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LBNL-57638

SOYBEAN RUST, A RISING STAR IN PHYTOPATHOLOGY

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Soybean Rust

Caused by two species of fungi:

Phakopsora pachyrhizi

aka “Old World” or “Asian” isolate

More aggressive pathogen.

Phakopsora meibomiae

aka “New World” or “American” isolate

Not as aggressive

Soybean rust hosts

LEGUMES (Papilionoideae)

Cultivated Crops:

Glycine max* (soybeans)

Phaseolus lunatus* (lima and butter beans)

***Phaseolus vulgaris* (green beans, kidney beans)**

Vigna unguiculata* (cowpeas)

***Cajanus cajan* (pigeon peas)**

Pachyrhizus erosus* (yam bean, jicama)

Ornamental plants:

Hyacinth bean, lupine, royal poinciana

Wild hosts:

Kudzu, sweet clover

Kudzu infected with soybean rust

Photos by Reid D. Frederick

Soybean Rust in the World

Phakopsora pachyrhizi

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Japan	**1904**	
Kenya	**1997/1998**	**Thought to be windborne from Asia**
Nigeria	**1997/1998**	
Rwanda	**1997/1998**	
Zimbabwe	**1997/1998**	
South Africa	**2001**	
Paraguay	**2001/2002**	**Thought to be windborne from Africa**
Brazil	**2002**	
Argentine	**2002**	
Bolivia	**2003**	
Colombia	**2004**	
USA	**Oct 2004**	**Hurricane Ivan**

Soybean Rust in the World

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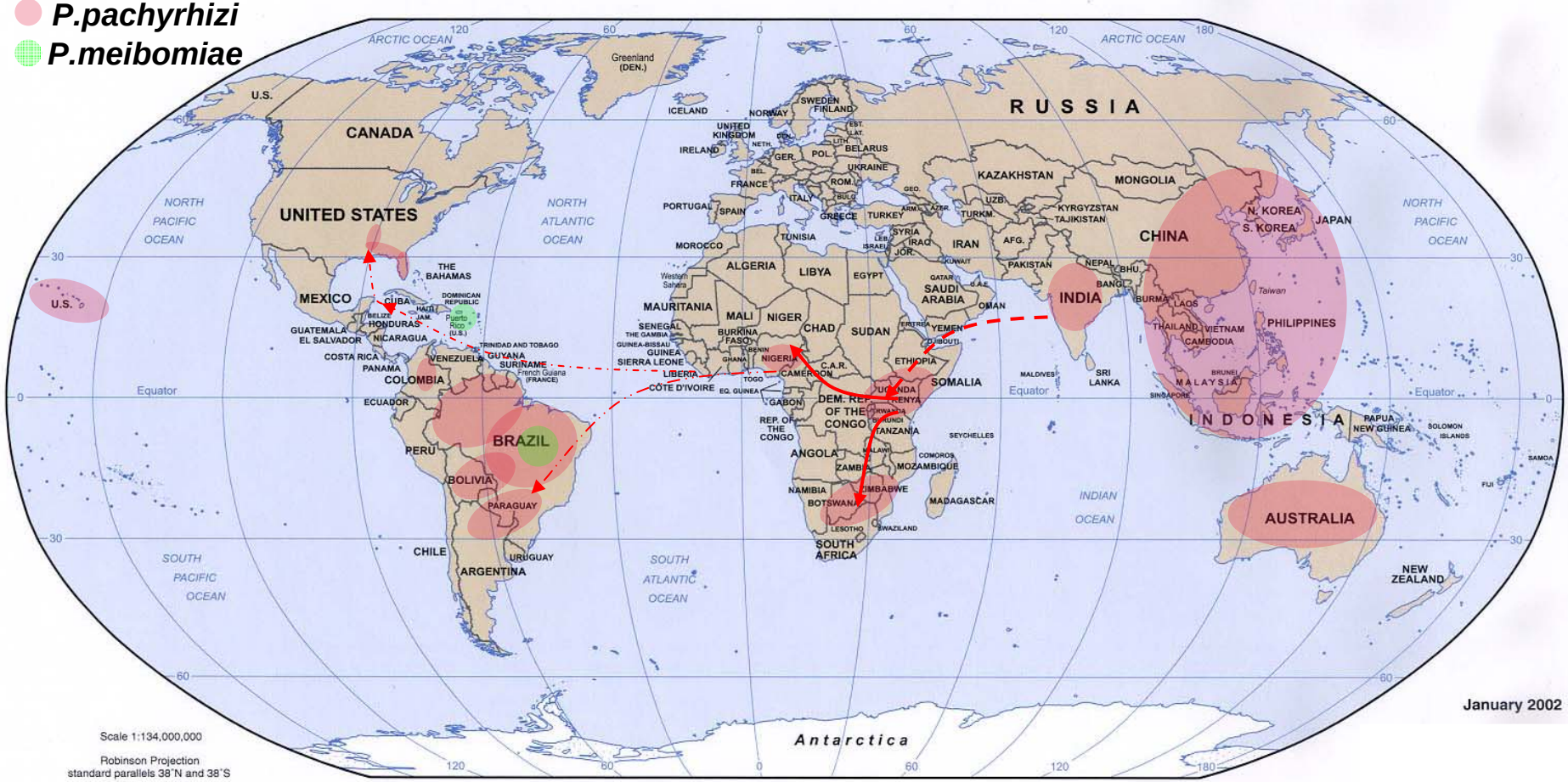
- *P.pachyrhizi*
- *P.meibomiae*



Soybean Rust Effects

Premature defoliation

Yield decrease characterized by:

- **Increase in number of unfilled pods/plant**
- **Decrease in number of normal pods/plant**
- **Decrease in number of seeds/plant**
- **Decrease in weight of seed/plant**
- **Decrease in 1000-seed weight**
- **Decrease in germinability of seed**

Soybean fields (Zimbabwee)

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Photos by Reid D. Frederick

Symptoms

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Symptoms

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Infected cotyledons




Infected stem





Infected pods


Photos by Christine Stone

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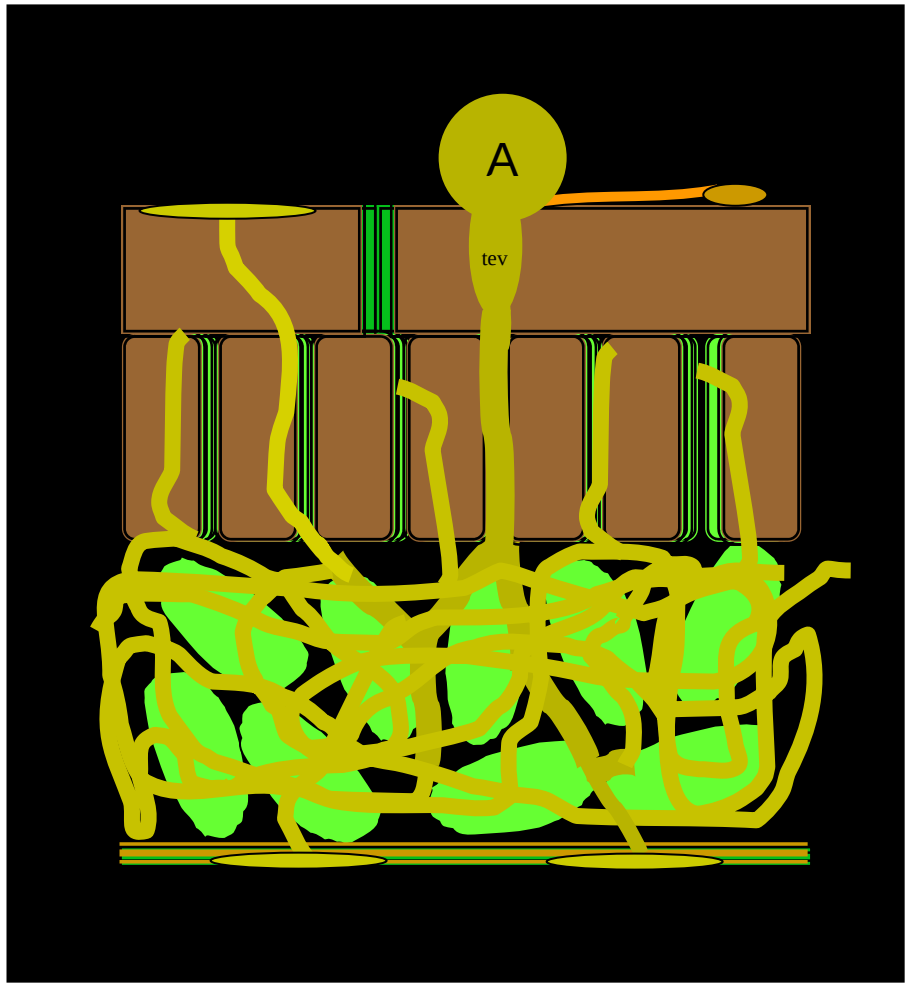
Interacción Susceptible

2 h	Appresoria begin developing
5 h	Appresoria expansion
7-12 h	Penetration through cuticle
12-16h	Increase in diameter
24 h	Primary hyphae emerging from tev
48 h	Intercellular hyphal growth (60µm from penetration site)
3-8 days	Intercellular hyphal growth (75-450 µm from penetration site)
9 days	Sporulation
14 days	Sporulation peak



(Based on Koch et al. 1983; Keogh et al. 1980)

Symptoms

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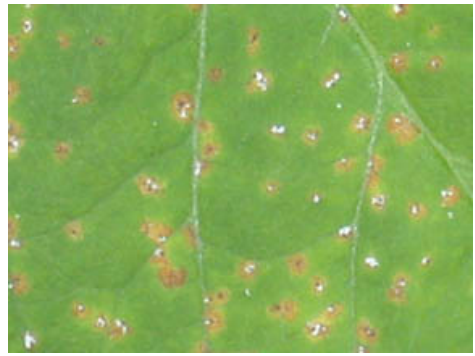
Infected leaves


9 dpi

12 dpi

15 dpi

18 dpi

Photos by Christine Stone

Genome Sequencing Project

Funded by

the U.S. Department of Agriculture/ Agricultural Research Service (USDA/ARS)

the U.S. Department of Energy/ Joint Genome Institute (DOE-JGI)

Genome Project Strategy

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Random shotgun libraries:
3kb insert size in vector pUC18,
Mid-size insert 8-10kb in vector p21
36-40kb insert size in pCC1FOS (Fosmids)
cDNA libraries from different stages of *P. pachyrhizi*

Sequencers:
ABI3730
MegaBACE 4000

Informatics:
Reads processing by Phred
Reads assembly by Phrap
Verification
Genome annotation

Sequencing at JGI

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	Library (Insert size)	Bases sequenced
P. pachyrhizi	3 Kb	146.60 Mb
	8 Kb	264.28 Mb
	40 Kb	5.75 Mb
Total		416.63 MB
P. Meibomiae	3 Kb	125.20 Mb
	8 Kb	5.97 Mb
Total		131.17 MB

DOE-JGI Data by 5.31.05

G+C content *in P. pachyrhizi* and *P. meibomiae*

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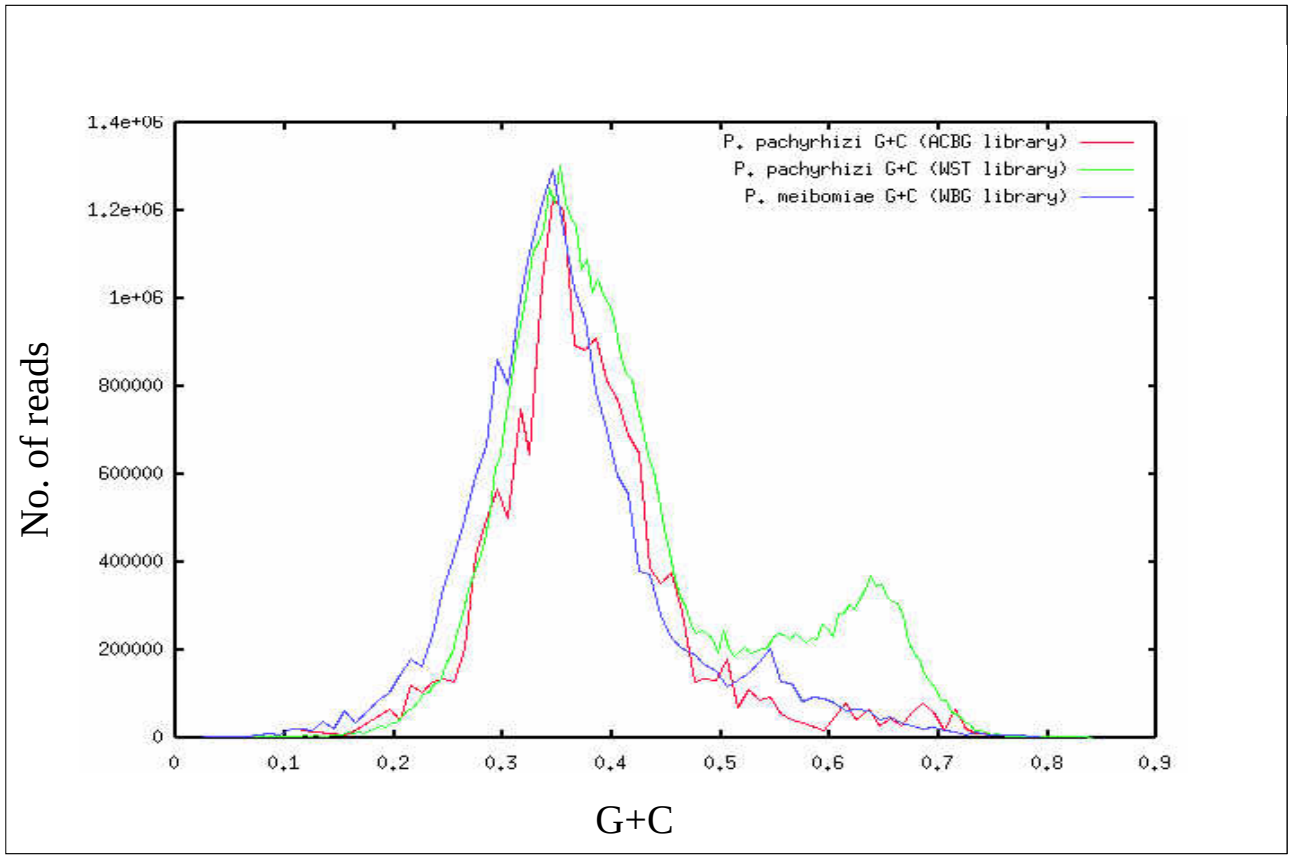

Phakopsora pachyrhizi and Phakopsora meibomiae G + C content estimation

The mean G+C content *in P. pachyrhizi* and *P. meibomiae* is 34-35%, estimated with the "G+C content program" (Chapman) on sequences from three different genomic libraries.

Several independent methods were used to estimate the genome size. Although there were considerable uncertainties associated with most of the methods, they consistently yielded a genome size above 500 MB.

Estimation Method	Genome Size
cDNA Coverage	720 Mb
All-Pairs Read Alignment	500-800 Mb
Gene Density	300-700 Mb
Shotgun Fosmid Coverage	600-950 Mb

Fosmid sequencing

Random fosmids

Finishing at Stanford :

Finished	87 (approx. 3.48 Mb x 8)
Incomplete	28 (approx. 1.12 Mb x 8)

Selected fosmids

Lawrence Livermore National Laboratory (LLNL):

Probes designed for 120 “genes”

Selected	50
To go	70
Sequenced	15
Finished	0

Probes designed based on ESTs selected by high similarity to “interesting” genes from other fungi and unknown genes highly expressed in germinating spores from *P. pachyrhizi.*

cDNA libraries

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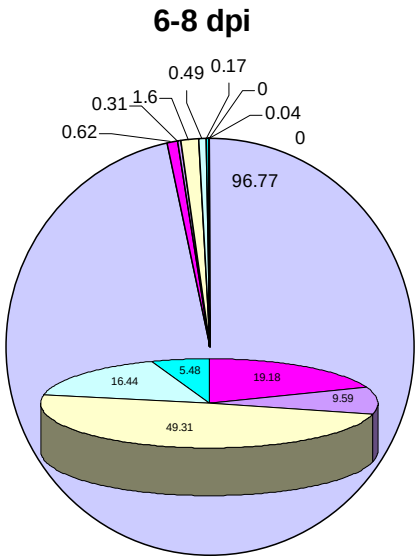
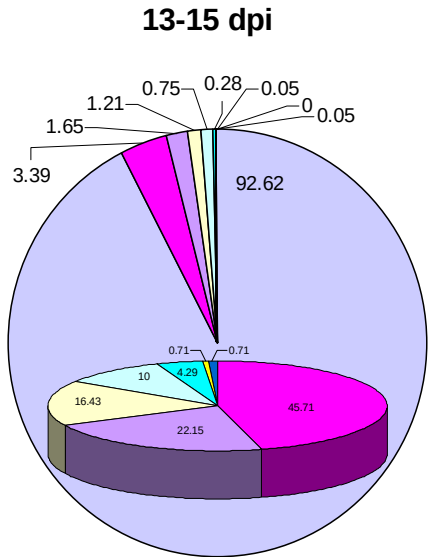
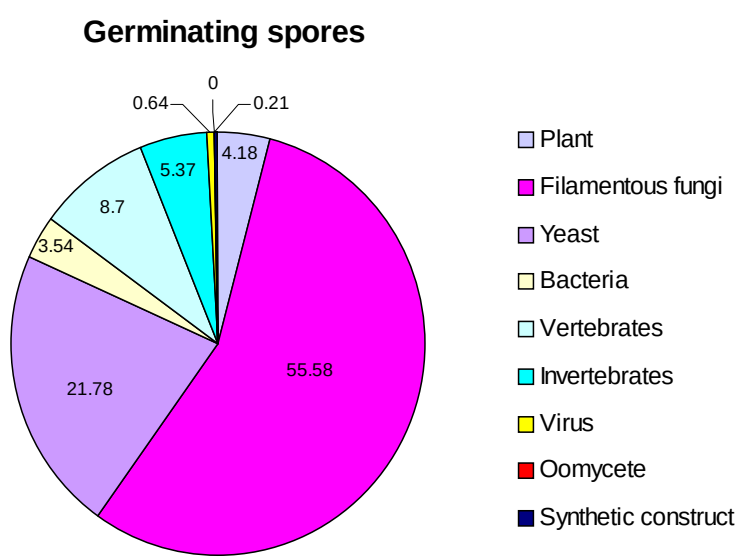
Germinating Spores	Resting spores	Hyphal growth*	High sporulation*
16 Hours on water surface	Kept at – 80°C	6 7 8 Days after inoculation	13 14 15 Days after inoculation

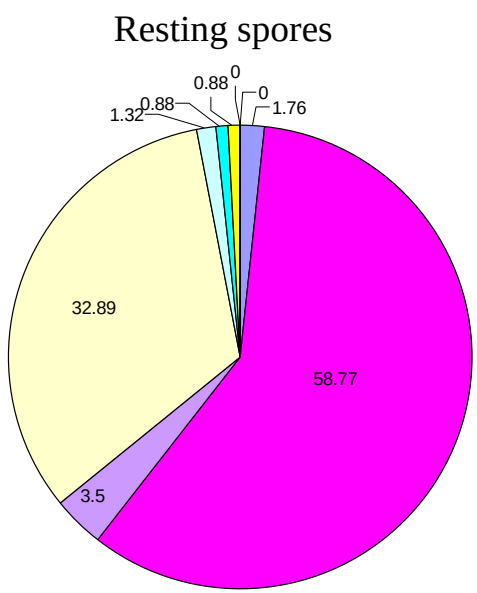
* :mRNA was extracted from infected leaf at each time point and pooled together for the construction of the cDNA libraries. Unidirectional cDNA libraries constructed in plasmid pSPORT1 (Invitrogen).

Description	ESTs	cDNAs	Libraries	Clusters	Consensus	Singlets
6-8 dpi	**6100**	**5374**	**1**	**1154**	**1278**	**1827**
13-15 dpi	**6023**	**4610**	**1**	**1291**	**1387**	**1356**
Resting urediniospores	**2295**	**1762**	**1**	**393**	**455**	**335**
Germinating urediniospores	**29601**	**18638**	**1**	**2686**	**3394**	**2142**
***Phakopsora pachyrhizi* v2.1**	**44019**	**30244**	**4**	**5105**	**6165**	**4961**









Percentage of similarity of cDNA clusters from the *Phakopsora pachyrhizi* germinating and resting spores libraries and the infected soybean leaf libraries (6-8 dpi and 13-15 dpi) to proteins from other organisms. Inner pies show the percentage of similarity of cDNA clusters to proteins from other organisms, excluding plant homologs.

cDNA functional categories

The cDNA clusters were classified into functional categories based on the BlastX hits and the Pfam hits, according to the Expressed Gene Anatomy database (EGAD, TIGR, Rockville, MD).

Approximately 23 % of the cDNA clusters from the 6-8 dpi and 13-15 dpi libraries and 40% from the germinating and resting spores libraries show similarity to hypothetical proteins or proteins of unknown function.

Several homologs to pathogenesis related proteins (PR proteins) and defense proteins were identified in the infected leaf tissue libraries (Apidaecin, Beta defensin, Thaumatin, etc). In the GS library several homologs to pathogenicity proteins were identified. All the libraries show a high percentage of metabolism related proteins.

MITOCHONDRIAL GENOMES

Known mitochondrial genome sequences were blasted against the entire set of reads from the genome project. Potential mitochondrial sequences were assembled with the Phred Phrap Package. This resulted in single contig assemblies for both fungal mitochondrial genomes, *P. pachyrhizi* and *P. meibomiae*.

Genome analysis and annotation:

DOGMA Dual Organellar GenoMe Annotator (http:// bugmaster.jgi-psf.org/dogma).

tRNAscan-SE 1.21 (http:// www.genetics.wustl.edu/eddy/tRNAscan-SE/)

MacVector 7.1 (Accelrys)

Blast algorithm

Mitochondrial Genomes

	P. Pachyrhizi	*P. meibomiae*
Size	**31.82 Kb**	**32.52 Kb**
G+C	**34.6 %**	**34.9 %**

These genomes contain:

- ATP synthase subunits 6, 8, and 9 (**atp6, atp8, and atp9**)

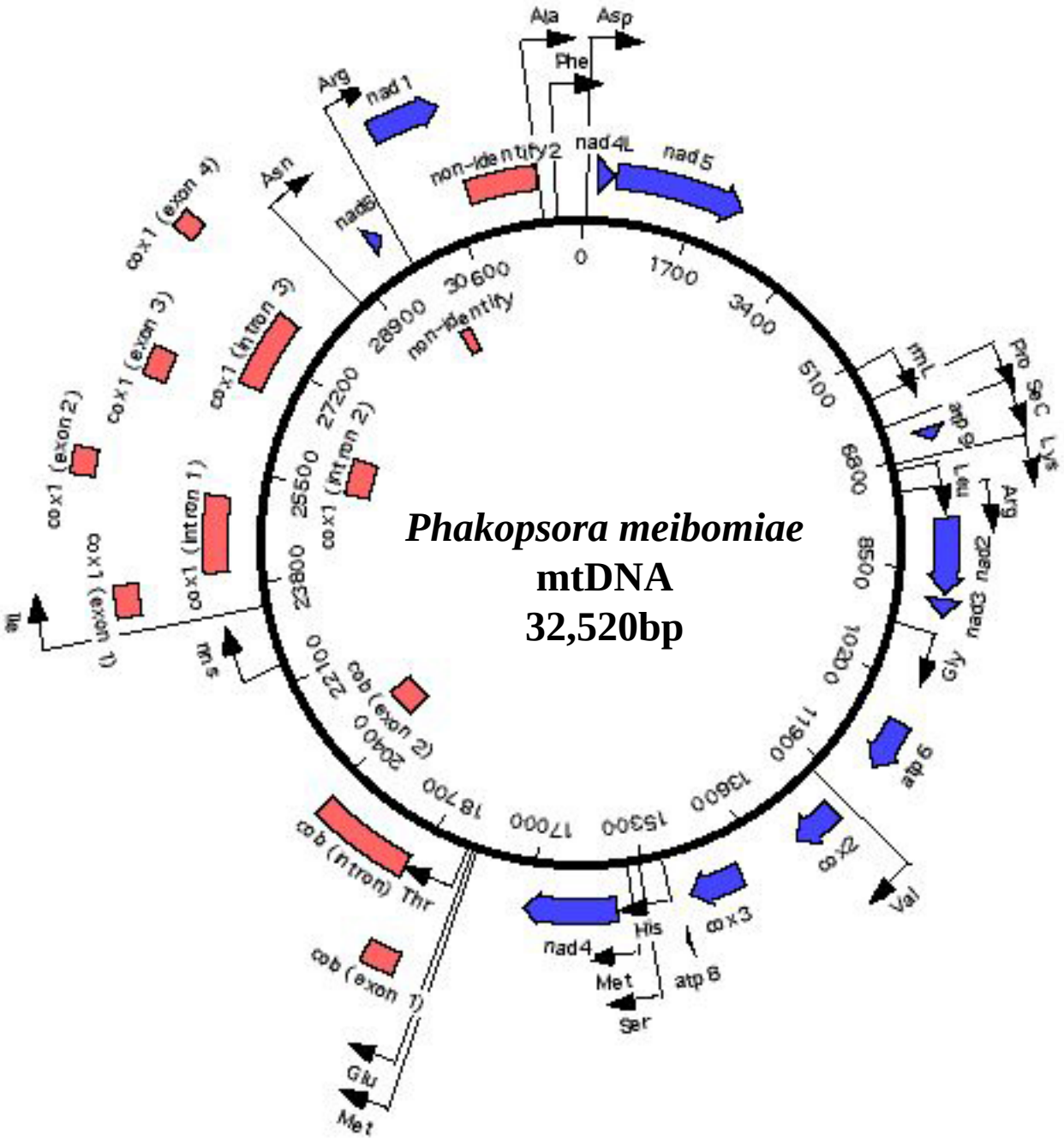
- cytochrome oxidase subunits I, II, and III (**cox1, cox2, and cox3**)

- apocytochrome b (**cob**)

- reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunits (**nad1, nad2, nad3, nad4, nad4L, nad5, and nad6**)

- the large and small mitochondrial ribosomal RNAs (**rnl** and **rns**).

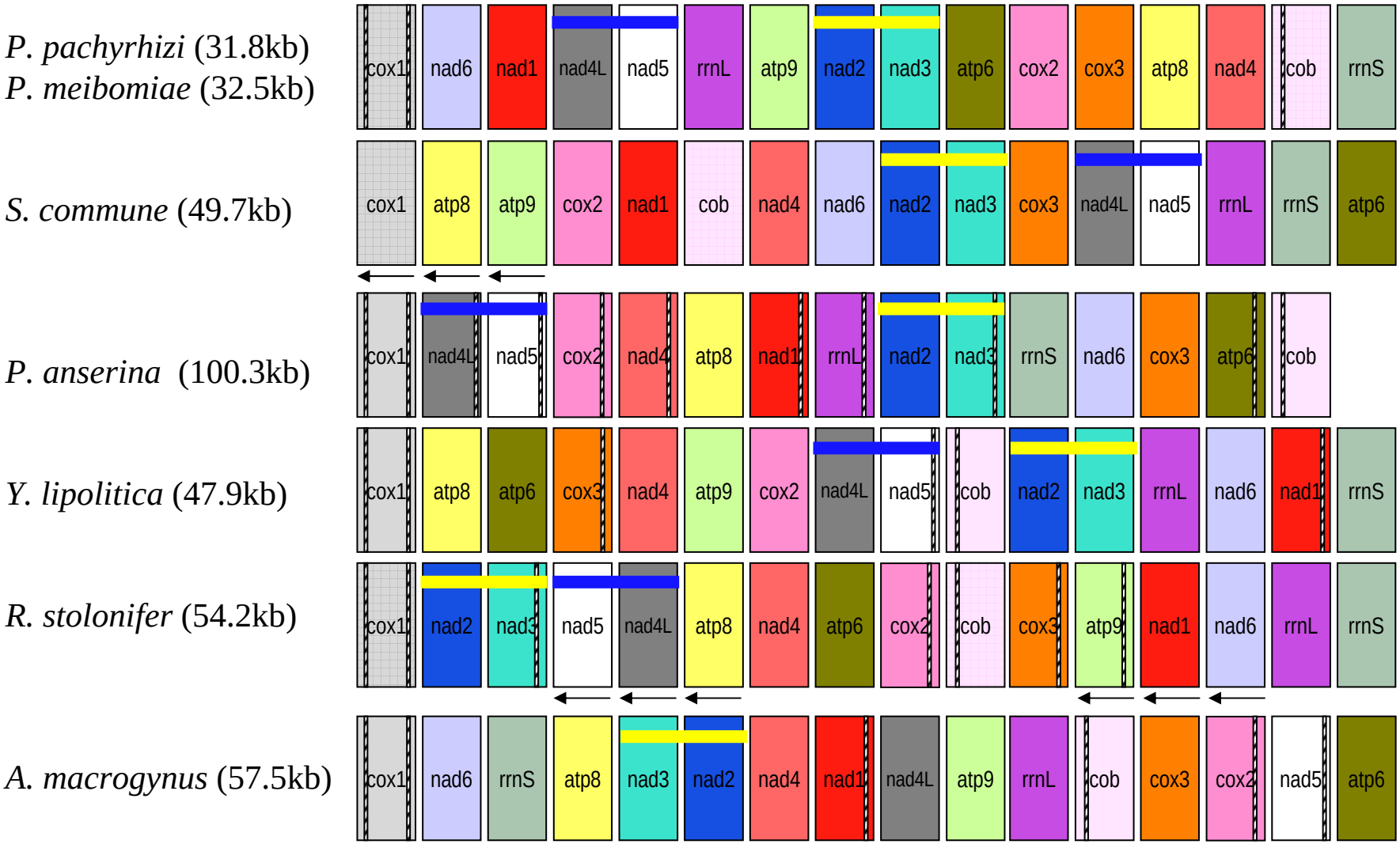
- **tRNAs** for all amino acids.



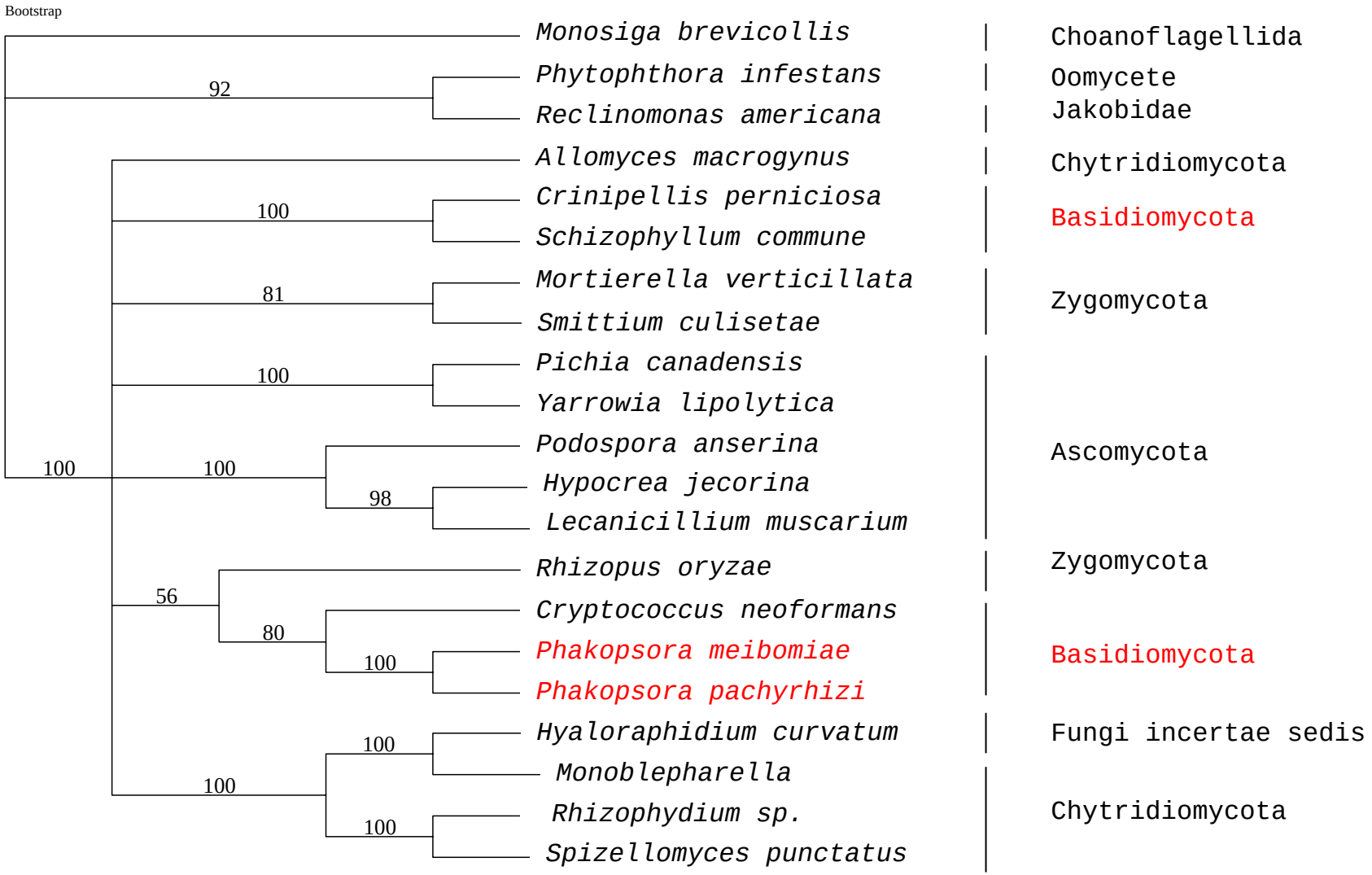
Phakopsora meibomiae

mtDNA

32,520bp



Comparison of mitochondrial genomes from the four phyla of fungi. Protein-coding and rRNA genes are represented by boxes; arrows indicate the direction of transcription. Lines within genes represent presence of intron(s).



Phylogenetic tree of 1582 amino acid position from seven mitochondrial-encoded proteins from 21 taxa, including 18 species from all fungal phyla and *Monosiga brevicollis*, *Phytophthora infestans* and *Reclinomonas americana* as outgroups. The genes encoding cob, cox1, cox2, cox3, nad1, nad4 and nad5 are present in all organisms compared. Parsimony-bootstrap support was calculated from 100 replicates using Paup 4.0b10.

Acknowledgements

USDA/ARS/FDWSRU

Reid D. Frederick
Jane J. Choi
Christine L. Stone
Craig Austin
Laura Ewing

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Jeffrey L. Boore
Peter Brokstein
Nick Putman
Harris Shapiro
Jarrod Chapman

Lawrence Livermore National Laboratory

Laurie Gordon

- This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48, Lawrence Berkeley National Laboratory under Contract No. DE-AC02-05CH11231 and Los Alamos National Laboratory under Contract No. W-7405-ENG-36.