Etiology of Switchgrass Rust (*Puccinia emaculata*)

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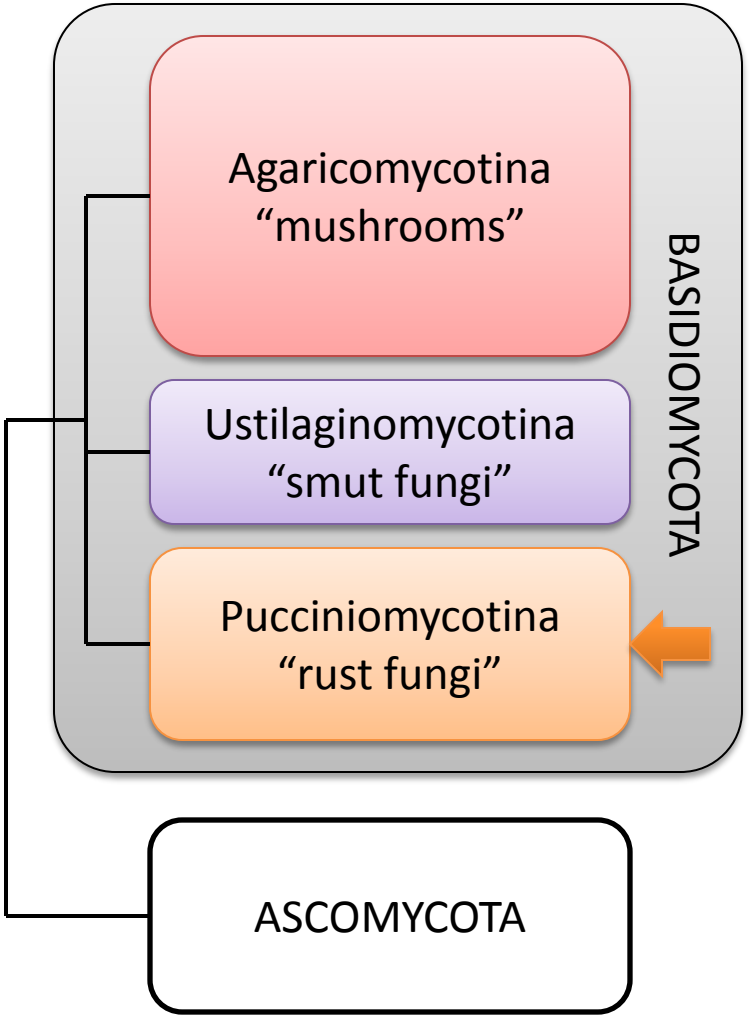
Outline

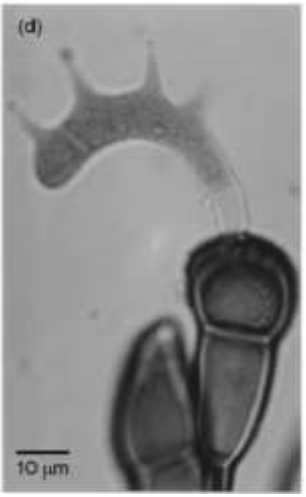
- Introduction to rust fungi
- Switchgrass rust(s): *Puccinia emaculata* et al.?
  - *Panicum*-infecting rust fungi (and alternate hosts)
- Defining etiology of switchgrass rust with phylogenetic "DNA barcodes"
  - Heterogenic alleles at barcode loci
- Efforts to identify alternate host
- "*Eudarluca*" sp., a possible mycoparasitic fungus of *P. emaculata*
  - Identification
  - Efforts to understand interaction on switchgrass

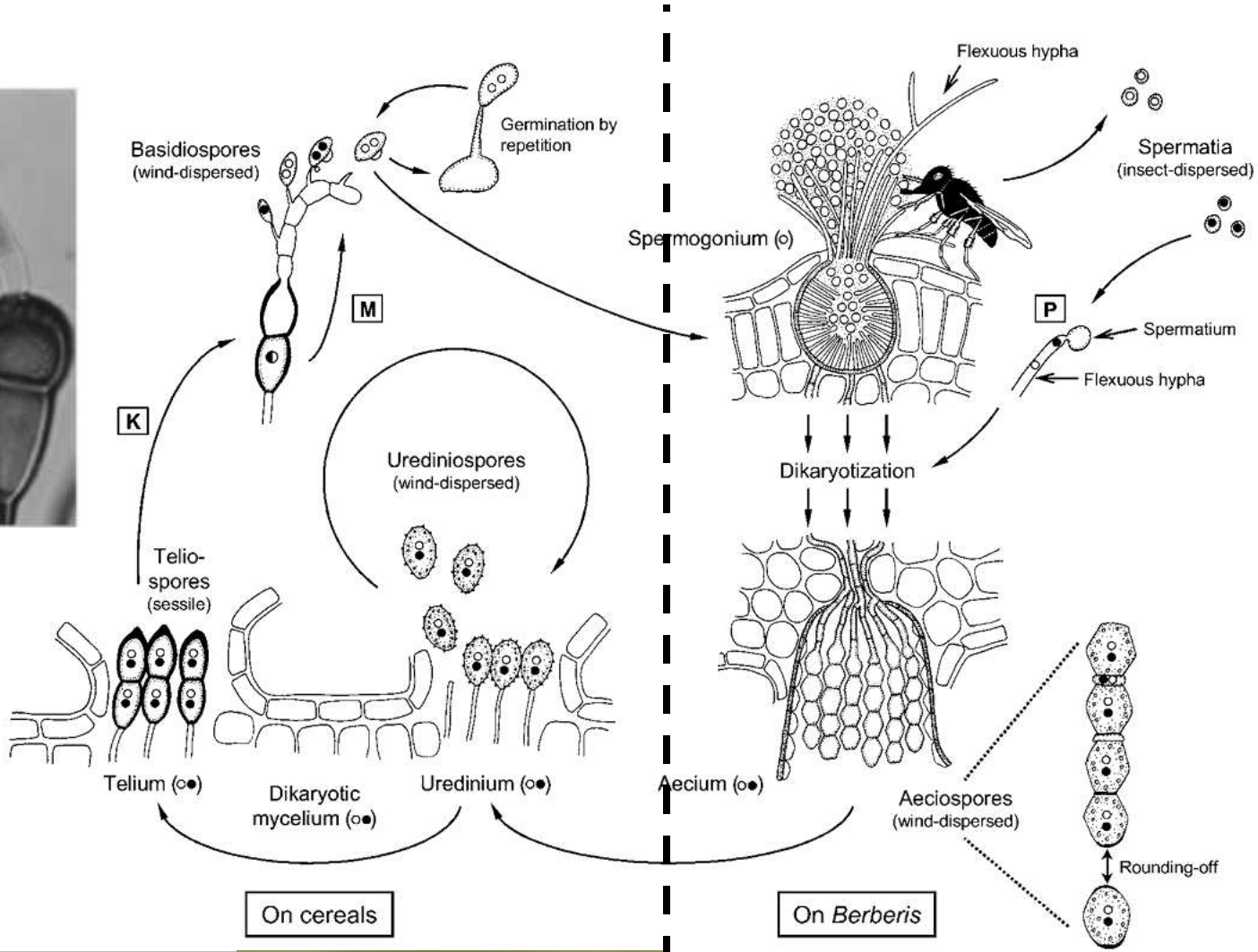
Introduction to rust fungi

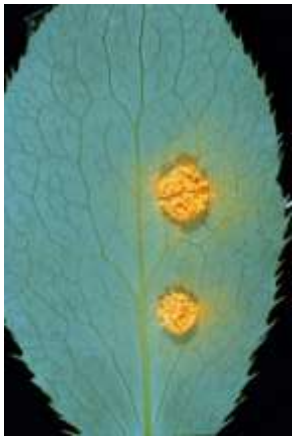
- Currently ~7,000 named species of rust fungi
- Obligate biotrophic parasites of plant hosts
  - Not culturable *in vitro*
  - Maintained on host plant
  - Can stored spores
- Complex life cycles with up to 5 spore stages



Life Cycle of *Puccinia graminis*

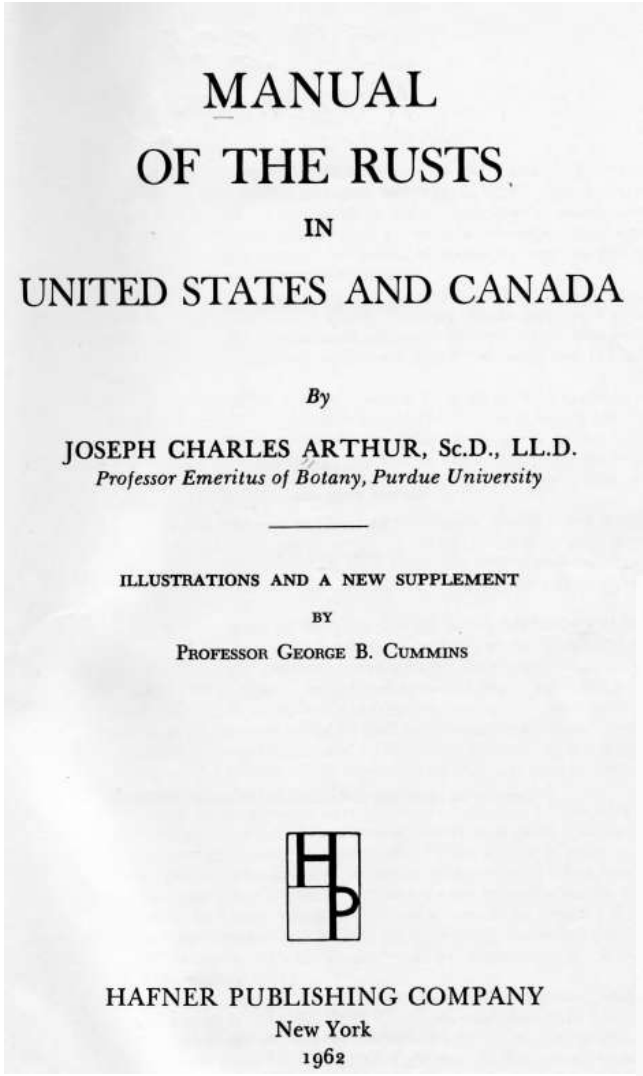






Switchgrass rust(s): *Puccinia emaculata* et al.? Identification of Rusts

- Know the host
  - *Panicum virgatum*
- Consult Manual of the Rusts (1962)
  - *Puccinia emaculata* (142)
  - *P. panici* (syn. *P. pammelii*) (143)
  - *Uromyces graminicola*
- Confirm morphological features match



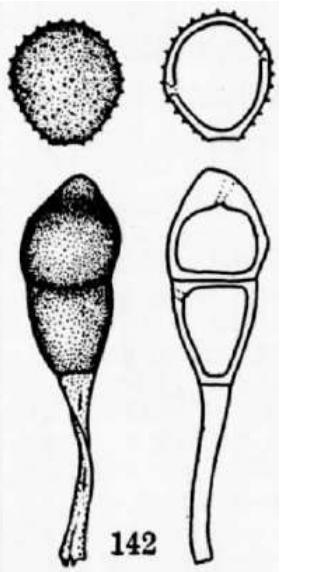
MANUAL
OF THE RUSTS
IN
UNITED STATES AND CANADA

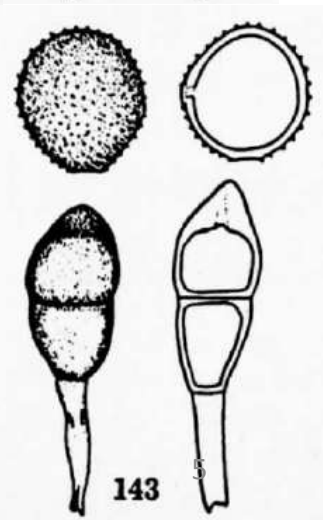
By

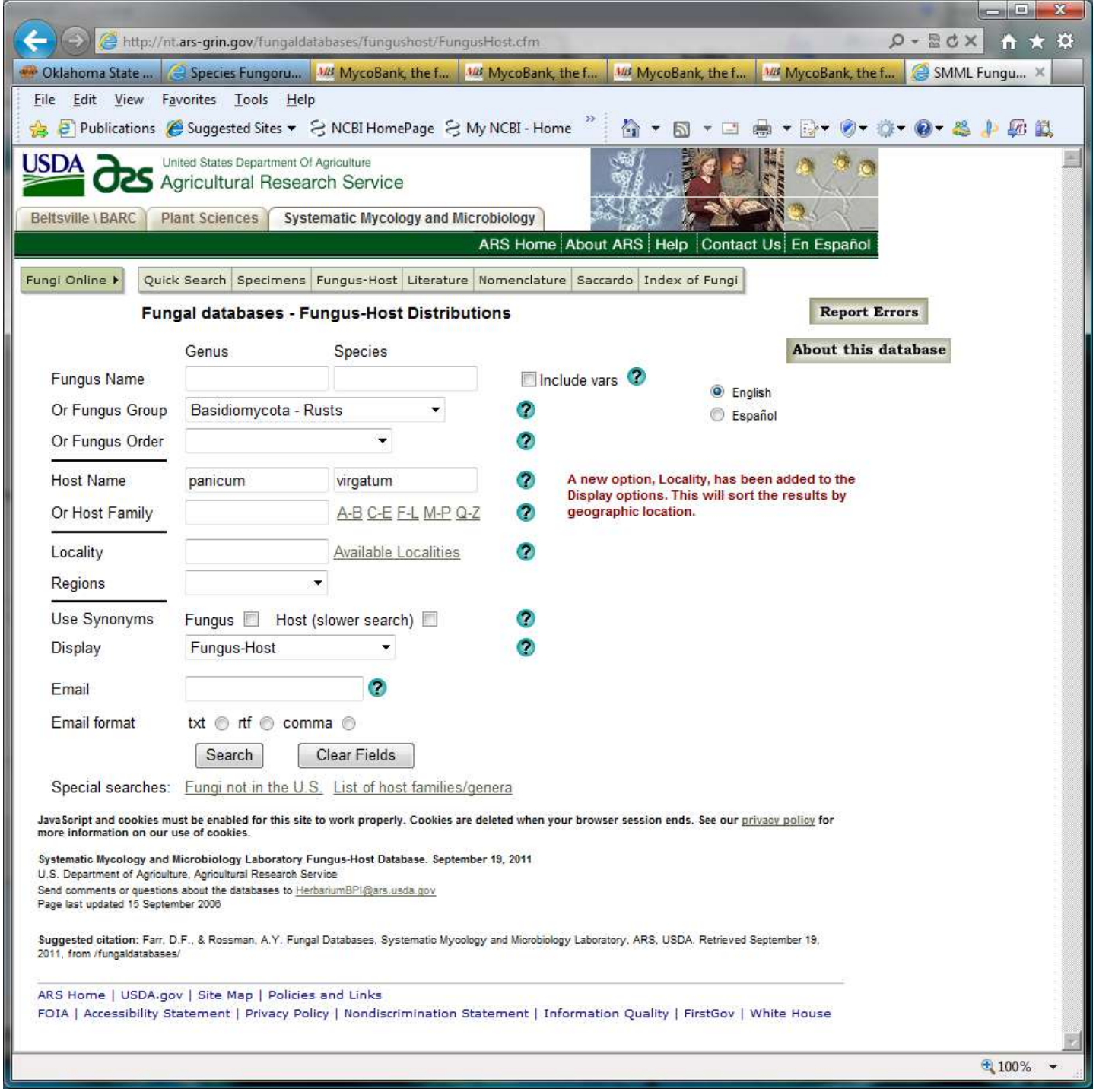
JOSEPH CHARLES ARTHUR, Sc.D., LL.D.
Professor Emeritus of Botany, Purdue University

ILLUSTRATIONS AND A NEW SUPPLEMENT
BY
PROFESSOR GEORGE B. CUMMINS

HAFNER PUBLISHING COMPANY
New York
1962



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NCBI HomePage
My NCBI - Home
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United States Department Of Agriculture
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Nomenclature
Saccardo
Index of Fungi
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Report Errors
About this database
Genus
Species
Fungus Name
Include vars
Or Fungus Group
Basidiomycota - Rusts
Or Fungus Order
English
Español
Host Name
panicum
virgatum
Or Host Family
A-B C-E F-L M-P Q-Z
A new option, Locality, has been added to the Display options. This will sort the results by geographic location.
Locality
Available Localities
Regions
Use Synonyms
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Host (slower search)
Display
Fungus-Host
Email
Email format
txt
rtf
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Search
Clear Fields
Special searches:
Fungi not in the U.S.
List of host families/genera
JavaScript and cookies must be enabled for this site to work properly. Cookies are deleted when your browser session ends. See our privacy policy for more information on our use of cookies.
Systematic Mycology and Microbiology Laboratory Fungus-Host Database. September 19, 2011
U.S. Department of Agriculture, Agricultural Research Service
Send comments or questions about the databases to HerbariumBPI@ars.usda.gov
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Suggested citation: Farr, D.F., & Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved September 19, 2011, from /fungaldatabases/
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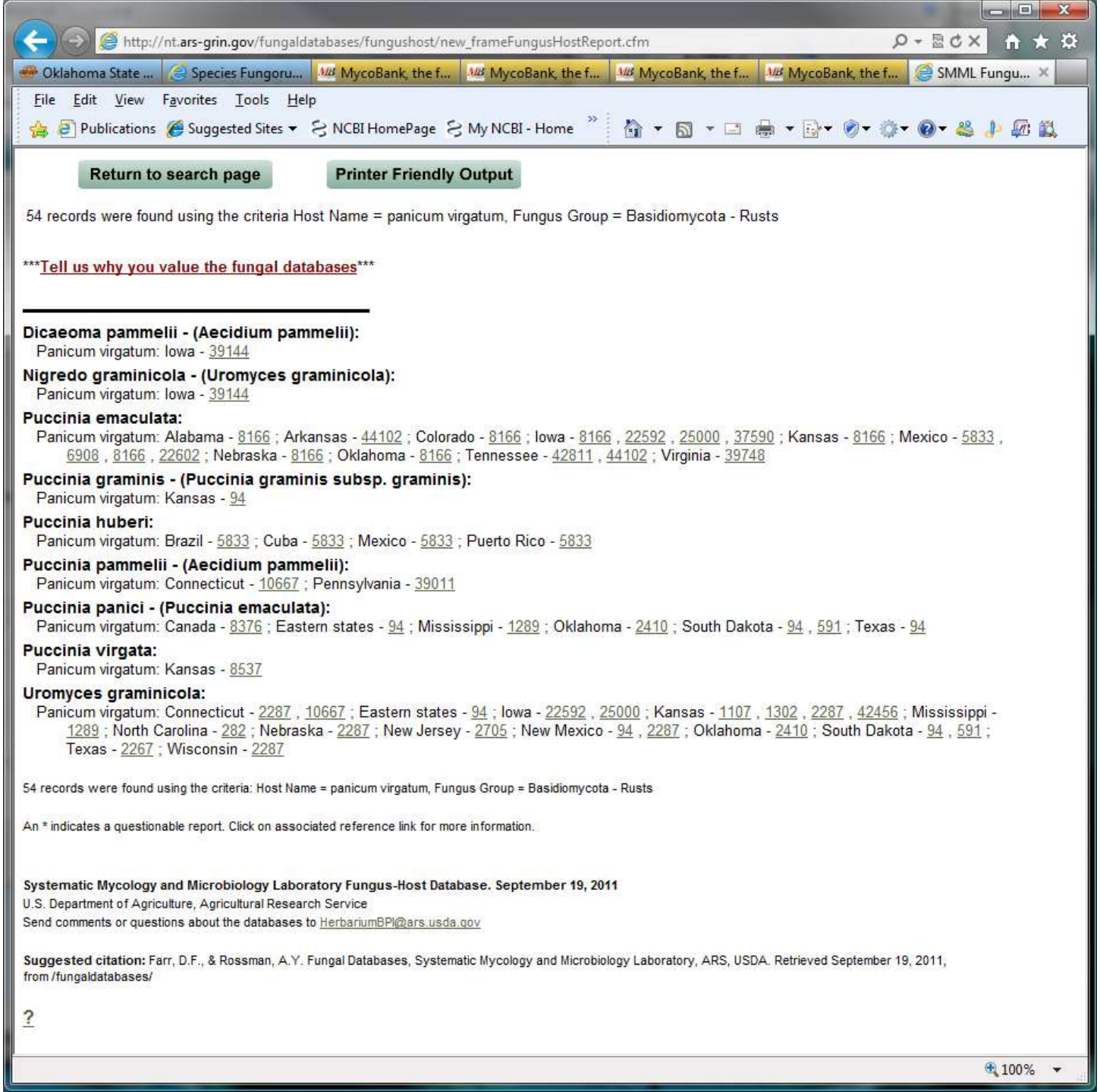
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MycoBank, the f...
SMML Fungu...
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54 records were found using the criteria Host Name = panicum virgatum, Fungus Group = Basidiomycota - Rusts
Tell us why you value the fungal databases
Dicaeoma pammelii - (Aecidium pammelii):
Panicum virgatum: Iowa - 39144
Nigredo graminicola - (Uromyces graminicola):
Panicum virgatum: Iowa - 39144
Puccinia emaculata:
Panicum virgatum: Alabama - 8166 ; Arkansas - 44102 ; Colorado - 8166 ; Iowa - 8166 , 22592 , 25000 , 37590 ; Kansas - 8166 ; Mexico - 5833 , 6908 , 8166 , 22602 ; Nebraska - 8166 ; Oklahoma - 8166 ; Tennessee - 42811 , 44102 ; Virginia - 39748
Puccinia graminis - (Puccinia graminis subsp. graminis):
Panicum virgatum: Kansas - 94
Puccinia huberi:
Panicum virgatum: Brazil - 5833 ; Cuba - 5833 ; Mexico - 5833 ; Puerto Rico - 5833
Puccinia pammelii - (Aecidium pammelii):
Panicum virgatum: Connecticut - 10667 ; Pennsylvania - 39011
Puccinia panici - (Puccinia emaculata):
Panicum virgatum: Canada - 8376 ; Eastern states - 94 ; Mississippi - 1289 ; Oklahoma - 2410 ; South Dakota - 94 , 591 ; Texas - 94
Puccinia virgata:
Panicum virgatum: Kansas - 8537
Uromyces graminicola:
Panicum virgatum: Connecticut - 2287 , 10667 ; Eastern states - 94 ; Iowa - 22592 , 25000 ; Kansas - 1107 , 1302 , 2287 , 42456 ; Mississippi - 1289 ; North Carolina - 282 ; Nebraska - 2287 ; New Jersey - 2705 ; New Mexico - 94 , 2287 ; Oklahoma - 2410 ; South Dakota - 94 , 591 ; Texas - 2267 ; Wisconsin - 2287
54 records were found using the criteria: Host Name = panicum virgatum, Fungus Group = Basidiomycota - Rusts
An * indicates a questionable report. Click on associated reference link for more information.
Systematic Mycology and Microbiology Laboratory Fungus-Host Database. September 19, 2011
U.S. Department of Agriculture, Agricultural Research Service
Send comments or questions about the databases to HerbariumBPI@ars.usda.gov
Suggested citation: Farr, D.F., & Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved September 19, 2011, from /fungaldatabases/
?
100%

Potential **alternate** (non-grass) hosts for switchgrass rust based on *Panicum*-infecting rusts in the SMML fungus-host distribution database and Arthur's Manual of the Rusts:

Rust fungus	Alternate host			
	Common name	**Scientific name(s)**	**Family**	**Duration**
Puccinia emaculata	**?**	**?**	**?**	**?**
P. panici (syn. *P. pammelii*; *Aecidium pammelii*; *Dicaeoma pammelii*)	Flowering spurge	*Euphorbia corollata*	*Euphorbiaceae*	P
	Snow on the mountain	*E. marginata*		A
	Sixangle spurge	*E. hexagona*		A
	American ipecac	*E. ipecacuanhae*		P
P. esclavensis	Sweet four o'clock	*Mirabilis longiflora*	*Nyctaginaceae*	P
	Colorado four o'clock	*M. multiflora*		P
P. substriata	Wright's ground-cherry	*Physalis acutifolia*	*Solanaceae*	A
	Ethiopian eggplant	*Solanum aethiopicum*		?
	Granadillo, potato vine	*S. bonariense*		P
	False Jerusalem cherry	*S. pseudocapsicum*		P
	Silverleaf nightshade	*S. elaeagnifolium*		P
	Turkey berry	*S. torvum*		P
	Eggplant	*S. melongena*		P
	Black nightshade	*S. nigrum*		P
	Hairy nightshade	*S. villosum*		A/P
	Jurubeba	*S. paniculatum*		?
	None	*S. sordidum*		?
	None	*S. subscandens*		?
	Fuzzyfruit nightshade	*S. candidum*		?
	Jurubeba velame	*S. variabile*		?
P. tubulosa (*A. tubulosum*)	Carolina horsenettle	*S. carolinense*	*Solanaceae*	P
	Eggplant	*S. melongena*		P
	None	*S. subscandens*		?
	Turkey berry	*S. torvum*		P
Uromyces graminicola	None	*Ditrysinia* (*Sebastiania*) *fruticosa*	*Euphorbiaceae*	P
	Queen's delight	*Stillingia sylvatica*		P
Aecidium crotonopsidis (*Uredo panici*)	Prairie tea, one seed croton	*Croton monanthogynus*	*Euphorbiaceae*	A

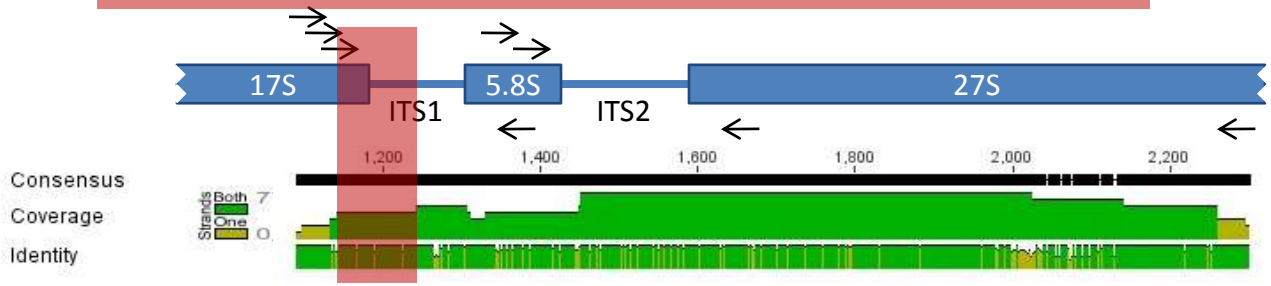
Defining etiology of switchgrass rust with phylogenetic "DNA barcodes"

- Multilocus "DNA barcodes" for identifying and classifying switchgrass rusts **(n rust sequences in NCBI)**
  - ITS-rDNA **(n = 1638)**
  - β-tubulin **(n = 218)**
  - Translation elongation factor (TEF)1-α **(n = 50)**
  - Mitochondrial cytochrome B (*cytB*) **(n = 28)**

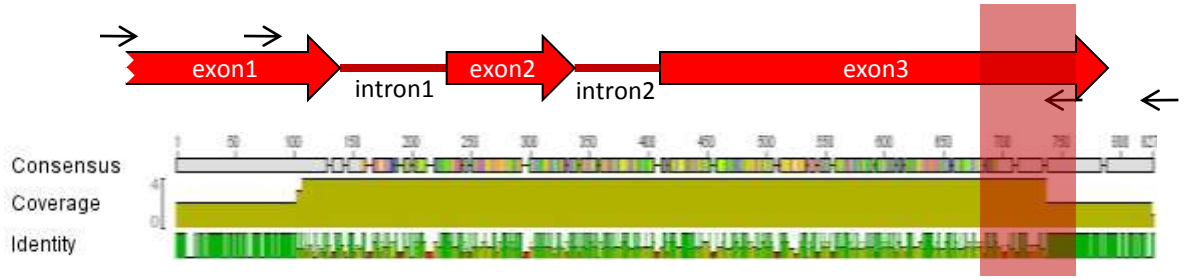
DNA barcode sequencing directly from *Puccinia emaculata* OSU09-1

homopolymeric indels disrupt PCR and sequencing

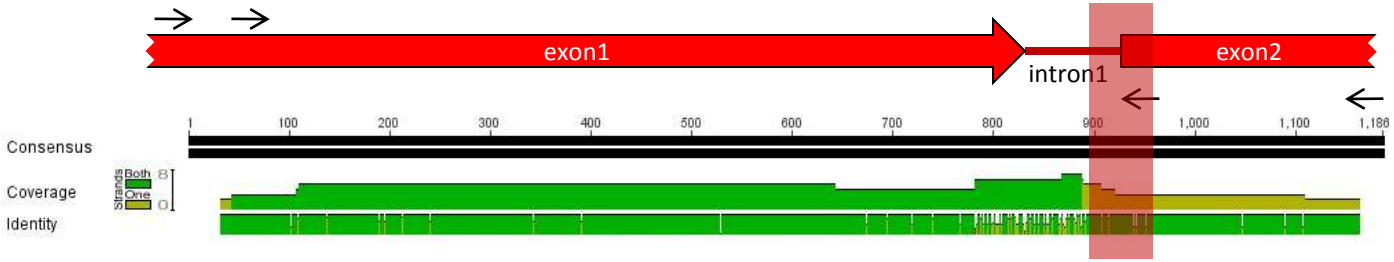
ITS-rDNA
(1,300bp)



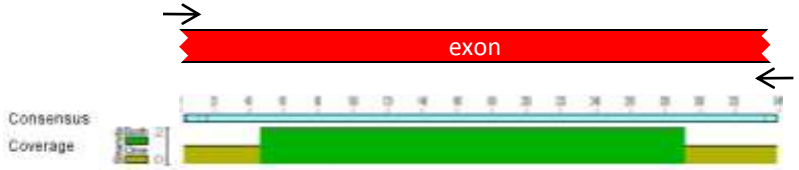
TEF1-α
(870bp)



β-tubulin
(1,000bp)



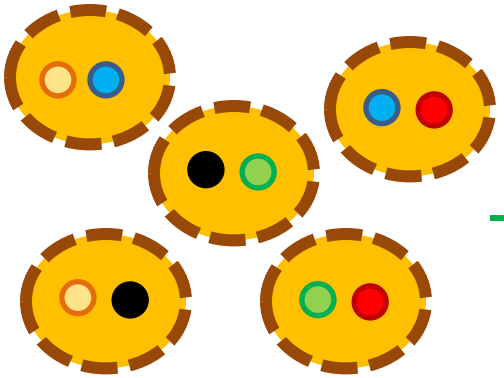
mitochondrial
cytochrome b
(330bp)



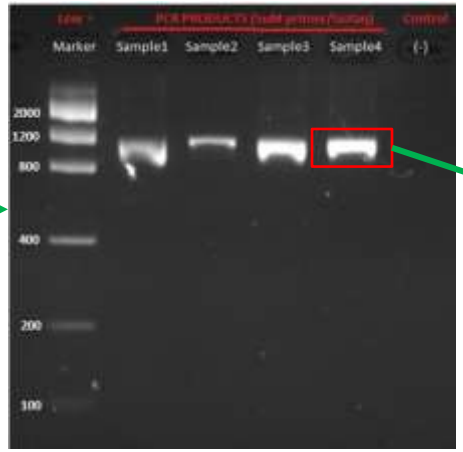
Heterogeneity of barcode alleles disrupt PCR and/or sequencing

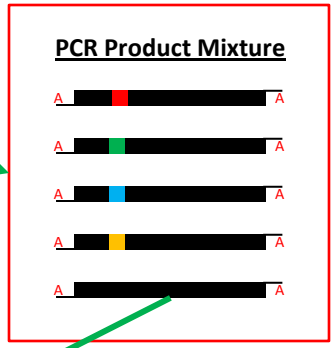
- Approach 1: Subclone PCR products prior to sequencing
- Approach 2: Isolate pure single-pustule rust cultures

Approach 1: Subclone amplified DNA barcodes prior to sequencing

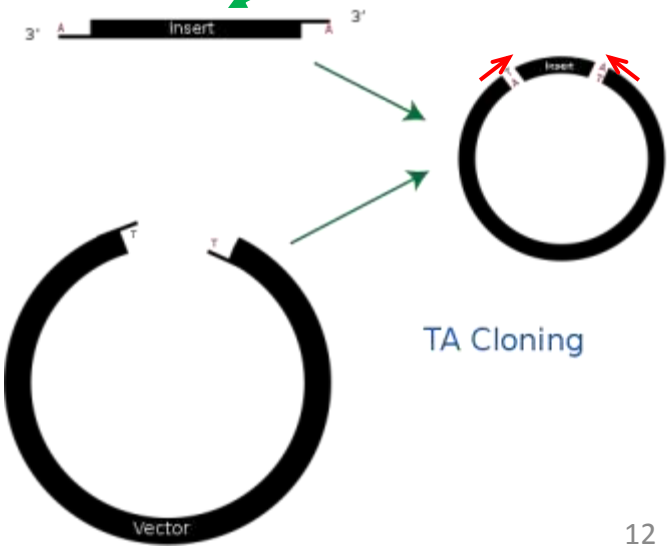


Dikaryotic urediniospores





- Multiple barcode alleles present in urediniospores
- Individual PCR products subcloned into TA vector
- ≥5 clones amplified and sequenced per barcode



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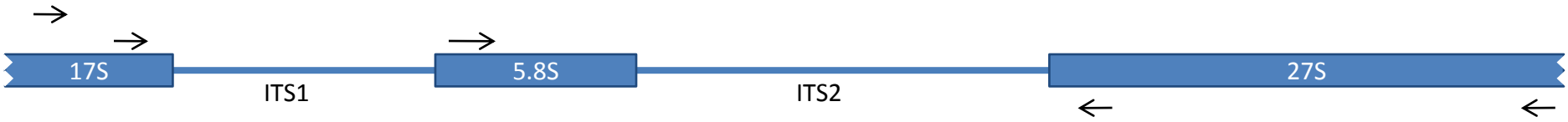
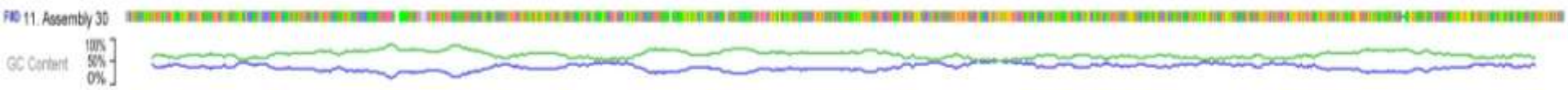
Approach 1: Subcloning DNA barcodes

ITS-rDNA

(1,300bp)



- 37 substitutions (SNPs)
- 8 INDELs in AT-rich regions
- Consensus 98.9% identical with *Puccinia emaculata* (EU915294) = most similar
- Clusters with *P. sorghi* (maize), *P. andropogonis* (big bluestem), and *P. asparagi* (asparagus)



- AT-rich composition likely disrupts PCR extension at 72°C

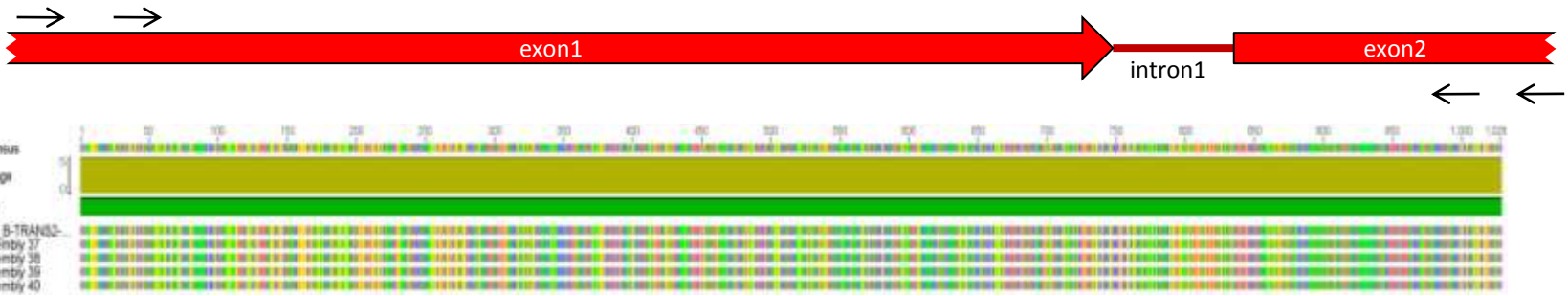
(Su et al. *NAR* 24:1574 1996)

Approach 1: Subcloning DNA barcodes

TEF1-α
(870bp)

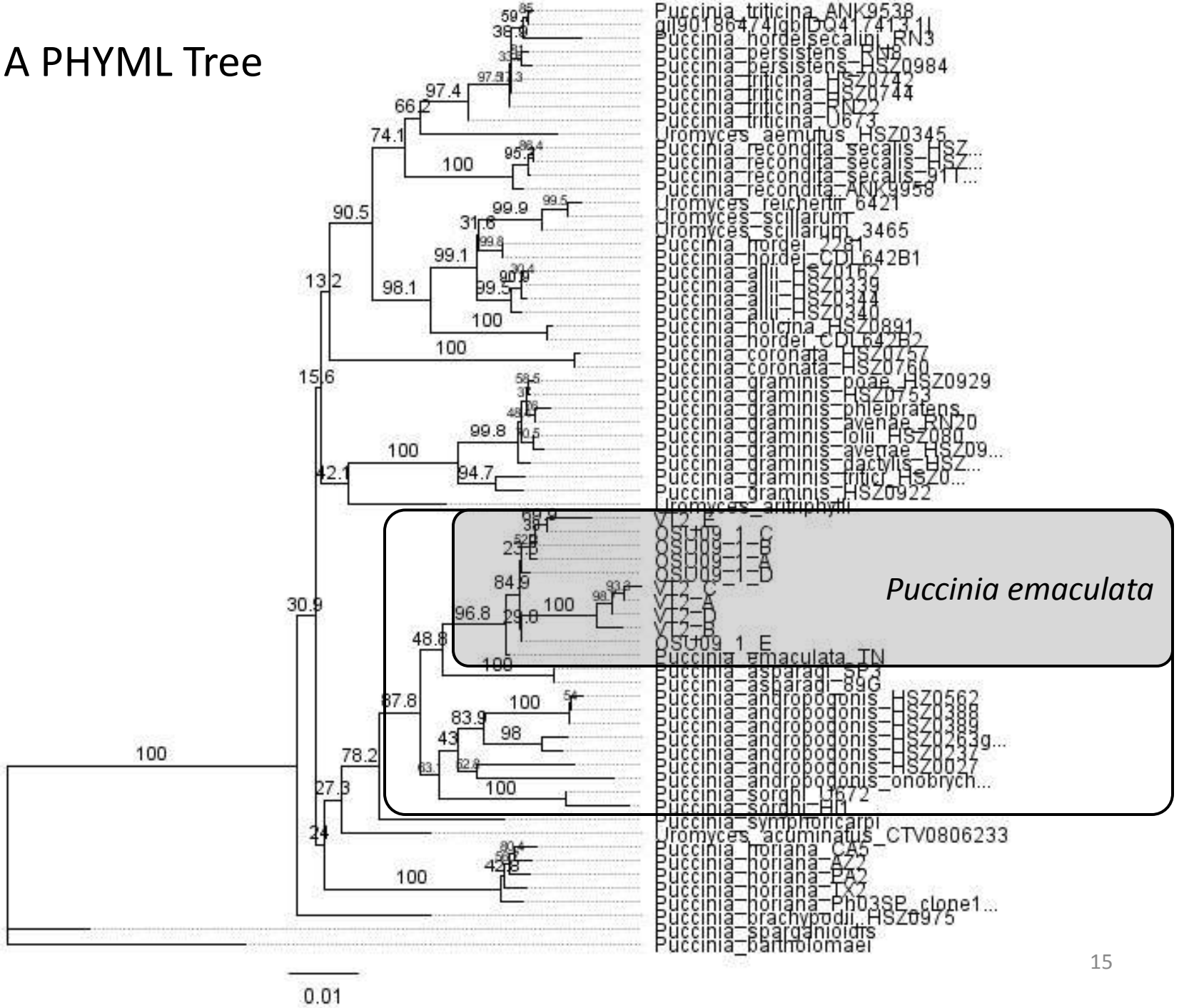
- Subcloning dramatically improved sequence quality
- No polymorphisms observed among subclones (i.e. monophyletic)
- Few TEF1α sequences from rusts available (93% ID *Uromyces polygoni-avicularis*)

β-tubulin
(1,000bp)

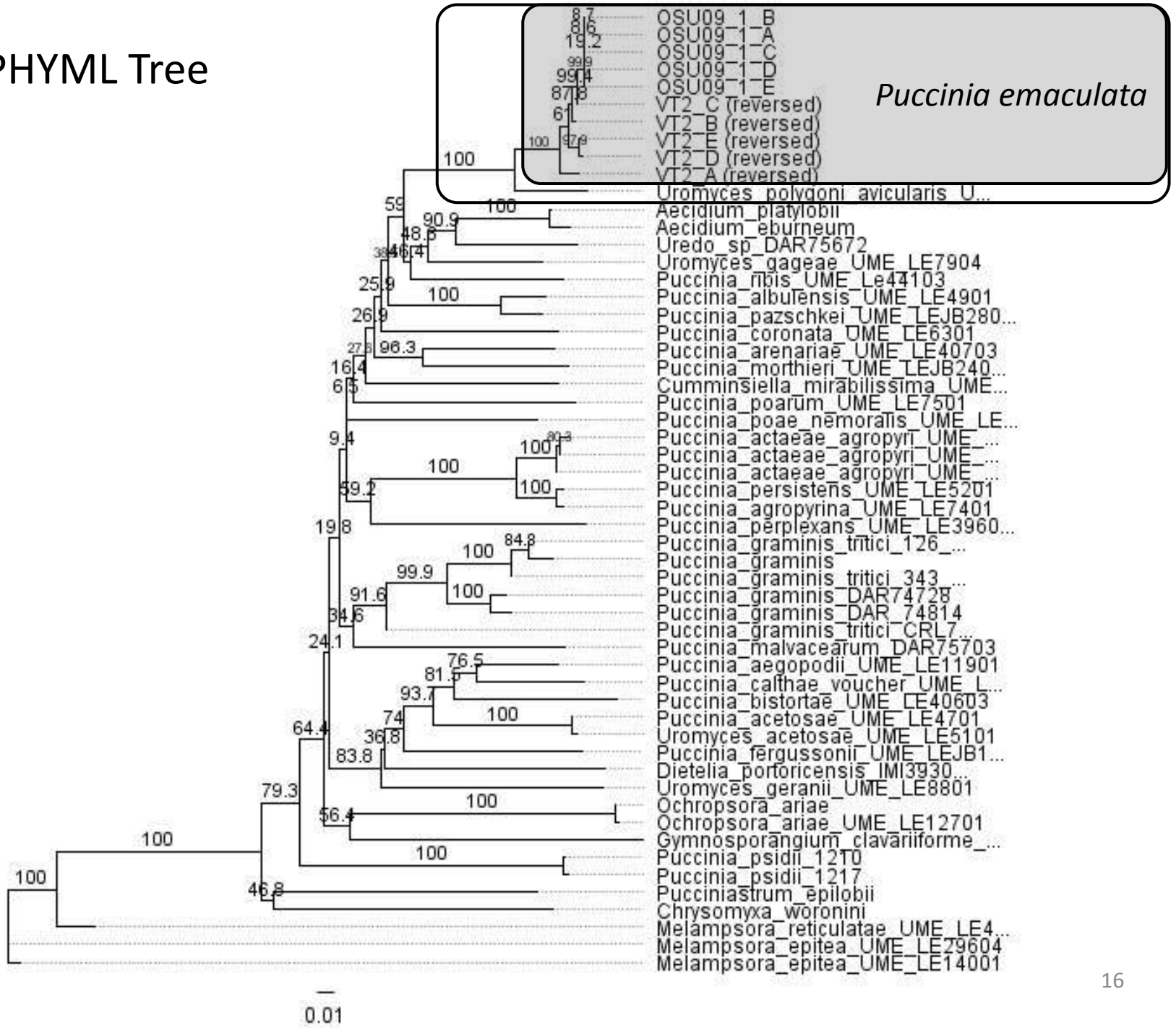


- Subcloning also dramatically improved sequence quality
- No polymorphisms observed among subclones (i.e. monophyletic)
- More β-tubulin sequences from rusts available
    - similar to *U. inaequialtus* (97%, on *Silene* spp.); *P. sorghi* (96%); *U. polygoni-avicularis* (95%, on *Polygonum* spp.)

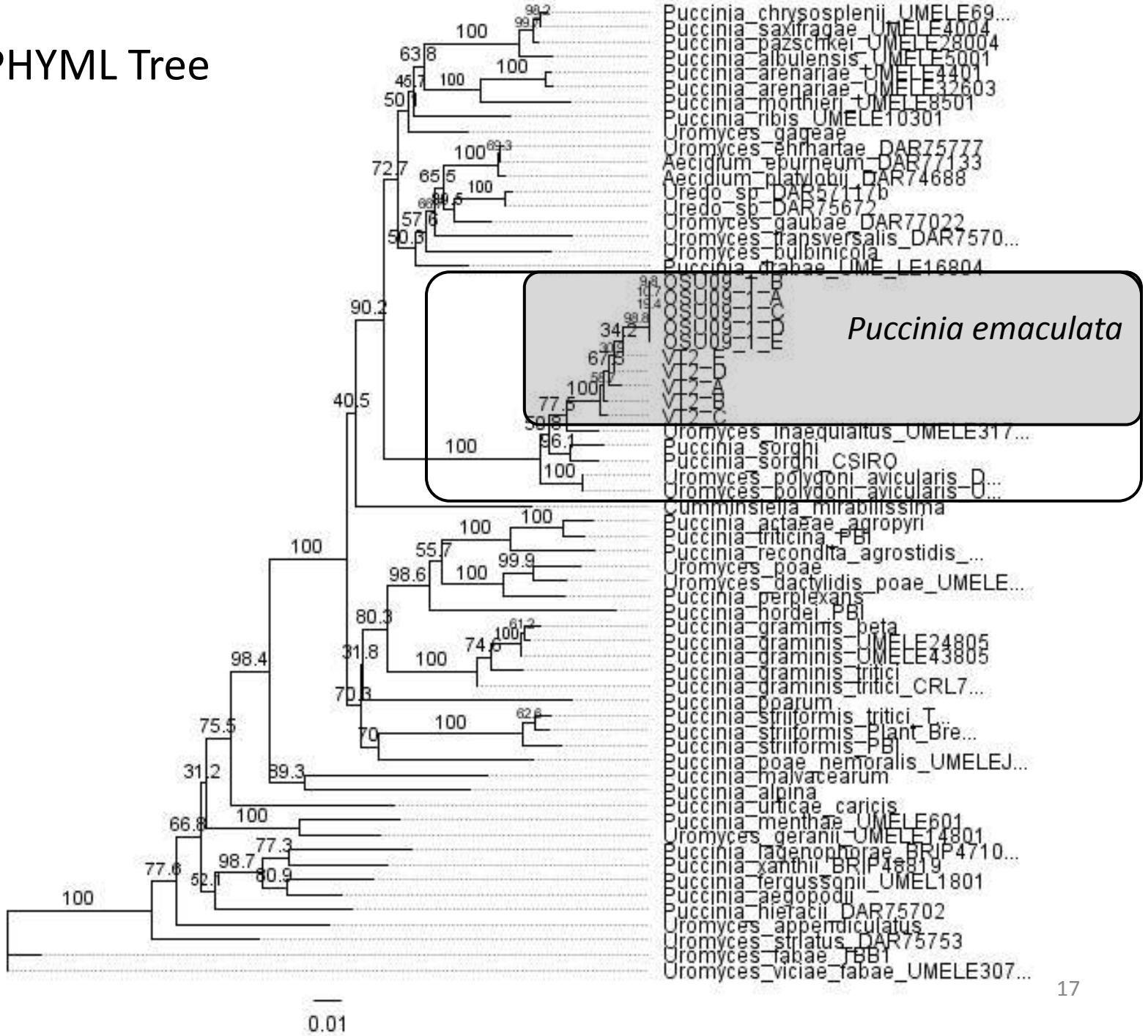
ITS-rDNA PHYML Tree



TEF1α PHYML Tree

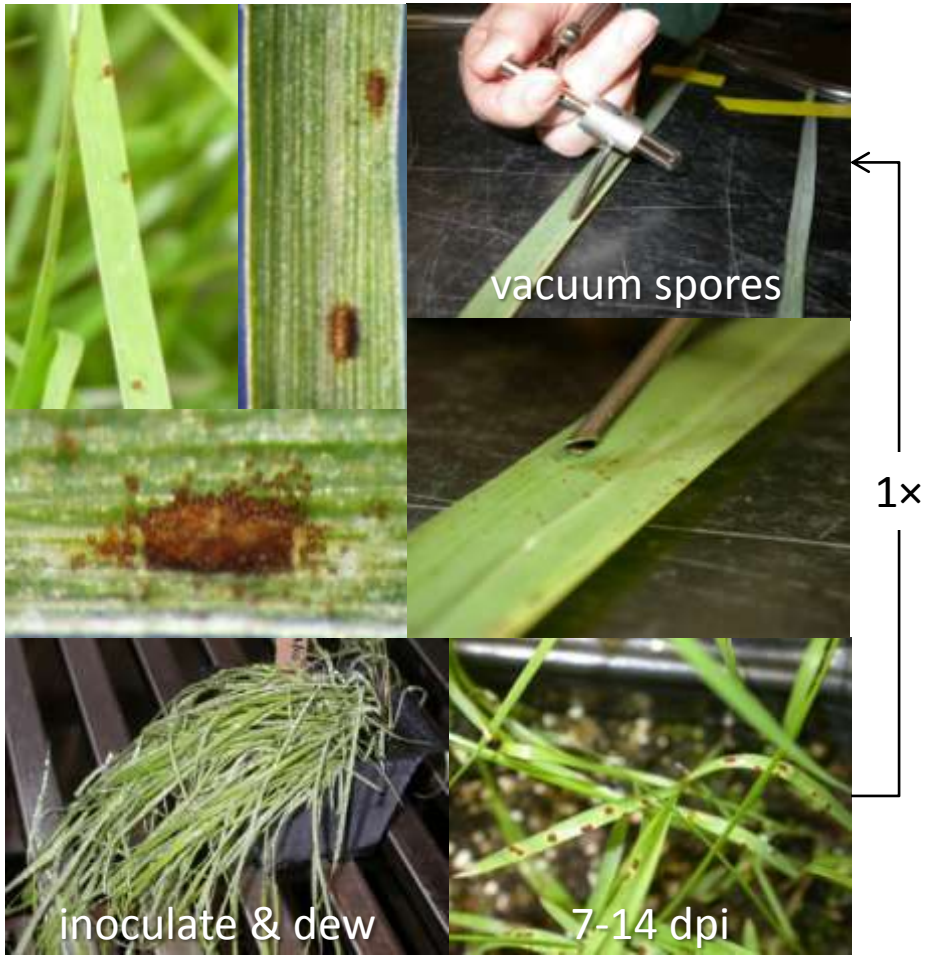


β-tubulin PHYML Tree



Approach 2: Isolate pure single-pustule rust cultures

- Protocol
  - Select single pustule
  - Microvacuum
  - Spray onto switchgrass seedlings
  - New pustules develop in 7 to 14 days post-inoculation
  - Repeat to purify
- Assumes single pustule from single urediniospore
- Reduces allelic variation of barcode loci to ~2
- 5 single pustule isolates
  - 4 barcodes from each were amplified and sequenced



Approach 2: Isolate pure single-pustule rust cultures

RESULTS

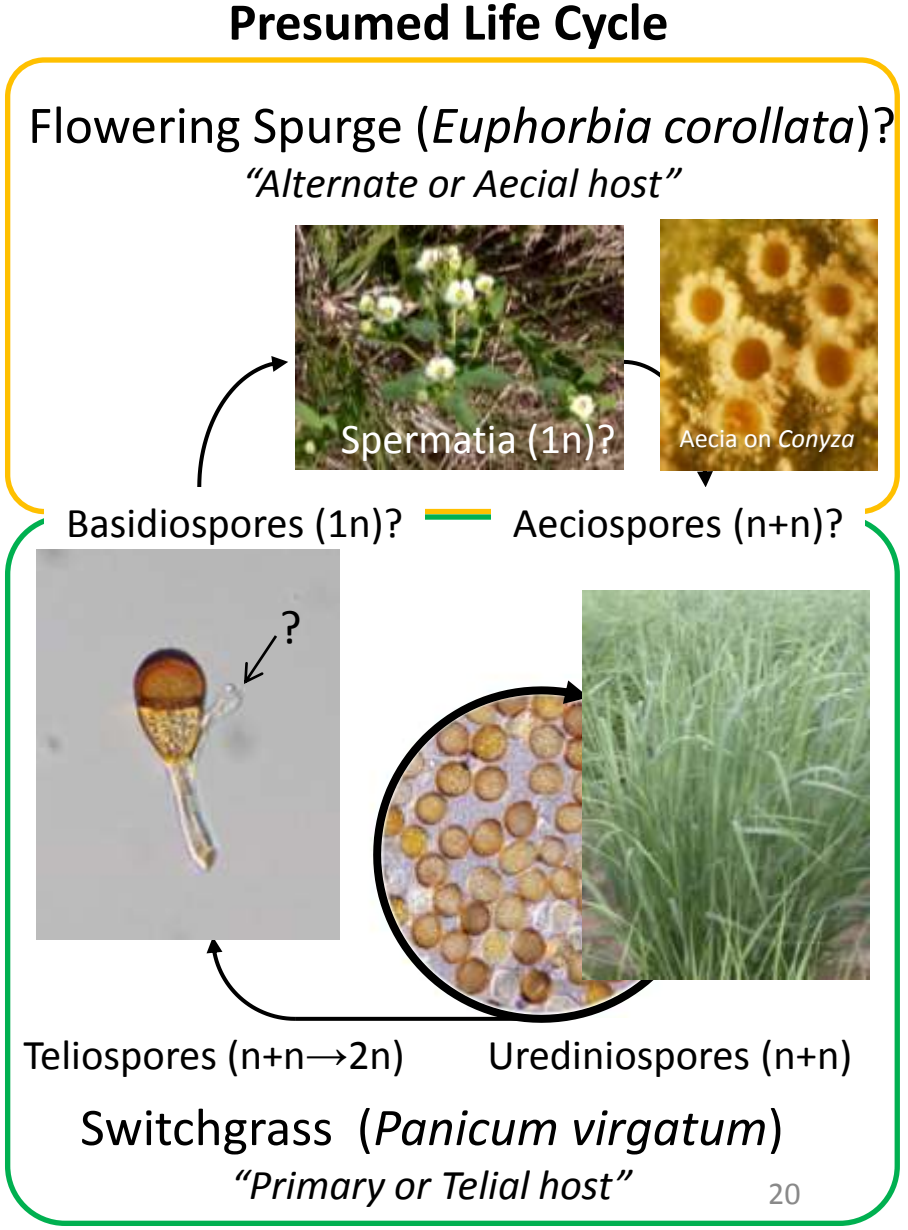
- All sequences (ITS-rDNA, TEF1α, βtub) from 5 single pustule isolates still of low quality, preventing contig assembly
- No improvement over direct amplification from mixed population of urediniospores

CONCLUSIONS

- Allelic variability within single pustule too high to permit direct amplification and sequencing of barcodes
- Barcodes will have to be subcloned

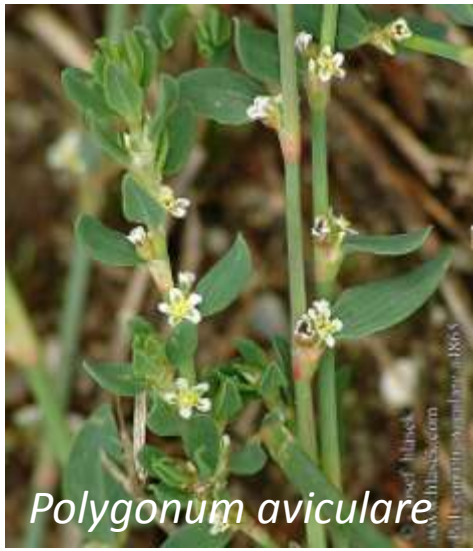
Efforts to identify alternate host

- Teliospore stratification to induce basidiospores
  - Float telia on water at 4°C for 2-4 weeks
  - Move to water agar at R.T.
  - Basidiospores in 2-4 days?
- Inoculated seedlings of:
  - *Euphorbia corollata*
  - *E. marginata*
  - *Solanum pseudocapsicum*
  - *Physalis alkekengi*
  - *Mirabilis jalapa*
- No aecia have formed
- DNA barcodes will be used to confirm identity if aecia develop



Efforts to identify alternate hosts: A clue?

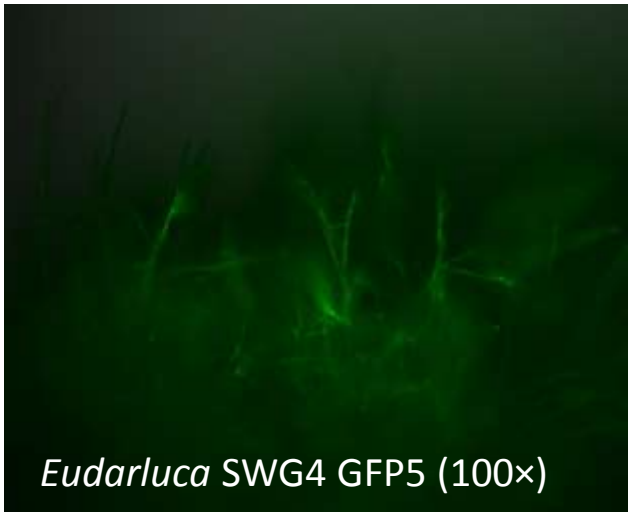
- Barcode sequences similar to *Uromyces polygoni-avicularis* and *U. inaequialtus* could imply alternate host of *P. emaculata* is a *Polygonum* sp. (smartweed) or a *Silene* sp. (catchfly), respectively
- Tranzschel's Law of Rust fungi
  - Related rust (*Uromyces* spp.) forms teliospores on aecial host of ancestor (*P. emaculata*), from which it evolved
- Will broaden survey to more potential alternate host families for aecia
  - 2011 drought not conducive for surveys


Polygonum aviculare


Silene

Possible "*Eudarluca*" mycoparasite of *Puccinia emaculata*

- Undescribed pycnidia-forming fungus 'infecting' telia of *P. emaculata*
- ≈ *Eudarluca* spp. (syn. *Sphaerellopsis* spp.), mycoparasites of rusts
- ITS-rDNA similar to uncultured endophytic and soilborne *Phoma* spp.
  - Not similar to *Eudarluca caricis* (mycoparasite of rust fungus, *Melampsora* spp.)
- Examining microscopic interaction with rust pustules
  - *Agrobacterium*-mediated transformation of isolate SWG-4 to express GFP or tdTomato fluorescent proteins



Eudarluca SWG4 GFP5 (100×)

Eudarluca SWG4 tdTom 1a (200×)

Gabriela Orquera collecting urediniospores

Thanks!

Any Questions?

"This work has been supported, in part, by the NSF EPSCoR award EPS 0814361."