Persistent viruses in plants and fungi: Molecular fossils?

Tracy Feldman
Prasenjit Saha
Guoan Shen
The Samuel Roberts Noble Foundation

Luis Márquez
Xiaodong Bao
Tony Stobbe
Penn State University

Felipe Chavarría
Area Conservación Guanacaste

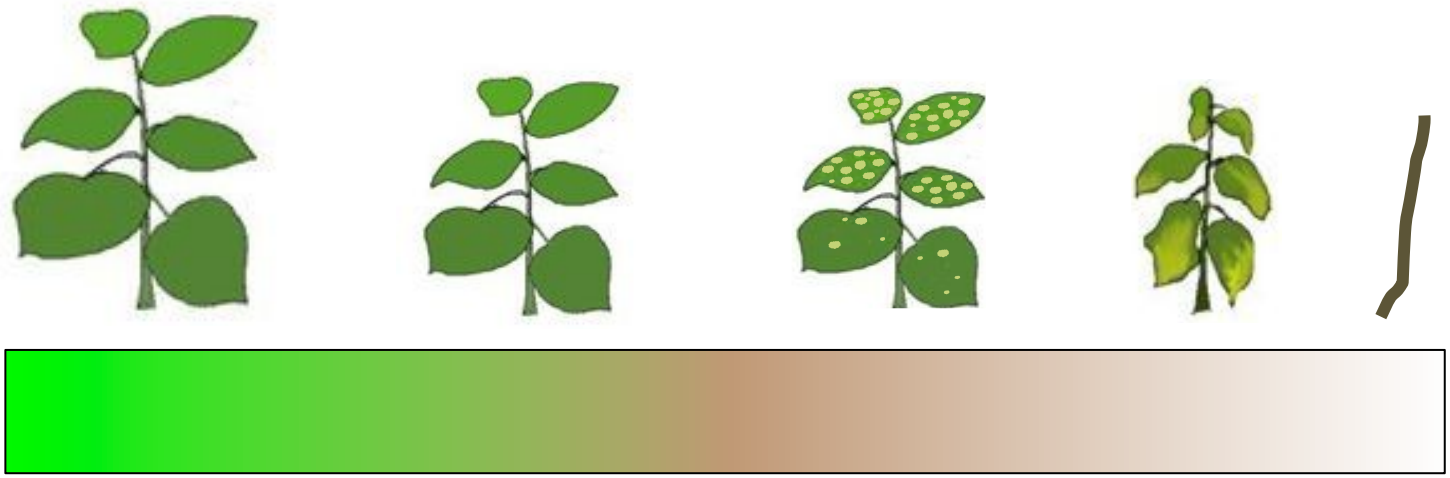
Viruses as Symbionts

- **Classic definition of symbiosis**
  - **From Frank and deBary in the 19th C.**
  - **Two or more dissimilar entities living in or on one another in an intimate relationship**

- **Lifestyle choices**
  - **Mutualistic**
  - **Commensal**
  - **Antagonistic**

- **Symbiosis occurs between a virus and its host, and among viruses in mixed infections.**

Lifestyle Choice Continuum



Mutualist

Antagonist

Persistent *versus* Acute Plant Viruses

- **Acute viruses initiate an infection, rapidly replicate and reach a high titer, and often cause disease or death.**
  - **Acute virus infections are resolved by recovery, death, or conversion to chronic infections**

- **Persistent viruses infect the host for long periods, probably many generations.**

Persistent Viruses in Plants

- Several families of viruses are known that are persistent, or cryptic.
- They have not been studied much.
- In general they are asymptomatic
  - not always possible to tell
- Most are double-stranded RNA viruses.
  - Endornaviruses are ss viruses but are only found as dsRNAs
- They are not thought to be transmitted horizontally.

More on persistent viruses…

- **In plants they do not move from cell-to-cell**
- **They may not be subjected to RNA silencing**
  - **They are found in meristems**
- **They are in the families *Partitiviridae*, *Endornaviridae* (*Chrysoviridae*, and *Totiviridae*)**
- **Members of these families are also found in fungi, including endophytic fungi and have similar lifestyles**

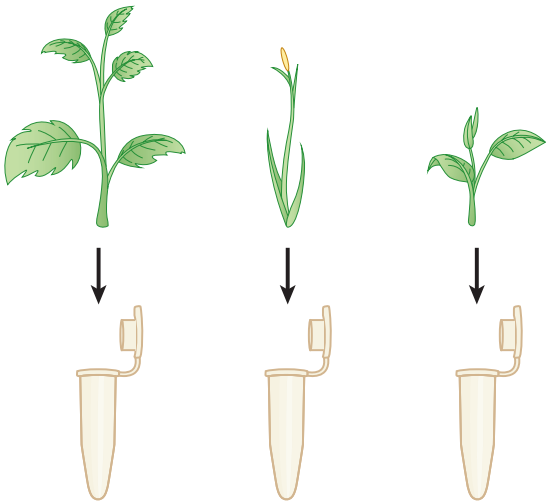
Exploring The Big Unknown: Biodiversity surveys of plant and fungal viruses

- Two major surveys have catalogued plant viruses from individual plants.
- Limited surveys have been done for fungal viruses.

a

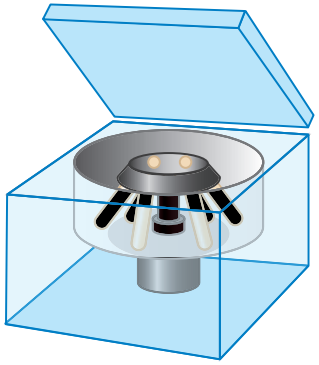
Metagenomics

Ecogenomics

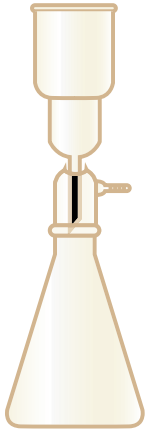


b

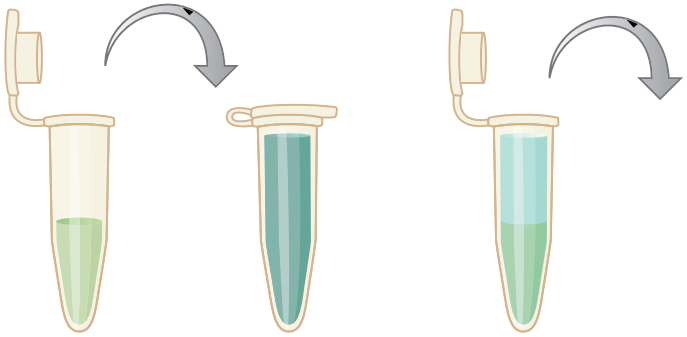
VLPs by centrifugation

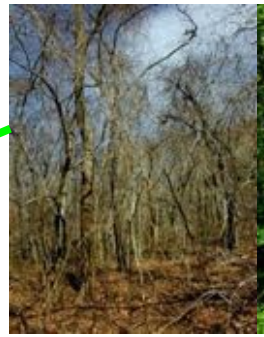


VLPs by filtration



Direct extraction of nucleic acids

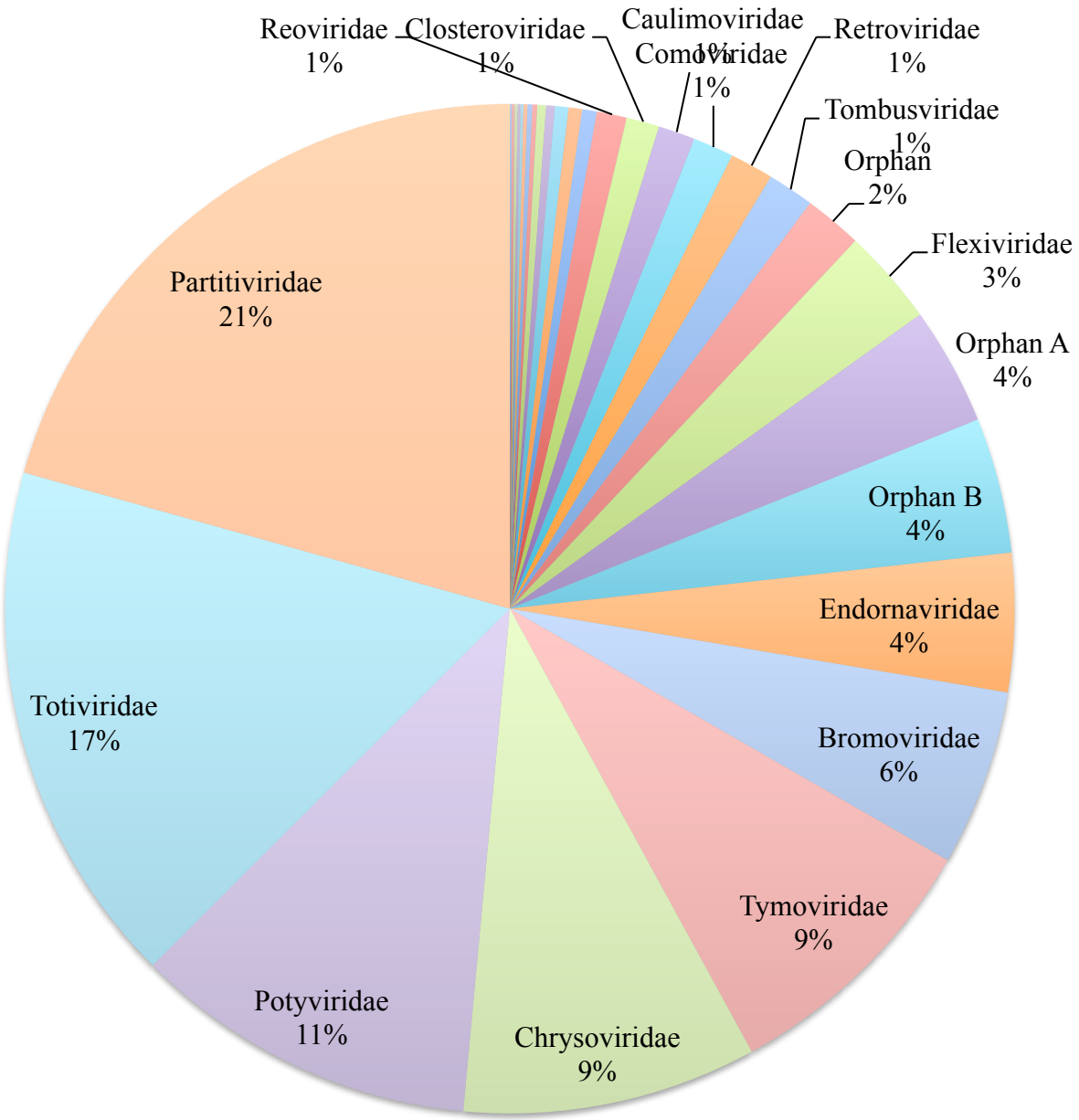




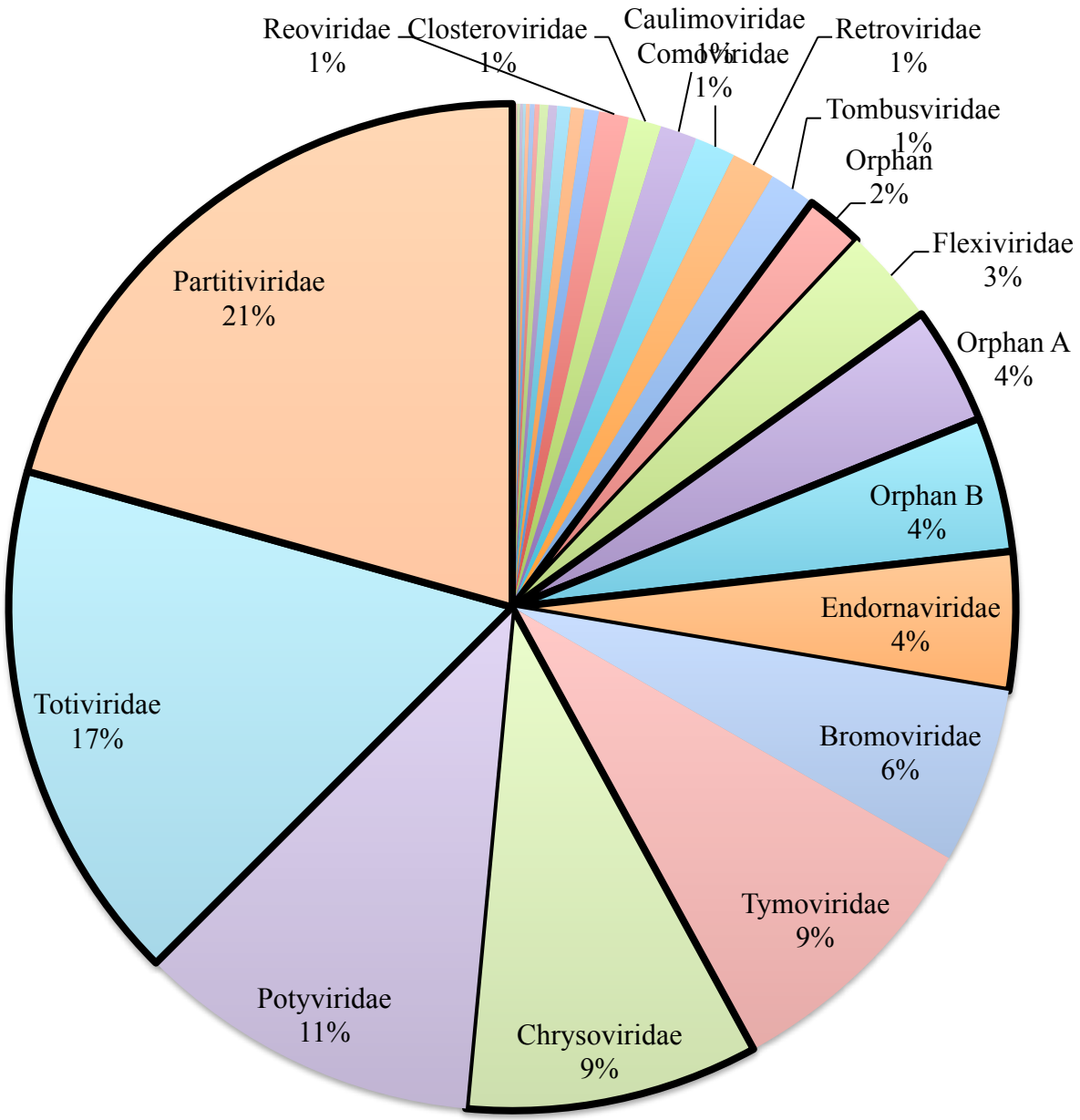
Plant Virus Biodiversity Inventories

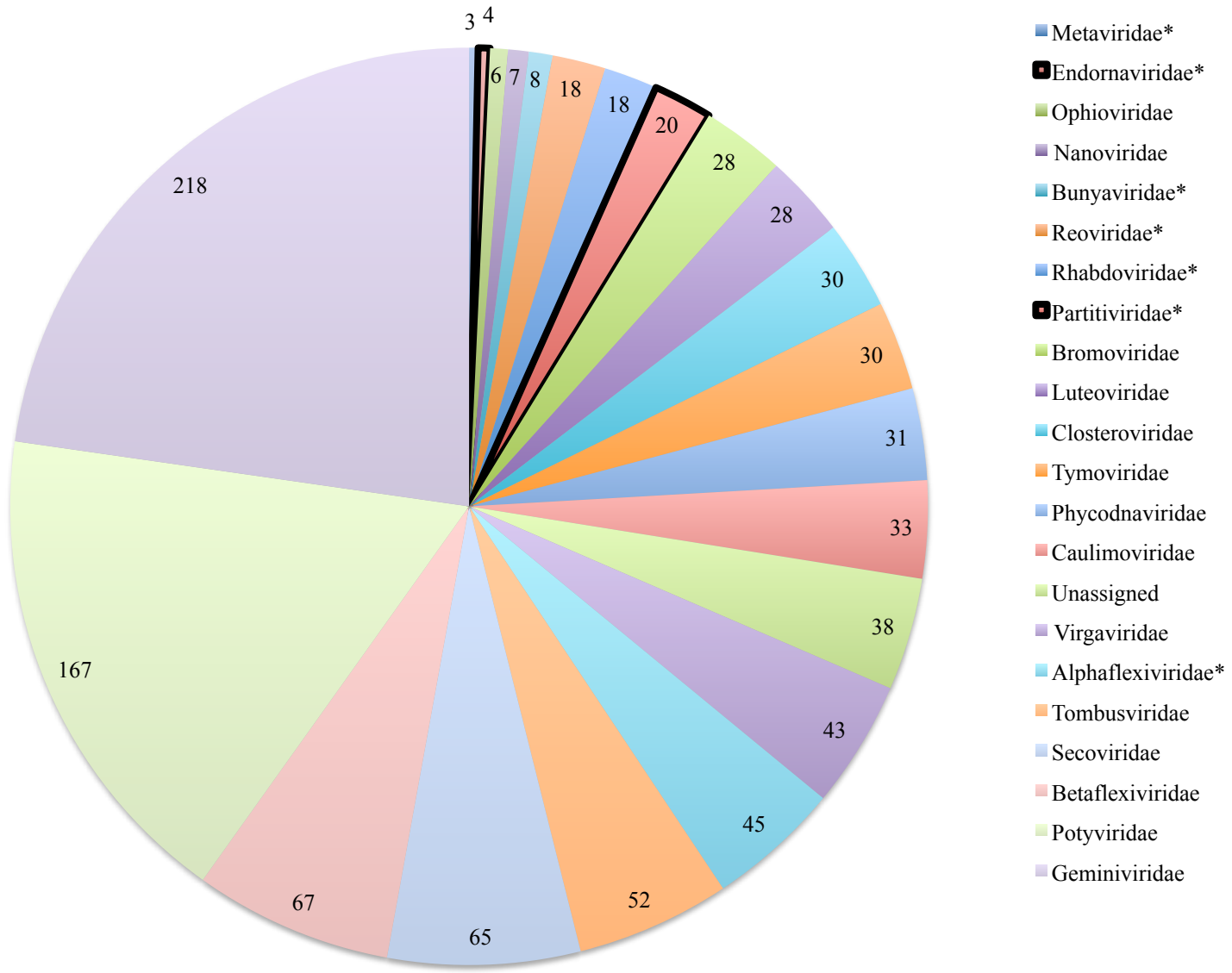
- Plants are collected and ID'd in the field and located by GPS
- Total nucleic acids and dsRNAs are isolated in the lab
- dsRNAs are converted to cDNA and sequenced by a multiplexing method on 454 (now switching to Illumina)
- Bioinformatics identifies the nearest relative (very few known viruses are found, but most can be identified to family)

Summary of virus families



Summary of virus families




3
4
6
7
8
18
18
20
28
28
30
30
31
33
38
43
45
52
65
67
167
218
Metaviridae*
Endornaviridae*
Ophioviridae
Nanoviridae
Bunyaviridae*
Reoviridae*
Rhabdoviridae*
Partitiviridae*
Bromoviridae
Luteoviridae
Closteroviridae
Tymoviridae
Phycodnaviridae
Caulimoviridae
Unassigned
Virgaviridae
Alphaflexiviridae*
Tombusviridae
Secoviridae
Betaflexiviridae
Potyviridae
Geminiviridae

Observations

- Unknowns ("Viral dark matter") makes up about 60% of contigs.
- Persistent viruses account for more than 60% of the viruses in wild plants, as opposed to the ICTV view of plant viruses where persistent viruses are rare.
- Totiviruses and Chrysoviruses, usually considered fungal viruses, are common.

Fungal Community:

1. How diverse is the community of fungal associates?
2. Do *C. cuspidata* and *A. psilostachya* share fungal species?

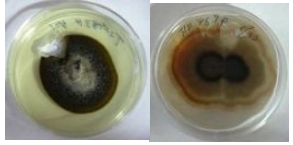
Prairie dodder:
Cuscuta cuspidata

Fungi??

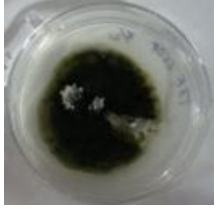
Western Ragweed:
Ambrosia psilostachya

photo by Guoan Shen

Fungal Diversity



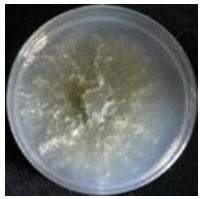
Cercospora *



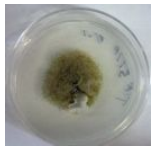
Septoria *

unknowns

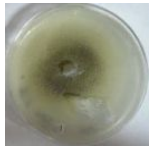
Cladosporium *



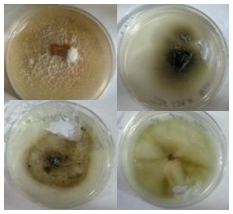
Alternaria alternata *



Pleosporales B *

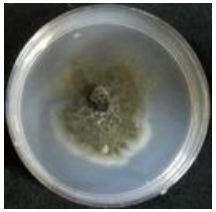


Pleosporales A *

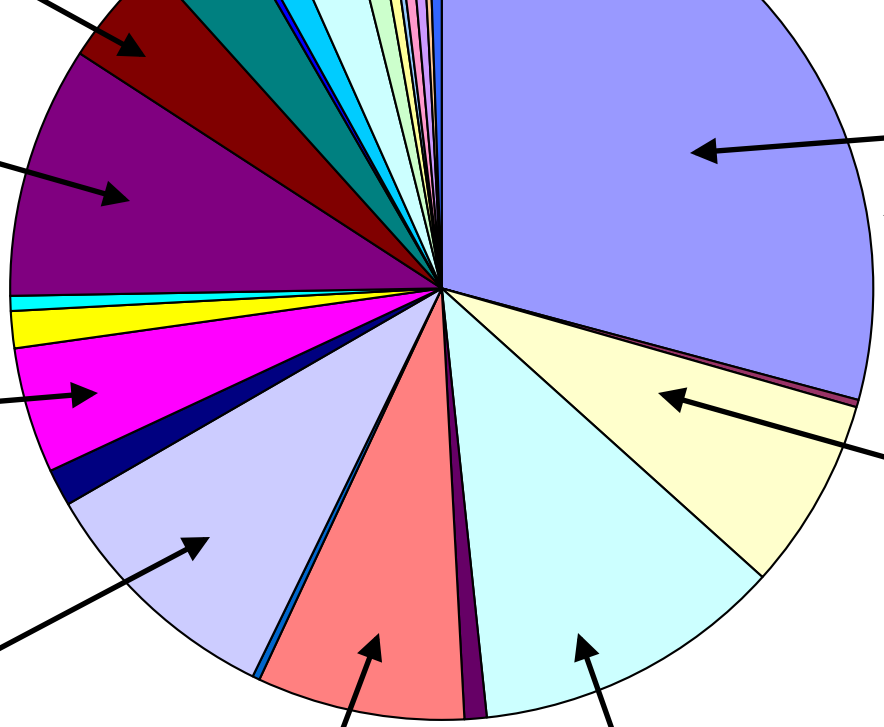


Phoma *

Curvularia *

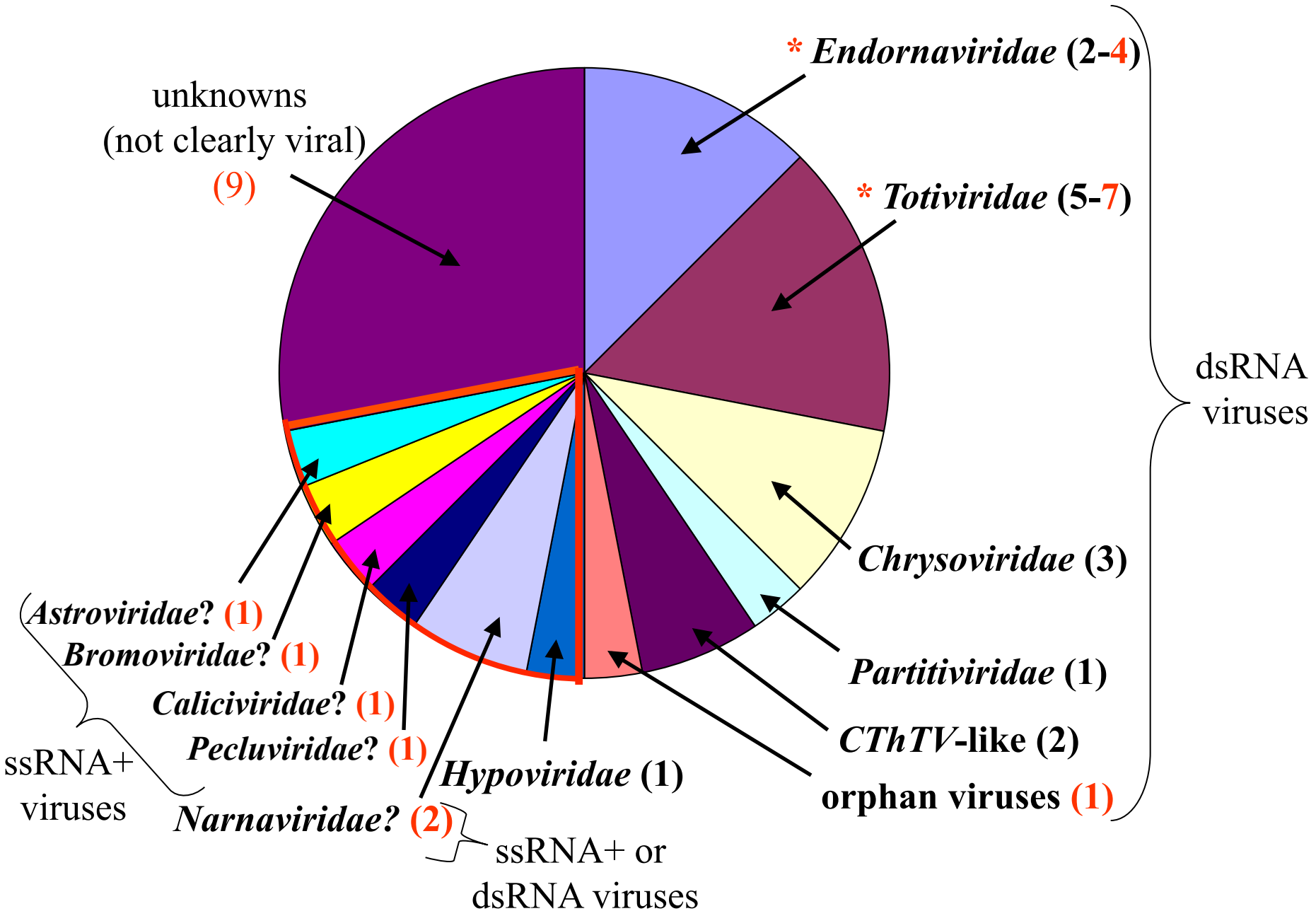


Stemphylium solani *

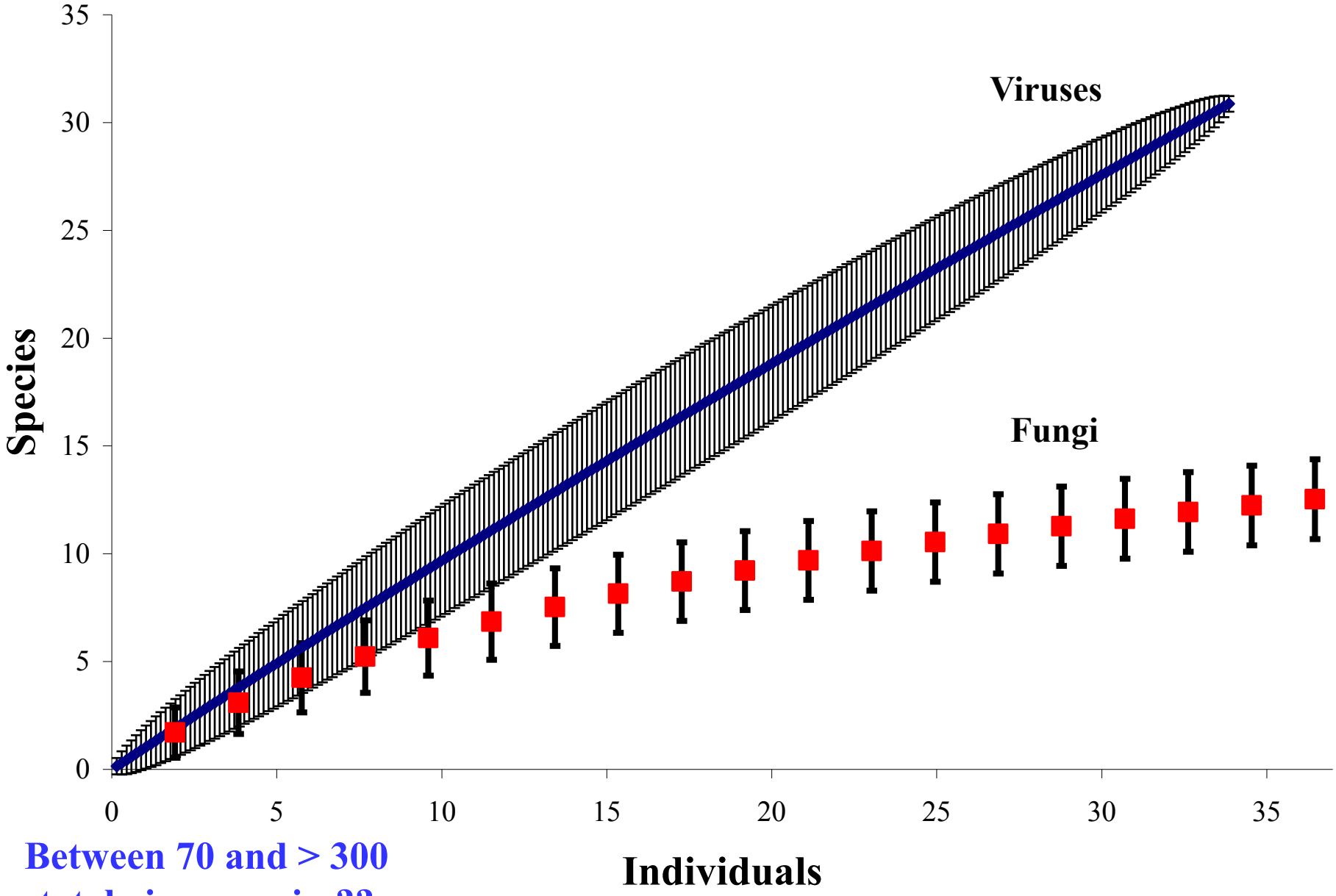


* In both *C. cuspidata* and *A. psilostachya*

Virus Diversity



Rescaled Fungal and Viral Rarefaction Curves



Between 70 and > 300 total virus species??

Diversity of Microbes

- **The smaller you go the greater the diversity?**
- **2 spp. of plants; 25 spp. of fungi; at LEAST 70 spp. of viruses**

Persistent Viruses

- Where do they come from?
- What do they do?
- Are they important for host biology?

Plant Adaptation to Extreme Environments

Photos courtesy of Joan Henson

Plant Adaptation to Extreme Environments

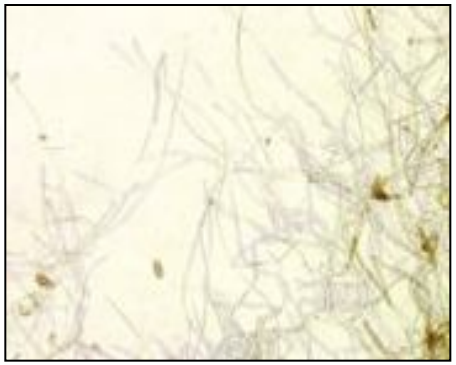
Group	Upper Temp Limits (°C)
Animals	**38-50**
Vascular Plants	**45**
Mosses	**50**
Algae	**55-60**
Fungi	**60-62**
Cyanobacteria	**70-73**
Hetero. Bacteria	**90**
Archaebacteria	**110-115**

From TD Brock Life at High Temperatures, 1994

Dichanthelium lanuginosum
Hot springs panic grass
Thermal western witchgrass

Plant/Fungal Symbiosis

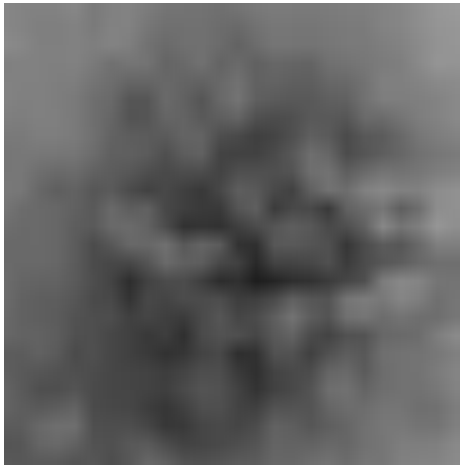
Curvularia protuberata



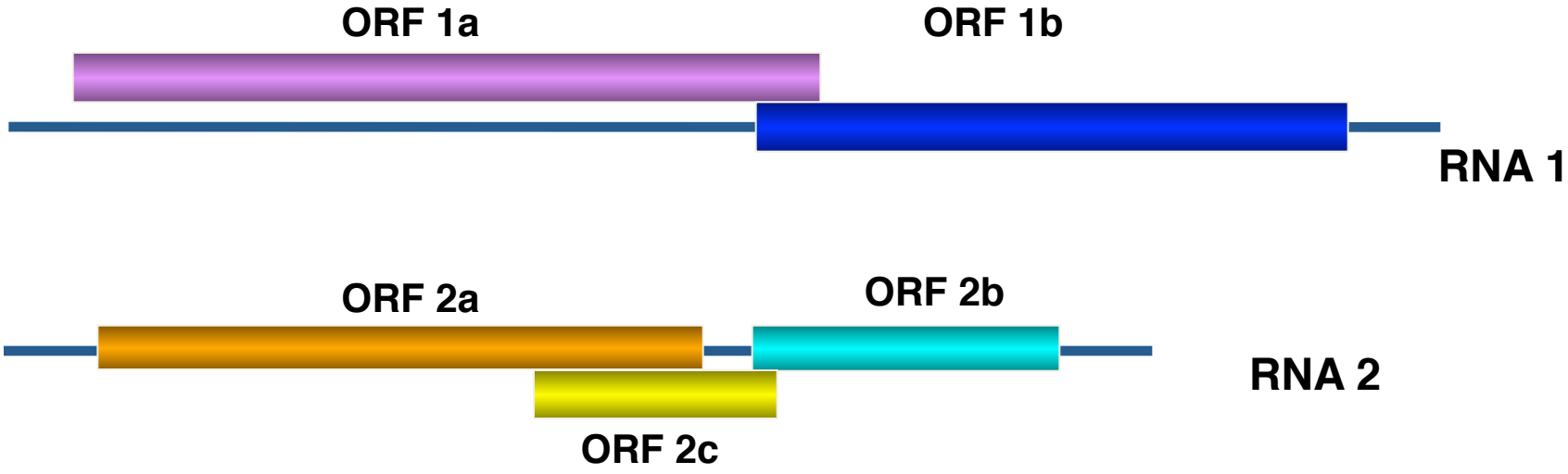
S = Symbiotic
NS = Non symbiotic

Redman et al. Science 2002

Characterization of the virus

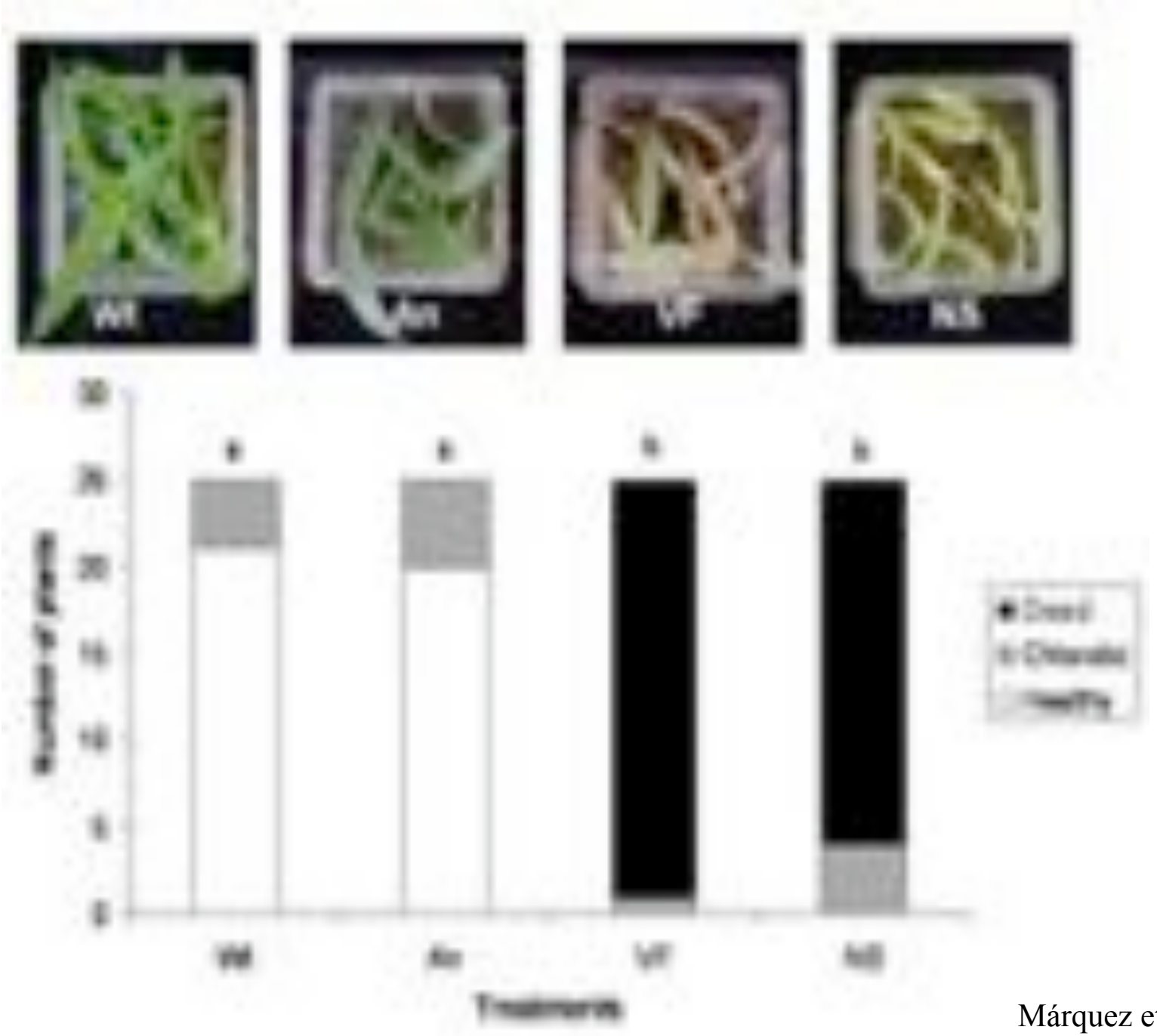


- Particles contain the 2 dsRNAs of 2.2 and 1.9 kb
- Encode 5 ORFs



Three strains of the fungus

- **WT is the original fungus from YNP**
- **VF is the original fungus cured of the virus**
- **An is the VF fungus where the virus was re-introduced**



Márquez et al
2007 Science

Why a virus?

- **Viruses may be the source of new genetic information.**
  - **Extreme diversity (mostly unknown).**
  - **Have clearly been the vehicles for genetic exchange in the past.**

- **There is little difference in terms of homologs among plant families.**
  - **Estimate less than 5% of genes are unique to a family**
  - **Similar for mammals and others?**

Endogenous RNA virus

- **Persistent lifestyle**
- **No evidence of encapsidation**
- **The RNA dependent RNA polymerase appears to be homologous, and shares closest similarity to closteroviruses.**
- **The remaining domains are variable by presence/absence, and by origin**

MeTr, methyltransferase
Hel, helicase
GT, glycosyltransferase
RdRp, RNA dependent RNA polymerase

Viruses used for this study

Virus	Host	Host type	Sigla
Tuber aestivum endornavirus	Truffle	Ascomycete	TaEV
Gremmeniella abietina type B RNA virus XL1	Pine pathogen	Ascomycete	GaBRV-XL1
Chalara elegans endornavirus 1	Root rot	Ascomycete	CeEV-1
Phytophthora endornavirus 1	Potato pathogen	Oomycete	PEV1
Oryza rufipogon endornavirus	Rice	Plant	ORV
Oryza sativa endornavirus	Rice	Plant	OSV
Bell pepper endornavirus-YW	Pepper	Plant	BPEV-YW
Helicobasidium mompa endornavirus 1-670	Root rot	Basidiomycete	HmEV1
Vicia faba endornavirus	Bean	Plant	VFV

Virus	MeTr*	Hel	Hel 2	GT	GT 2	RdRp	Total Length°
TaEV	none	cl12029		none		cl030409	9,760
GaBRVXL-1	cl03298	cl12029	cl14126	none		cl030409	10,374
CeEV-1	none	cl14126		cl10013		cl030409	11,602
PEV-1	none	cl14126		cl10013		cl030409	13,883
ORV	none	none		cl12292	cl10013	cl030409	13,936
OSV	none	none		cl12292	cl10013	cl030409	13,952
BPEV-KS	cl03298	cl14126		cl10013		cl030409	14,727
HmEV-1	none	none		cl07328		cl030409	16,614
VFV	none	cl14126		none		cl030409	17,635

* MeTr, methyl transferase; Hel, helicase; GT, Glycosyltransferase; RdRp, RNA dependent RNA polymerase; domains ar in the same order as found in the ORF.
° length in bp of the viral genomic RNA

Color coding: plant host | oomycete host | fungal host

Helicase domains

- cl 12029
  - DEAD/DEXD type of helicase
- cl 14126
  - UVRd repair
  - Related to RNA alphavirus helicase

Gylcosyltransferases

- cl10013
  - GTB topology
  - Most similar to GTs for antibiotic maturation
  - (also found in some hypoviruses)
- cl12292
  - DXD motif, use nt-sugars as donors and require divalent cation
- cl07328
  - 28-N family, membrane associated

Plants reported with *Endornavirus*

bell pepper	***Capsicum annuum***
melon	***Cucumis melo***
barley	***Hordeum vulgare***
mulberry	***Morus spp.***
wild rice	***Oryza rufipogens***
rice	***Oryza sativa***
avocado	***Persea americana***
green bean	***Phaseolus vulgaris***
turtle bean	***Phaseolus vulgaris***
broad bean	***Vicia faba***

Plants reported with *Partitivirus*

fig	***Ficus carica***
beet	***Beta vulgaris***
green algae	***Bryopsis cinicola***
hemp	***Cannabis sativa***
jalapeño pepper	***Capsicum annuum***
carrot	***Daucus carota***
Scot pine	***Pinus sylvestris***
Japanese mock orange	***Pittosporum tobira***
primrose	***Primula malacoides***
Chinese pear	***Pyrus pyrifolia***

Plants reported with Orphan persistent viruses

strawberry	***Fragalia chiloensis***
rose	***Rosa multiflora***
blueberry	***Vaccinium corymbosum***
bean	***Vicia faba***

Plants reported with integrated partitivirus sequences

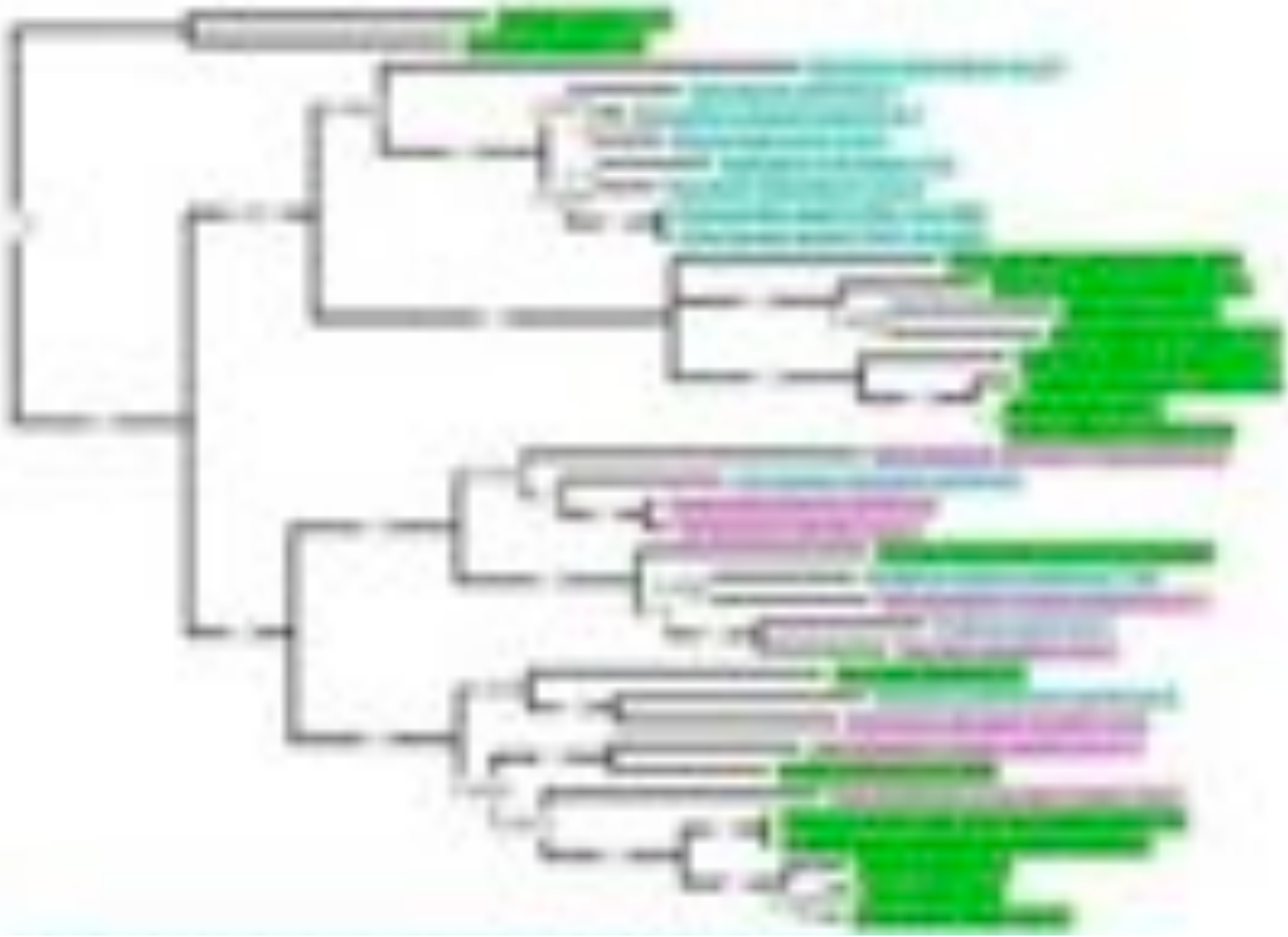
tomato	***Solanum lycopersicum***
cabbage	***Brassica oleracea***
turnip	***Brassica rapa***
rock cress	***Arabidopsis thaliana***
	Arabidopsis arenosa
	Olimarabidopsis pumil
shepherd's purse	***Capsella bursa-pastoris***
potato	***Solanum tuberosum***
rapeseed	***Brassica napus***
	Olimarabidopsis cabulica
tower mustard	***Turritis glabra***
	Capsella rubella

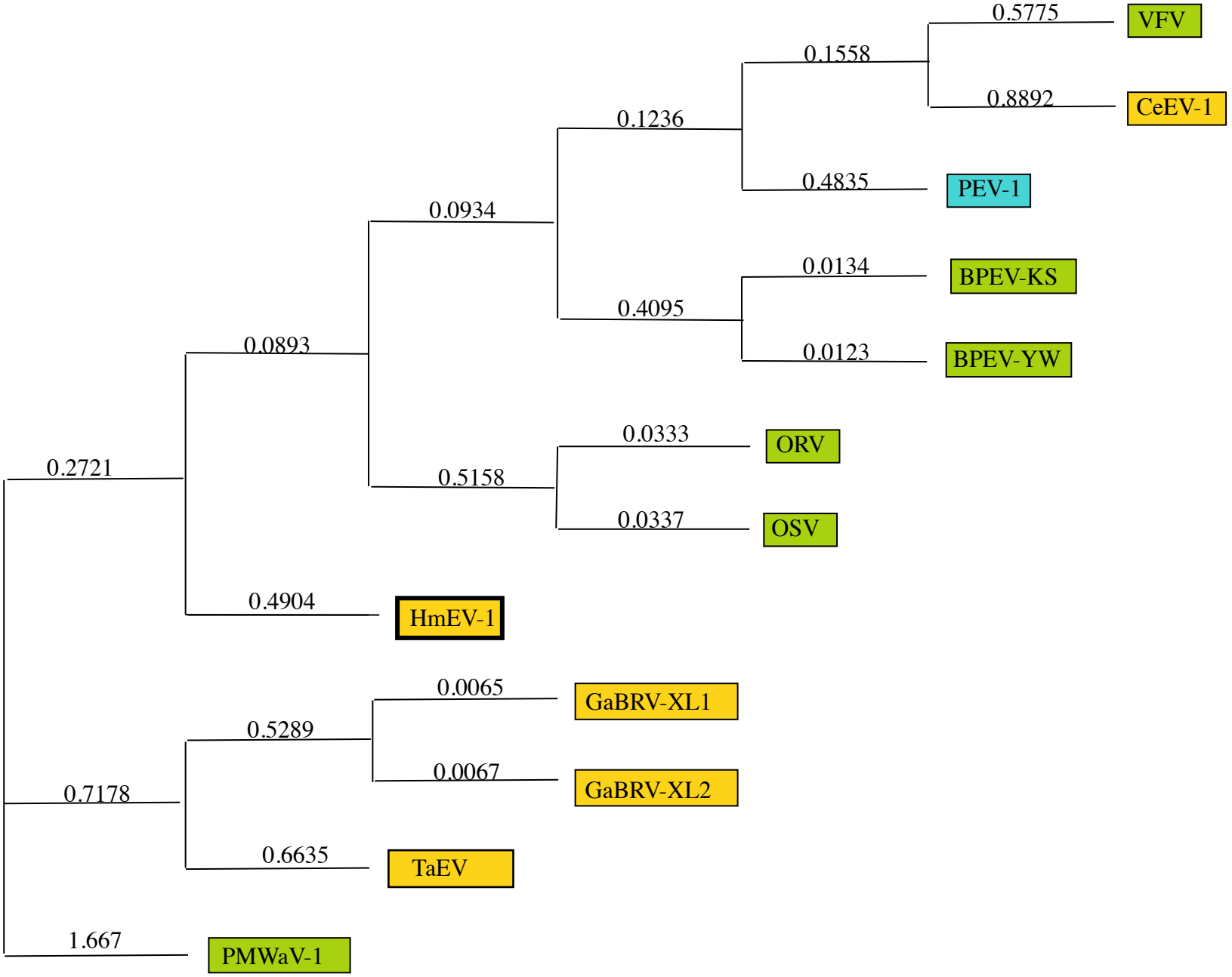
Plants reported with *Partitivirus*

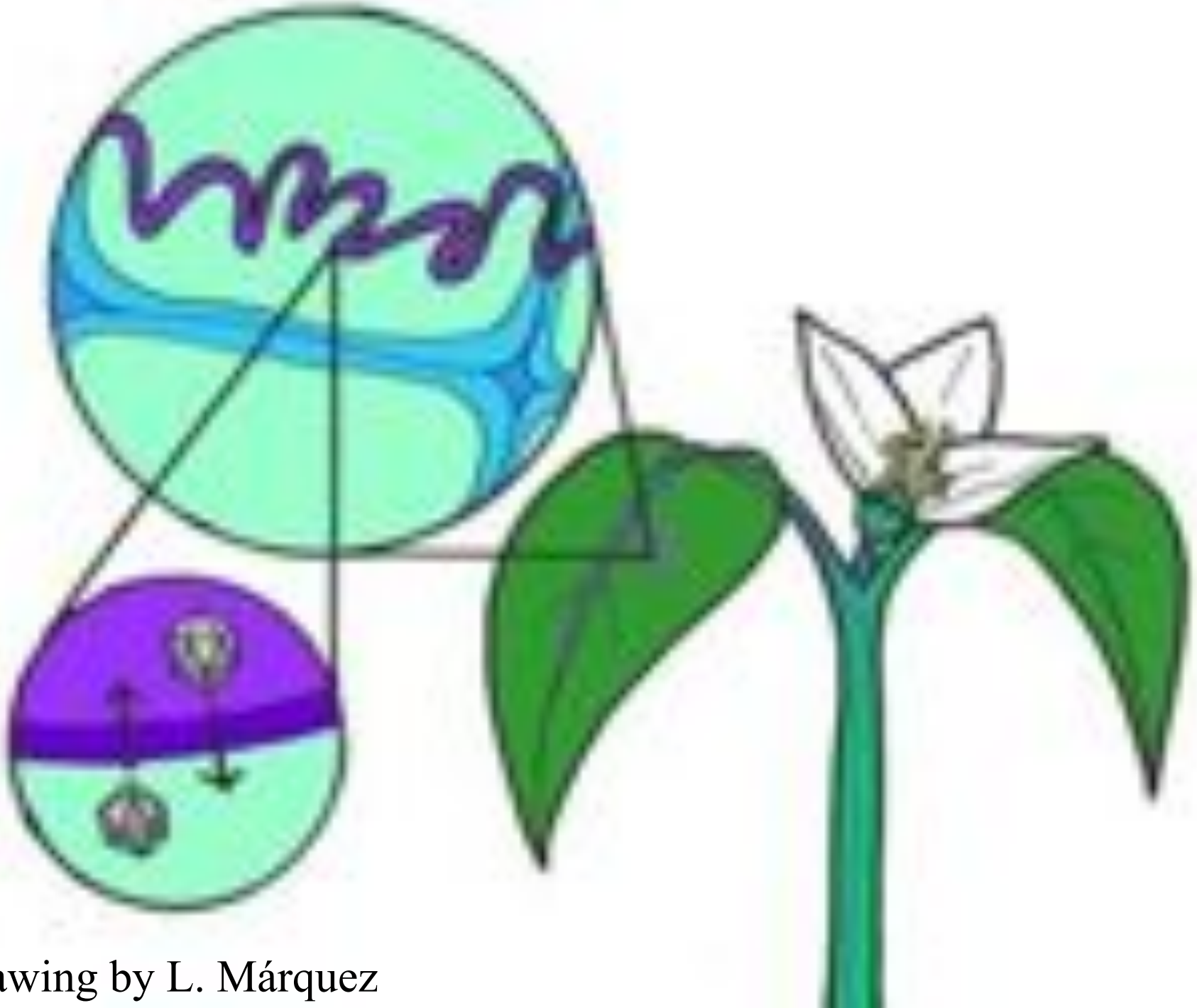
fig	***Ficus carica***
beet	***Beta vulgaris***
green algae	***Bryopsis cinicola***
hemp	***Cannabis sativa***
jalapeño pepper	***Capsicum annuum***
carrot	***Daucus carota***
Scot pine	***Pinus sylvestris***
Japanese mock orange	***Pittosporum tobira***
primrose	***Primula malacoides***
Chinese pear	***Pyrus pyrifolia***

Are Plant and Fungal Persistent Viruses Related?

- **Phylogenetic studies suggest that they are.**




0.5775
VFV
0.1558
0.8892
CeEV-1
0.1236
0.4835
PEV-1
0.0934
0.0134
BPEV-KS
0.4095
0.0893
0.0123
BPEV-YW
0.0333
ORV
0.2721
0.5158
0.0337
OSV
0.4904
HmEV-1
0.0065
GaBRV-XL1
0.5289
0.0067
GaBRV-XL2
0.7178
0.6635
TaEV
1.667
PMWaV-1



Drawing by L. Márquez

What are they doing?

- **Vertical transmission**
  - **98% to >99%**
  - **Through pollen or ovules, so could be reintroduced if lost in outcrossing plants.**
- **Without selection they would get lost overtime.**
- **Plants may recognize them as "self"**
  - **No immune reponse.**

Involved in Habitat-Specific Adaptation

- **Curvularia thermal tolerance virus**
  - **Required for heat tolerance of panic grass and its fungal endophyte in YNP**

- ***White clover cryptic virus***
  - **Regulates nodulation in clover in response to nitrogen**

Molecular fossils?

- **Persistent viruses may be remnants of an ancient mutualistic symbiosis between an RNA entity and a DNA entity, that reside in the cytoplasm of their hosts.**
  - **their lifestyle implies mutualism**
  - **at least 2 are clearly mutualistic**
  - **MUCH MORE STUDY REQUIRED!**

- **In some cases they may have integrated into host genomes, negating the need for the cytoplasmic form.**

Conclusions

- **Biodiversity studies of plant and fungal viruses indicate that persistent viruses are the most common types of viruses in these hosts.**
- **They are maintained through very long periods of time with very high levels of vertical transmission.**
- **Plant and fungal persistent viruses are related and phylogenetic studies indicate they have been transmitted between host and across kingdoms.**
- **At least some persistent viruses confer important functions for the plant or fungal hosts.**

My Mutualistic Network

- My lab

- Rusty Rodriguez
- Regina Redman

- Felipe Chavarría
- My lab in Costa Rica

CIDD Center for Infectious Disease Dynamics

The Huck Institutes of the Life Sciences

Penn State

Area de Conservación Guanacaste — ACG — Fuente de Vida y Desarrollo — MINAE · SINAC

CSREES — Education · Research · Extension · USDA

NSF

The Samuel Roberts Noble Foundation