  
 CH063\_0351 AASGVTALTKVLMQKNAIPPHVGIK-----  
 CS\_35 AASGVTALTKVLMQKNAIPPHVGIKKEINKTFPKDLSERNVNIAFHLPFKRRDQKPR  
 GLRG\_04203 AASGVTALTKVLMQKNAIPPHVGIKKEINKTFPKDLSERNVNIAFHLPFKRRDQKPR  
 GLRG\_11956 AASGVTALIKVLLMMQKNEIPPHCGIK-----  
 CH063\_1223 AASGVTIAKVLMMMKHSIIPPHIGVKTEFNRRLPNLAQRNARIAMVPTAWPRPPHGGSR  
 GLRG\_08620 AAAGVTSLAKALMMMKHSTLPPHAGIK-----  
 CH063\_0250 AAAGVTSLAKVLMMMKHSTIPPHIGIK-----  
 CS\_39 AAAGITSIIKALLMFQHGKVPVPHVGIK-----  
 GLRG\_03507 AGGNTCMVI-----  
 GLRG\_03509 AAAGVTSVIKALMMFRENAIPPHAGIKTRINSKFPPELEKANIHIARELKPFDMDKEPRRI

GLRG\_10537 -----  
 CS\_23 -----  
 CH063\_1191 -----  
 CS\_16 -----  
 GLRG\_06162 -----  
 GLRG\_09075 -----  
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 CH063\_1411 SFGIGGSNAHVILEA-----  
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 GLRG\_11893 -----  
 CS\_19 -----  
 CH063\_0663 -----  
 GLRG\_00918 -----  
 CH063\_0821 -----  
 CS\_13 -----  
 CH063\_0053 -----  
 CH063\_1006 -----  
 GLRG\_07171 -----  
 CS\_22 -----  
 CS\_14 -----  
 CS\_26 -----  
 GLRG\_11435 -----  
 GLRG\_08640 -----  
 GLRG\_10827 -----  
 GLRG\_11425 -----  
 CH063\_0013 -----  
 GLRG\_08482 -----  
 CH063\_1130 -----  
 GLRG\_03511 -----  
 CS\_18 -----  
 GLRG\_05714 -----  
 CH063\_0922 -----  
 CH063\_1282 VCVTNYGLGGANSAILLEQ-----  
 CS\_28 -----  
 GLRG\_11563 -----  
 C.P.NRPS\_6 ASVN-----SFGFGGTNAHVILEAY-----  
 CH063\_0617 ASVN-----SFGFGGTNAHVILES-----  
 C.P.NRPS\_1 ASVN-----SFGFGGTNSHVILES-----  
 GLRG\_09842 ASVN-----SFGFGGTNAHVILES-----  
 GLRG\_09715 ASIN-----SFGFGGTNAHAILES-----  
 C.P.NRPS\_8 ASVNKVFPFVANDDDSFSGFGGTNAHAILES-----  
 GLRG\_10367 ASVN-----NFGFGGTNAHAILES-----  
 CS/1 TSVN-----SFGFGGANAHAIILE-----  
 CH063\_0489 -----  
 CH063\_0392 -----  
 GLRG\_07434 ASVN-----SFGFGGTNAHIILES-----  
 CS/20 ASVN-----SFGFGGANAHAIIES-----  
 CH063\_0306 ASVN-----SFGFGGANAHAIIES-----  
 CH030B VSVN-----SFGFGGANACHAIILES-----

CS\_30 -----  
 CS\_29 -----  
 CH063\_0199 -----  
 C.P.NRPS\_2 ASIN-----GFGSGGTNVHVIVES-----  
 CH063\_0288 -----  
 CS\_4 -----  
 GLRG\_08212 -----  
 CS\_15 -----  
 CH063\_1445 -----  
 GLRG\_02583 -----  
 CS\_32 -----  
 GLRG\_03360 -----  
 CH063\_1222 -----  
 CH063\_0134 NYGYGGTNGHVIIVAICSIGL-----  
 CS\_21 NFGYGGANSHIIMEE-----  
 GLRG\_09267 -----  
 CH0190 -----  
 CH063\_0873 -----  
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 GLRG\_01860 -----  
 CS\_25 -----  
 CS\_34 -----  
 GLRG\_11770 -----  
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 CS\_11 -----  
 GLRG\_11840 -----  
 CH063\_0781 -----  
 CH063\_1357 -----  
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 CH063\_1471 SFGYGGTNAHCILDD-----  
 CH063\_0506 -----  
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 CS\_3 -----  
 GLRG\_11878 -----  
 CH063\_0051 -----  
 CH0135 -----  
 CS\_43 -----  
 GLRG\_09268 -----  
 GLRG\_11857 -----  
 CH063\_0213 -----  
 CH063\_0477 -----  
 CH063\_0313 -----  
 CS\_44 -----  
 GLRG\_11575 NYGAAGSNAAIMIRQ-----  
 CH063\_1347 RASLVNNYGAAGSNAAILVRQ-----  
 C.PKS -----  
 CS\_45 -----  
 GLRG\_11798 -----  
 GLRG\_11778 -----  
 CH0398 -----  
 CS\_37 -----  
 GLRG\_11836 -----  
 CH063\_1255 VLINNFDAAGGNTALVLED-----  
 CS\_40 -----  
 CS\_38 LAVVNSFGAHGGNTTVLL-----  
 CS\_36 -----  
 GLRG\_08632 -----  
 CH063\_0351 -----  
 CS\_35 RVFVNNFSAAGGNTGLLLED-----  
 GLRG\_04203 RIFVNNFSAAGGNTGLLLED-----  
 GLRG\_11956 -----



CH063\_1223 RVLLNNFSAAGGNTSIVMED-----  
GLRG\_08620 -----  
CH063\_0250 -----  
CS\_39 -----  
GLRG\_03507 -----  
GLRG\_03509 MIGNFGATGGNTCMVIEE-----

GLRG\_10537 -----  
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CS\_16 -----  
GLRG\_06162 -----  
GLRG\_09075 -----  
CH063\_1187 -----  
CH063\_1411 -----  
CS\_5 -----  
GLRG\_11893 -----  
CS\_19 -----  
CH063\_0663 -----  
GLRG\_00918 -----  
CH063\_0821 -----  
CS\_13 -----  
CH063\_0053 -----  
CH063\_1006 -----  
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CS\_22 -----  
CS\_14 -----  
CS\_26 -----  
GLRG\_11435 -----  
GLRG\_08640 -----  
GLRG\_10827 -----  
GLRG\_11425 -----  
CH063\_0013 -----  
GLRG\_08482 -----  
CH063\_1130 -----  
GLRG\_03511 -----  
CS\_18 -----  
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CH063\_0922 -----  
CH063\_1282 -----  
CS\_28 -----  
GLRG\_11563 -----  
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CH063\_0617 -----  
C.P.NRPS\_1 -----  
GLRG\_09842 -----  
GLRG\_09715 -----  
C.P.NRPS\_8 -----  
GLRG\_10367 -----  
CS/1 -----  
CH063\_0489 -----  
CH063\_0392 -----  
GLRG\_07434 -----  
CS/20 -----  
CH063\_0306 -----  
CH030B -----  
CS\_30 -----  
CS\_29 -----  
CH063\_0199 -----  
C.P.NRPS\_2 -----  
CH063\_0288 -----

CS\_4 -----  
 GLRG\_08212 -----  
 CS\_15 -----  
 CH063\_1445 -----  
 GLRG\_02583 -----  
 CS\_32 -----  
 GLRG\_03360 -----  
 CH063\_1222 -----  
 CH063\_0134 -----  
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 CS\_25 -----  
 CS\_34 -----  
 GLRG\_11770 -----  
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 CS\_11 -----  
 GLRG\_11840 -----  
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 CH063\_1357 -----  
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 CH063\_1471 -----  
 CH063\_0506 -----  
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 CS\_3 -----  
 GLRG\_11878 -----  
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 CH0135 -----  
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 GLRG\_09268 -----  
 GLRG\_11857 -----  
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 CH063\_0477 -----  
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 CS\_44 -----  
 GLRG\_11575 -----  
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 C.PKS -----  
 CS\_45 -----  
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 GLRG\_11778 -----  
 CH0398 -----  
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 GLRG\_11836 -----  
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 CS\_40 -----  
 CS\_38 -----  
 CS\_36 -----  
 GLRG\_08632 -----  
 CH063\_0351 -----  
 CS\_35 -----  
 GLRG\_04203 -----  
 GLRG\_11956 -----  
 CH063\_1223 -----  
 GLRG\_08620 -----  
 CH063\_0250 -----  
 CS\_39 -----  
 GLRG\_03507 -----

GLRG\_03509 -----

## Appendix D - Alignments used to create the rooted phylogenetic trees in chapter 5

### PART 1: ITS sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

```
CgS11      CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGTAGGGTCCGCC
JG34.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG42.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG7.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS1.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
CBS_129661
CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
E031      CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
FS13.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
FS34.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
FS37.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
FS5.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
FS8.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
GS1.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG102.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG18.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG2.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG75.00   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG88.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
JG9.001   CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
S3.001_NR CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS100.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS107.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS124.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS159.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS171.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS204.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS240.001 CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS26.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS29.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS41.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS45.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS63.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
SS78.001  CTCCGGATCCCGATGCGAGACGTTAGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCC
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CgS11      ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG34.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG42.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG7.001   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
SS1.001   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
CBS_129661
ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
E031      ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
FS13.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
FS34.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
FS37.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
FS5.001   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
FS8.001   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
GS1.001   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG102.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG18.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG2.001   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG75.00   ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
JG88.001  ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT
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SS107.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS124.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS159.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS171.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS204.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS240.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS26.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS29.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS41.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS45.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS63.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT  
SS78.001 ACTACTTTTGAGGGCCTACGTCAACCGTAGTGCCCCAACACCAAGCGAAGCTTGAGGGTT

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JG34.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
JG42.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
JG7.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS1.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
CBS\_129661 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
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FS13.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
FS34.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
FS37.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
FS5.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
FS8.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
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JG9.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
S3.001\_NR GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS100.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS107.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS124.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS159.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS171.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
SS204.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
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SS26.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
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SS41.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
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SS63.001 GAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAA  
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JG34.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
JG42.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
JG7.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
SS1.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
CBS\_129661 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
E031 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
FS13.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
FS34.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
FS37.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT

FS5.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
FS8.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
GS1.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
JG102.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
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S3.001\_NR GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
SS100.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
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SS63.001 GATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTT  
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CgS11 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
JG34.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
JG42.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
JG7.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS1.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
CBS\_129661 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
E031 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
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FS34.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
FS37.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
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JG102.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
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JG2.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
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JG88.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
JG9.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
S3.001\_NR CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS100.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS107.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS124.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS159.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS171.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS204.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS240.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS26.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS29.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
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SS45.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS63.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT  
SS78.001 CATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTATTTGCTTGTGCCACT

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JG34.001 CAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGCGACCCCTCC

JG42.001 CAGAAGAAACGTCGTAAATCAGAGTTTGGTTATCCTCCGGCGGGCGCCGCGACCCCTCC  
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CgS11 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
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 CBS\_129661 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
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 FS13.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
 FS34.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
 FS37.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
 FS5.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
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 SS100.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
 SS107.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC  
 SS124.001 CGGAGGAGGAGGGCGCGCCGGGAGGGCGTCCCCGGGGGGACCCCTAACC CGCCGAAGC

SS159.001  
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SS171.001  
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SS204.001  
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SS240.001  
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SS26.001 CGGAGGAGGAGGGCGCGGCCGGGAGGGCGTCCCCGGGGGGACCCCCTAACCCGCCGAAGC  
SS29.001 CGGAGGAGGAGGGCGCGGCCGGGAGGGCGTCCCCGGGGGGACCCCCTAACCCGCCGAAGC  
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SS63.001 CGGAGGAGGAGGGCGCGGCCGGGAGGGCGTCCCCGGGGGGACCCCCTAACCCGCCGAAGC  
SS78.001 CGGAGGAGGAGGGCGCGGCCGGGAGGGCGTCCCCGGGGGGACCCCCTAACCCGCCGAAGC

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CgSI1 AACAGTTAGGTATGTTCA  
JG34.001 AACAGTTAGGTATGTTCA  
JG42.001 AACAGTTAGGTATGTTCA  
JG7.001 AACAGTTAGGTATGTTCA  
SS1.001 AACAGTTAGGTATGTTCA  
CBS\_129661 AACAGTTAGGTATGTTCA  
E031 AACAGTTAGGTATGTTCA  
FS13.001 AACAGTTAGGTATGTTCA  
FS34.001 AACAGTTAGGTATGTTCA  
FS37.001 AACAGTTAGGTATGTTCA  
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FS8.001 AACAGTTAGGTATGTTCA  
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JG102.001 AACAGTTAGGTATGTTCA  
JG18.001 AACAGTTAGGTATGTTCA  
JG2.001 AACAGTTAGGTATGTTCA  
JG75.001 AACAGTTAGGTATGTTCA  
JG88.001 AACAGTTAGGTATGTTCA  
JG9.001 AACAGTTAGGTATGTTCA  
S3.001\_NR AACAGTTAGGTATGTTCA  
SS100.001 AACAGTTAGGTATGTTCA  
SS107.001 AACAGTTAGGTATGTTCA  
SS124.001 AACAGTTAGGTATGTTCA  
SS159.001 AACAGTTAGGTATGTTCA  
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SS240.001 AACAGTTAGGTATGTTCA  
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SS29.001 AACAGTTAGGTATGTTCA  
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SS45.001 AACAGTTAGGTATGTTCA  
SS63.001 AACAGTTAGGTATGTTCA  
SS78.001 AACAGTTAGGTATGTTCA

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## PART 2: Concatenated sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

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GS1.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
SS63.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
E031 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
FS13.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
FS34.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
FS37.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
FS5.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
JG88.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
SS100.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
SS107.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
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SS78.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
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JG7.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
SS1.001 TCGTTTGGTCATCGCTTCTGATCAGCTCGCCAGGCAATGTTTCGAAACCTTGGAGGCGGAT
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JG2.001 GCTGTGCCTTTGTTCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCTCGACGGGT  
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S3.001\_CBS

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JG2.001 ATGGTCTTGTTTCAGATTTGTAACGTATGCTTGGTGGCT

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### PART 3: APN2 sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

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CBS129661  
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JG34.001 GGTGAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
JG42.001 GGTGAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
JG7.001 GGTGAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
SS1.001 GGTGAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
JG102.001 GGTGAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
JG18.001 GGTGAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC

JG2.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 JG75.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 JG9.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 CgS11 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 E031 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 FS13.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 FS34.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 FS37.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 FS5.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 FS8.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 GS1.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 JG88.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS100.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS107.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS124.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS159.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS171.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS204.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS240.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS26.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS29.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS41.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS45.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS63.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 SS78.001 GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC  
 S3.001\_CBS GGTGAAAGGGATGAAGGACGAGAGACACCAGTGCTTTACGACCTGACGCGAATATTCCAC

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CBS129661 CCCACAA  
 JG34.001 CCCACAA  
 JG42.001 CCCACAA  
 JG7.001 CCCACAA  
 SS1.001 CCCACAA  
 JG102.001 CCCACAA  
 JG18.001 CCCACAA  
 JG2.001 CCCACAA  
 JG75.001 CCCACAA  
 JG9.001 CCCACAA  
 CgS11 CCCACAA  
 E031 CCCACAA  
 FS13.001 CCCACAA  
 FS34.001 CCCACAA  
 FS37.001 CCCACAA  
 FS5.001 CCCACAA  
 FS8.001 CCCACAA  
 GS1.001 CCCACAA  
 JG88.001 CCCACAA  
 SS100.001 CCCACAA  
 SS107.001 CCCACAA  
 SS124.001 CCCACAA  
 SS159.001 CCCACAA  
 SS171.001 CCCACAA  
 SS204.001 CCCACAA  
 SS240.001 CCCACAA  
 SS26.001 CCCACAA  
 SS29.001 CCCACAA  
 SS41.001 CCCACAA  
 SS45.001 CCCACAA  
 SS63.001 CCCACAA  
 SS78.001 CCCACAA  
 S3.001\_CBS CCCACAA

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**PART 4: MAT1/APN2sequences**

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

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CBS129661 CCGCGGTTCCACAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG34.001 CCGCGGTTCCAAAGGATCTAAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG7.001 CCGCGGTTCCAAAGGATCTAAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
SS1.001 CCGCGGTTCCAAAGGATCTAAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG42.001 CCGCGGTTCCAAAGGATCTAAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG9.001 CCGCGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
CgS11 CCGCGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGACAAGA
JG102.001 CCGCGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG18.001 CCGCGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG2.001 CCGCGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
JG75.001 CCGCGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
S3.001_CBS CCACGGTTCCAAAGGATCTGAAAGGGCGTGAACACCCATGACGGCTCCAGAGGGA----
E031 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
FS13.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
FS34.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
FS37.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
FS5.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
GS1.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
JG88.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS100.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS107.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS124.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS159.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS171.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS204.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS240.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS26.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS29.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS41.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS45.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS63.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
SS78.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
FS8.001 CCACGGTTCCAAAGGATCTGAAAGGGCTTGAACACCCATGACGGCTCCAGAGGGA----
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CBS129661 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG34.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG7.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
SS1.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG42.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG9.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
CgS11 AATGGTAGGTCAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG102.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG18.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG2.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
JG75.001 -----CAAGAAATGGTAGGTGAGAACGGGGGAGAGAGGGGAGAGCAACATACCAC
S3.001_CBS -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
E031 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
FS13.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
FS34.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
FS37.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
FS5.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
GS1.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
JG88.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
SS100.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
SS107.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
SS124.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
SS159.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC
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SS171.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS204.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS240.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS26.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS29.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS41.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS45.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS63.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
SS78.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC  
FS8.001 -----CAAGAAATGGTAGGTGAGAACGGTGGAGAGAGGGGAGAGCAACATACCAC

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CBS129661 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG34.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG7.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS1.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG42.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG9.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
CgS11 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG102.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG18.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG2.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
JG75.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
S3.001\_CBS AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
E031 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
FS13.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
FS34.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
FS37.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
FS5.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
GS1.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
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SS100.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS107.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS124.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS159.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS171.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS204.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS240.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS26.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS29.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS41.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS45.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS63.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
SS78.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG  
FS8.001 AGTTGACACCCGGCTTCTTGGTCTGGAGGATGATGCAGGGCTCCCCATGCTCGCACCTTG

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CBS129661 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG34.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG7.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS1.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG42.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG9.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
CgS11 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG102.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG18.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG2.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
JG75.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
S3.001\_CBS GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
E031 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
FS13.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
FS34.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA

FS37.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
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SS171.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS204.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS240.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS26.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS29.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS41.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS45.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS63.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
SS78.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA  
FS8.001 GCGCGACCCTCTTCCCAAGGTTGAACTTGGACCAAGACTCCTTGACCTCGATGGGATCAA

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CBS129661 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
JG34.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
JG7.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
SS1.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
JG42.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
JG9.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
CgS11 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
JG102.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
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S3.001\_CBS AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
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FS13.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
FS34.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
FS37.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
FS5.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
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SS124.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
SS159.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
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SS240.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
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SS63.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
SS78.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG  
FS8.001 AGACTCTTTCCGAGGTATCGATTCCAGGCACCGTAGTGGTGGCGGAAGAGCCTGCCAAGG

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JG34.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCGCTGCGCCTGCGA  
JG7.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCGCTGCGCCTGCGA  
SS1.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCGCTGCGCCTGCGA  
JG42.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCGCTGCGCCTGCGA  
JG9.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCGCTGCGCCTGCGA  
CgS11 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCGCTGCGCCTGCGA



JG102.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 JG18.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 JG2.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 JG75.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 S3.001\_CBS GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 E031 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 FS13.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 FS34.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 FS37.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 FS5.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 GS1.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
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 SS107.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS124.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS159.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS171.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS204.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS240.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS26.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
 SS29.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
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 SS63.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
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 FS8.001 GGGACGGAGAAGGTTCTATTGGCGGCGGAGCGTCTACTGTTGCGCCCCTGCGCCTGCGA  
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CBS129661

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 JG9.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
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 JG18.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
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 FS34.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
 FS37.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
 FS5.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
 GS1.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
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 SS107.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC  
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 SS78.001 GAGGAGGAGGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCCTGCGGCTGCTGTGC

FS8.001 GAGGAGGAGGGTCTTTGATACCCCCGAGAGAGGTGGGCCACCGCTGCGGCTGCTGTGC  
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JG34.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCCGACGGGTGGCGGCT  
JG7.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCCGACGGGTGGCGGCT  
SS1.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCCGACGGGTGGCGGCT  
JG42.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCCGACGGGTGGCGGCT  
JG9.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCTCGACGGGTGGCGGCT  
CgSII CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCTCGACGGGTGGCGGCT  
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SS78.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCTCGACGGGTGGCGGCT  
FS8.001 CTTTGTTCCTTTGTGGGCGACGGCTGGACTACCGCCGTATCGGCCTCGACGGGTGGCGGCT  
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JG34.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
JG7.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
SS1.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
JG42.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
JG9.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
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JG102.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
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E031 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
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FS37.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
FS5.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
GS1.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
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SS78.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT  
FS8.001 TCGAGGGCGGTTGCTTGGGTTTGAAGAAGCCTGTGAGGGTGCTTTGTTTGGATAAAGGAT

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JG7.001 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
SS1.001 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
JG42.001 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
JG9.001 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
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JG102.001 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
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JG2.001 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
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S3.001\_CBS CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
E031 CTGATTCGCTGGCCTTGGTCTTCTTTGAGGGACGTCTTGAGGGCATTGCTGTGTCCGGATG  
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E031 ACACTTGGGATGTTGGTGGCTCCAAAGTTTCTTCTGAGAGAATTCTCCGTCCTCTTTCT  
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SS63.001 ACACTTGGGATGTTGGTGGCTCCAAAGTTTCTTCTGAGAGAATTCTCCGTCCTCTTTCT  
SS78.001 ACACTTGGGATGTTGGTGGCTCCAAAGTTTCTTCTGAGAGAATTCTCCGTCCTCTTTCT  
FS8.001 ACACTTGGGATGTTGGTGGCTCCAAAGTTTCTTCTGAGAGAATTCTCCGTCCTCTTTCT

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JG7.001 CTTGGGGGGTGGCCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS1.001 CTTGGGGGGTGGCCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
JG42.001 CTTGGGGGGTGGCCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCCATG  
JG9.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCCATG  
CgS11 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
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JG2.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
JG75.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
S3.001\_CBS CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
E031 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
FS13.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
FS34.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
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FS5.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
GS1.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
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SS100.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS107.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS124.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
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SS171.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS204.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS240.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS26.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS29.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
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SS63.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
SS78.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCCAATG  
FS8.001 CTTGGGGGGTGGTCAGCTCCTCGGAGACGAGACTCTGCGACTGTGACTCC-ATG

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## PART 5: SOD2 sequences

CLUSTAL FORMAT: MUSCLE (3.8) multiple sequence alignment

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## References

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## Vita

### Katia Viana Xavier

#### EDUCATION

1. University of Kentucky (USA) PhD Plant Pathology. 2012 - present
2. Universidade Federal de Lavras (Brazil) M. Sc. Plant Pathology. 2011
3. Universidade Federal de Viçosa (Brazil) B. Sc. Agronomy Engineer. 2009

#### PROFESSIONAL EXPERIENCE

1. Chromatin Inc. Collaborative Project: “Response of ten parental sorghum lines to 16 isolates of *Colletotrichum*”, University of Kentucky, USA. 2015
2. Graduate Research Assistant, Fungal Genetics, University of Kentucky. 2012-present
3. Graduate Research Assistant, Plant Pathology, Universidade Federal de Lavras, Brazil. 2009-11
4. Graduate Research Assistant, Genetics, Universidade Federal de Viçosa, Brazil. 2006
5. Volunteer trainee, Crop protection. 2004-05

#### ORAL PRESENTATIONS AT CONFERENCES:

1. The potential impact of anthracnose on sweet sorghum production. APS Southern Division meeting. Balm, Florida, February 20–22, 2016.
2. Genetic diversity of *Colletotrichum* isolates from *Sorghum bicolor* and *S. halepense* in the Southeastern United States. APS Annual meeting. Tampa, Florida, July 3 - August 3, 2016.
3. Controle da Ferrugem do Cafeeiro com Produtos Alternativos. 31º Congresso Brasileiro de Pesquisas Cafeeiras, 2005, Guarapari, ES.

**SCIENTIFIC ACTIVITIES:** Reviewer, Plant Disease.

#### ABSTRACTS AND PROCEEDINGS:

1. **Xavier, K.**, Pfeiffer, T., Parreira, D., & Vaillancourt, L. (2016, April). The potential impact of anthracnose on sweet sorghum production. In PHYTOPATHOLOGY (Vol. 106, No. 2, pp. 16-16). 3340 PILOT KNOB ROAD, ST PAUL, MN 55121 USA: AMER PHYTOPATHOLOGICAL SOC.

2. **Xavier, K. V.**, M. F. Torres, E. A. Buiate, I. Gaffoor, S. Chopra, and L. J. Vaillancourt. "Comparison of putative secondary metabolite genes and gene clusters of *Colletotrichum graminicola* and *C. sublineolum*." In PHYTOPATHOLOGY, vol. 102, no. 7, pp. 138-139. 3340 PILOT KNOB ROAD, ST PAUL, MN 55121 USA: AMER PHYTOPATHOLOGICAL SOC, 2012.
3. Waquim, A. P.; **Xavier, K. V.**; Sakiyama, N. S.; Caixeta, E. T.; Zambolim, E. M.; Zambolim, L. Identificação de marcadores moleculares ligados aos genes de resistência do cafeeiro à ferrugem. In: XVII SIC, 2007, Viçosa-MG. XVII SIC, 2007.
4. **Xavier, K. V.**; Caixeta, E. T.; Barbosa, J. C.; Zambolim, E. M.; Zambolim, L.; Sakiyama, N. S. Escolha de primers para identificação de marcadores rapd ligados a resistência do cafeeiro à ferrugem. In: Simpósio de Iniciação Científica (XVI SIC), 2007, Vicosa-MG. Simpósio de Iniciação Científica (XVI SIC), 2007.
5. Braga, P. P.; Zambolim, Laércio; Sousa, A. F.; **Xavier, K. V.**; Souza Neto, P. N. Controle Químico da Ferrugem do Cafeeiro (*Hemileia vastatrix* Berk.et Br) pela Aplicação de Fungicidas Sistêmicos via Solo e Foliar, Associados com a Aplicação de Fungicida Cuprico. In: XIV Simpósio de Iniciação Científica VI Simpós - Mostra Científica da Pós-graduação e IV Simpósio de Extensão Universitária, 2006, Viçosa. XIV Simpósio de Iniciação Científica, 2006.
6. **Xavier, K. V.**; Zambolim, Laércio; Sousa, A. F.; Braga, P. P.; Souza Neto, P. N. Controle da Ferrugem do Cafeeiro *Hemileia vastratrix*, com Produtos Alternativos. In: XV Simpósio de Iniciação Científica, V Mostra Científica da Pós-graduação e III Simpósio de Extensão Universitária Pesquisa, Extensão e Desenvolvimento, 2006, Viçosa, MG. XV Simpósio de Iniciação Científica, V Mostra Científica da Pós-graduação e III Simpósio de Extensão Universitária Pesquisa, Extensão e Desenvolvimento, 2006.
7. Braga, P. P.; Zambolim, Laércio; Sousa, A. F.; **Xavier, K. V.** O Contole Químico da Ferrugem do Cafeeiro pela Aplicação de Fungicidas Sistêmicos Via Solo e Foliar, Associados com a Aplicação de Fungicidas Cuprico. In: 31º Congresso Brasileiro de Pesquisas Cafeeiras, 2005, Guarapará, ES. 31º Congresso Brasileiro de Pesquisas Cafeeiras. Guarapará, ES: Gráfica e Editora Bom Pastos, 2005. p. 353-354.
8. **Xavier, K. V.**; Zambolim, Laércio; Sousa, A. F.; Braga, P. P.; Souza Neto, P. N. Controle da Ferrugem do Cafeeiro com Produtos Alternativos. In: 31º Congresso Brasileiro de Pesquisas Cafeeiras, 2005, Guarapará, ES. 31º Congresso Brasileiro de Pesquisas Cafeeiras. Guarapará, ES: Gráfica e Editora Bom Pastor, 2005. p. 388-390.
9. Barbosa, J. C.; Zambolim, Laércio; Duarte, H. S. S.; **Xavier, K. V.**. Controle Químico da Ferrugem do Feijoeiro nos Sistemas de Plantio Direto e Convencional. In: XIV Simpósio de Iniciação Científica, IV Mostra Científica da Pós-graduação e II Simpósio de Extensão Universitária Pesquisa, Extensão e Desenvolvimento, 2004, Viçosa, MG. XIV Simpósio de Iniciação Científica, 2004. p. 130-130.

### **FIRST AUTHOR PUBLICATIONS IN PROGRESS:**

1. A comparative genomic analysis of putative pathogenicity genes in two host-specific *Colletotrichum* sibling species. Journal: BMC Genomics
2. Impact of *Colletotrichum* strains from cultivated and wild *Sorghum* spp. on the sweet sorghum variety Sugar Drip in field, greenhouse, and laboratory assays. Journal: Plant disease
3. Genetic diversity of *Colletotrichum* isolates from *Sorghum bicolor* and *S. halepense* in the Southeastern United States. Journal: Phytopathology
4. Identification of two new species of *Colletotrichum* infecting *Sorghum bicolor* and *S. halepense* in the Southeastern United States. Journal: Phytopathology

### **RELEVANT EXTRACURRICULAR ACTIVITIES AND FELLOWSHIPS**

1. Reviewer, Plant Disease.
2. Conference/ meetings Leadership (2).
3. Administrative Coordinator of NEFIT (Association of Plant Pathology Scholars – UFLA). 2009-11
4. Fellowships CAPES. 2005-2006; 2009-11

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