Supplemental Information

Article: Narnaviruses: novel players in fungal-bacterial symbioses

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This supplemental information includes:

Table S1. Ribosomal RNA gene sequences included in the genomic organism reference database.

Table S2. *Rhizopus microsporus* and *Burkholderia* sensu lato [1] genomes included in the genomic organism reference database.

Table S3. Viral genomes used for the phylogenetic analyses performed in this study. Sequences reported as type viral strains are highlighted in bold [2].

Figure S1. Phylogenetic tree of selected viral RdRp proteins employing MrBayes.

Figure S2. Phylogenetic tree of selected viral RdRp proteins employing the Maximum Likelihood method based on the General Reverse Transcriptase model.

Figure S3. RT-PCR employing cDNA shows that strain 814 wt does harbor RmNV-20S and RmNV-23S, while strain 813 wt does not (above).

Figure S4. Genotypic characterization of ATCC 52814 narnaviruses-free strains.

Figure S5. Microscopy of ATCC 52814 narnaviruses-free (nv-), bacteria-free (b-) and bacteria-reinfected (b*) strains employing Syto9 to detect symbiotic *Mycetohabitans* sp. B7 within *R. microsporus*.

Figure S6. Relative quantification of symbionts in *R. microsporus*.

Figure S7. Fungal dry biomass generated by all ATCC 52814 strains used in this study.

Table S1. Ribosomal RNA gene sequences included in the genomic organism reference database.

Sequence id	Database	source	Organism
AB250178	LSU	silva	*Rhizopus microsporus* var microsporus
AB250188	LSU	silva	*Rhizopus microsporus* var microsporus
AF113479	LSU	silva	*Rhizopus microsporus* var microsporus
AY803932	LSU	silva	*Rhizopus microsporus* var microsporus
AB250155	SSU	silva	*Rhizopus microsporus* var microsporus
AB250165	SSU	silva	*Rhizopus microsporus* var microsporus
AF113438	SSU	silva	*Rhizopus microsporus* var microsporus
FN182237	SSU	silva	*Rhizopus microsporus* var microsporus

Table S2. *Rhizopus microsporus* and *Burkholderia* sensu lato [1] genomes included in the genomic organism reference database.

#	Genome name	Org tag	link
1	*Rhizopus microsporus* ATCC52813	Rhizopus	https://genome.jgi.doe.gov/portal/Rhimi1_1
2	*Rhizopus microsporus* ATCC52814	Rhizopus	https://genome.jgi.doe.gov/Rhimi_ATCC52814_1/Rhimi_ATCC52814_1.home.html
Burkholderia sensu lato			
Mycetohabitans			
3	*Burkholderia rhizoxinica* HKI 454	Burkholderia (since 11.2018 *Mycetohabitans rhizoxinica*)	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/198/775/GCF_000198775.1_ASM19877v1
Unassigned – endofungal bacteria			
4	*Candidatus* Glomeribacter gigasporarum BEG34	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/227/585/GCF_000227585.1_ASM22758v1
5	*Candidatus* Glomeribacter endosymbiont of *Mortierella elongata* strain AG77	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/003/966/915/GCF_003966915.1_ASM396691v1
Burkholderia and Paraburkholderia			
6	*Burkholderia* sp. 383	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/012/945/GCF_000012945.1_ASM1294v1
7	*Burkholderia* sp. CCGE1001	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/176/935/GCF_000176935.3_ASM17693v3
8	*Burkholderia* sp. CCGE1002	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/092/885/GCF_000092885.1_ASM9288v1
9	*Burkholderia* sp. CCGE1003	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/148/685/GCF_000148685.1_ASM14868v1
10	*Burkholderia* sp. KJ006	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/262/695/GCF_000262695.1_ASM26269v1
11	*Burkholderia* sp. RPE64	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/402/035/GCF_000402035.1_ASM40203v1
12	*Burkholderia* sp. YI23	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/236/065/GCF_000236065.1_ASM23606v1
13	*Burkholderia ambifaria* AMMD	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/203/915/GCF_000203915.1_ASM20391v1
14	*Burkholderia ambifaria* MC40-6	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/019/925/GCF_000019925.1_ASM1992v1
15	*Burkholderia cenocepacia* AU 1054	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/014/085/GCF_000014085.1_ASM1408v1
16	*Burkholderia cenocepacia* HI2424	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/203/955/GCF_000203955.1_ASM20395v1
17	*Burkholderia cenocepacia* J2315	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/009/485/GCF_000009485.1_ASM948v1
18	*Burkholderia cenocepacia* MC0-3	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/019/505/GCF_000019505.1_ASM1950v1
19	*Burkholderia cepacia* GG4	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/292/915/GCF_000292915.1_ASM29291v1
20	*Burkholderia gladioli* BSR3	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/194/745/GCF_000194745.1_ASM19474v1
21	*Burkholderia glumae* BGR1	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/022/645/GCF_000022645.2_ASM2264v1
22	*Burkholderia mallei* ATCC 23344	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/011/705/GCF_000011705.1_ASM1170v1
23	*Burkholderia mallei* NCTC 10229	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/015/605/GCF_000015605.1_ASM1560v1
24	*Burkholderia mallei* NCTC 10247	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/015/625/GCF_000015625.1_ASM1562v1
25	*Burkholderia mallei* SAVP1	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/015/465/GCF_000015465.1_ASM1546v1

#	Genome name	Org tag	link
26	*Burkholderia multivorans* ATCC 17616	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/010/545/GCF_000010545.1_ASM1054v1
27	*Burkholderia phenoliruptrix* BR3459a	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/300/095/GCF_000300095.1_ASM30009v1
28	*Burkholderia phymatum* STM815	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/020/045/GCF_000020045.1_ASM2004v1
29	*Burkholderia phytofirmans* PsJN	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/020/125/GCF_000020125.1_ASM2012v1
30	*Burkholderia pseudomallei* 1026b	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/260/515/GCF_000260515.1_ASM26051v1
31	*Burkholderia pseudomallei* 1106a	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/015/925/GCF_000015925.1_ASM1592v1
32	*Burkholderia pseudomallei* 1710b	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/012/785/GCF_000012785.1_ASM1278v1
33	*Burkholderia pseudomallei* 668	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/015/905/GCF_000015905.1_ASM1590v1
34	*Burkholderia pseudomallei* BPC006	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/294/635/GCF_000294635.1_ASM29463v1
35	*Burkholderia pseudomallei* K96243	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/011/545/GCF_000011545.1_ASM1154v1
36	*Burkholderia pseudomallei* MSHR305	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/439/695/GCF_000439695.1_ASM43969v1
37	*Burkholderia pseudomallei* MSHR346	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/182/585/GCF_000182585.2_ASM18258v2
38	*Burkholderia pseudomallei* NCTC 13179	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/494/855/GCA_000494855.1_ASM49485v1
39	*Burkholderia thailandensis* E264	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/152/285/GCF_000152285.1_ASM15228v1
40	*Burkholderia thailandensis* MSMB121	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/385/525/GCF_000385525.1_ASM38552v1
41	*Burkholderia vietnamiensis* G4	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/016/205/GCA_000016205.1_ASM1620v1
42	*Burkholderia xenovorans* LB400	Burkholderia	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/013/645/GCF_000013645.1_ASM1364v1
43	*Burkholderia terrae* BS001	Burkholderia	https://www.ncbi.nlm.nih.gov/nuccore/AKAU00000000.1

Table S3. Viral genomes used for the phylogenetic analyses performed in this study. Sequences reported as type viral strains are highlighted in bold [2].

Id or Acronym	Accession	Description in phylogeny	Reference
Family Narnaviridae Genus *Narnavirus*			
RmNV-20S	MK204624	*Rhizopus microsporus* 20S RNA narnavirus	This work
RmNV-23S	MK204625	*Rhizopus microsporus* 23S RNA narnavirus	This work
ScNV-20S	**AAC98925.1**	***Saccharomyces* 20S RNA narnavirus**	[2, 3]
ScNV-23S	AAC98708.1	*Saccharomyces* 23S RNA narnavirus	[3]
PiRV-4	JN400241.	*Phytophthora infestans* RNA virus 4	[4]
LsNLV1	ANI86034	*Leptomonas seymouri* Narna-like virus 1	[5]
FpNV1	BAV56295	*Fusarium poae* narnavirus 1	[6]
AaNV	QBA55490.1	Aedes angustivittatus narnavirus	[7]
WeNLV8	APG77263.1	Wenling narna-like virus 8	[8]
BeNLV24	YP_009333245.1	Beihai narna-like virus 24	[8]
WeNLV7	YP_009337166.1	Wenling narna-like virus 7	[8]
Family Narnaviridae Genus *Mitovirus*			
OMV6	NC_004054	*Ophiostoma* novo-ulmi mitovirus 6	[9]
OMV4	NC_004052	*Ophiostoma* novo-ulmi mitovirus 4	[9]
BcMV1	NC_011372	*Botrytis cinerea* mitovirus 1	[10]
CpMV1	**NC_004046**	***Cryphonectria parasitica* mitovirus 1**	[2, 10]
TcMV	TCU51331	*Thanatephorus cucumeris* mitovirus	[3]
SsMV1	NC_026510	*Sclerotinia sclerotiorum* mitovirus 1	[11]
SsMV2	JQ013378	*Sclerotinia sclerotiorum* mitovirus 2	[12]
ShMV	AY172454	*Sclerotinia homoeocarpa* mitovirus	[3]
RhMV	AB558120	*Rhizophagus* sp. RF1 mitovirus	[13]
33086	MG256173	*Gigaspora margarita* mitovirus 1	[14]
34036	MG256174	*Gigaspora margarita* mitovirus 2	[14]
34470	MG256175	*Gigaspora margarita* mitovirus 3	[14]
34875	MG256176	*Gigaspora margarita* mitovirus 4	[14]
Family Unassigned Genus *Ourmiavirus*			
CsVC	ACI03053.1	Cassava virus C	[3]
OuMV	**ACF16360.1**	**Ourmia melon virus**	[2, 3]
EpCV	ACF16357.1	Epirus cherry virus	[3]
BOlV	NC_028476	*Botrytis* ourmia-like virus	[15]
Family Leviviridae Genus *Levivirus*			
EnP GA	CAA27499	Enterobacteria phage GA	[16]
EnP MS2	**CAA23991**	**Enterobacteria phage MS2**	[2, 16]
Family Leviviridae Genus *Allolevirus*			
EnP SP	CAA30375	Enterobacteria phage SP	[16]
EnP MX1	AAC14701	Enterobacteria phage MX1	[16]

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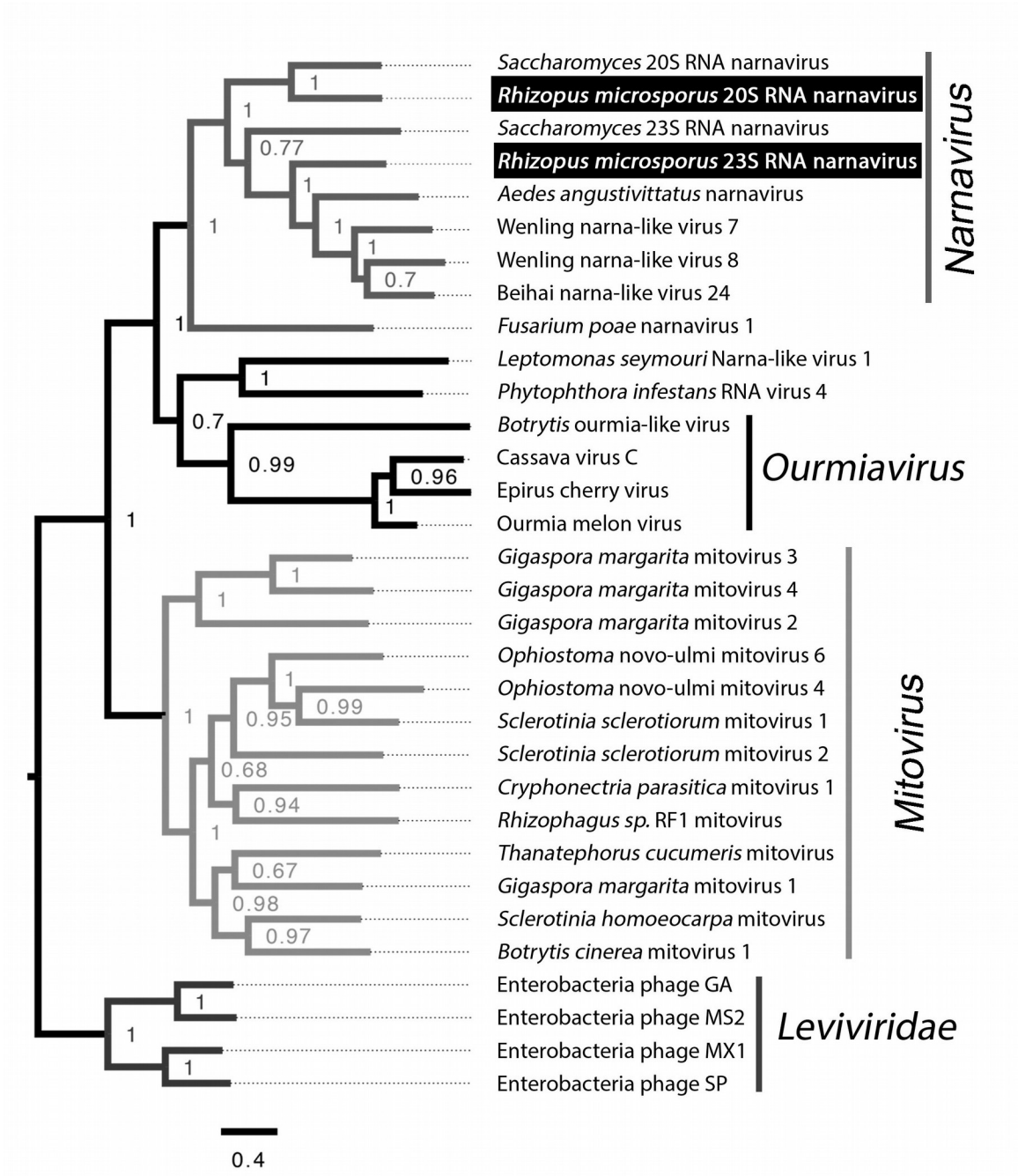

Figure S1. Phylogenetic tree of selected viral RdRp proteins. The evolutionary history was inferred using MrBayes under a mixed amino acid model. The posterior probability support is shown next to each node. Two independent chains were used along with 10M Monte Carlo Markov chain generations. The analysis involved 32 amino acid sequences.

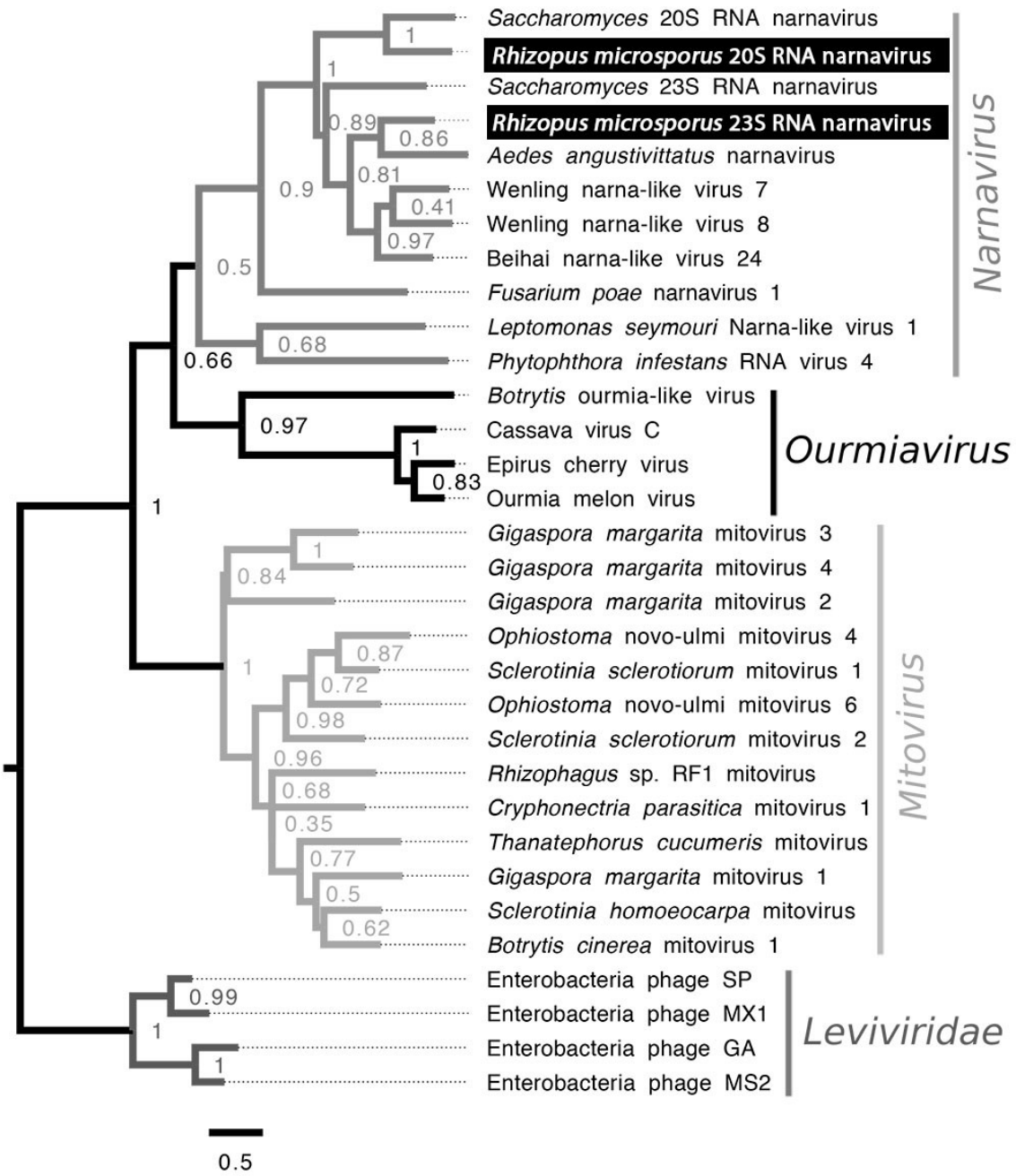

Figure S2. Phylogenetic tree of selected viral RdRp proteins employing the Maximum Likelihood method based on the General Reverse Transcriptase model. The percentage of trees in which the associated taxa clustered together in 1000 bootstrap replicates is shown next to the branches. The tree is drawn to scale, with branch lengths representing the number of substitutions per site. The analysis involved 32 amino acid sequences.

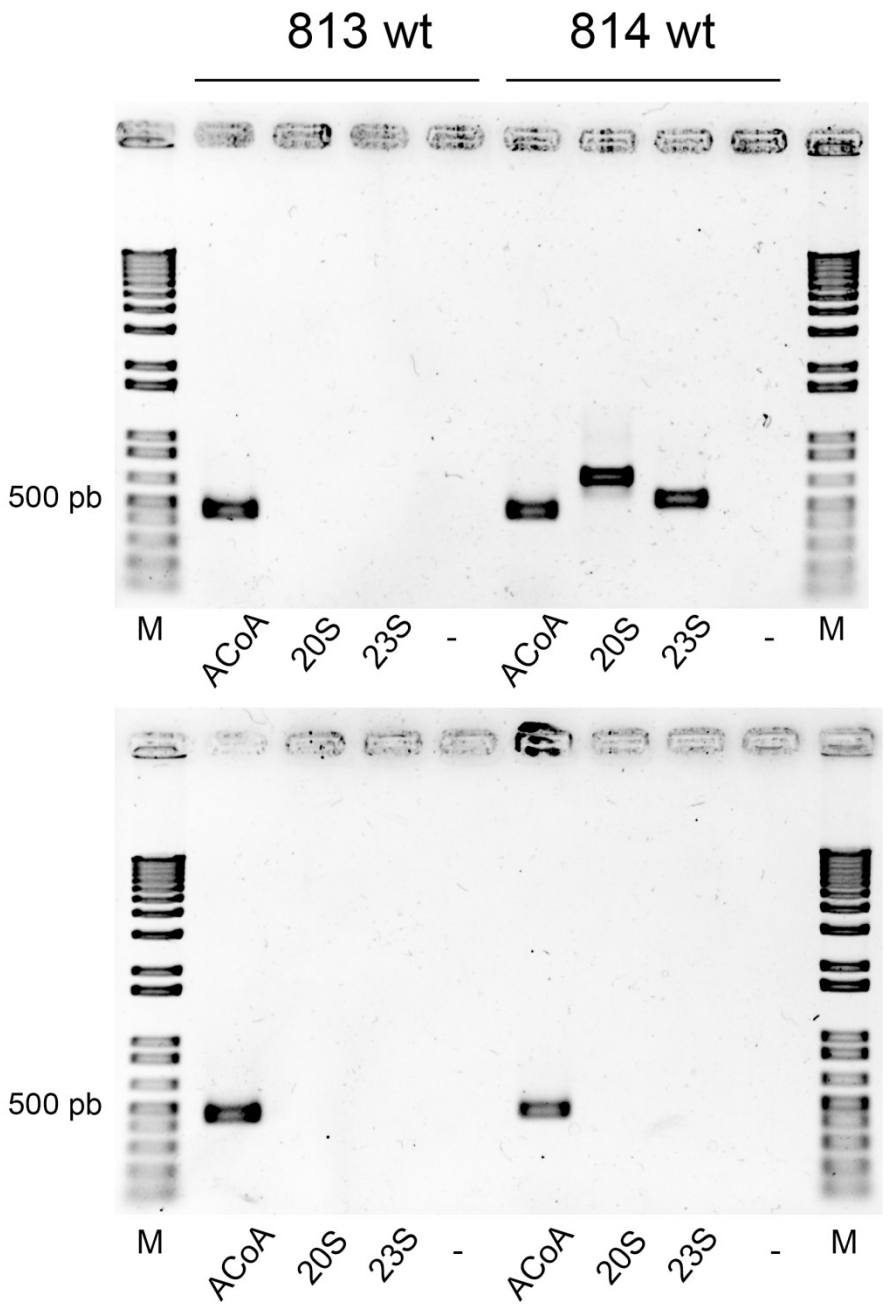

Figure S3. RT-PCR employing cDNA shows that strain 814 wt does harbor RmNV-20S and RmNV-23S, while strain 813 wt does not (above). Potential endogenization of viral sequences was analyzed by final point PCR using genomic DNA (below). M molecular marker, ACoA positive control for fungal acetyl-CoA transporter gene (392 pb), 20S RmNV-20S (683 pb) and 23S RmNV-23S (537 pb).

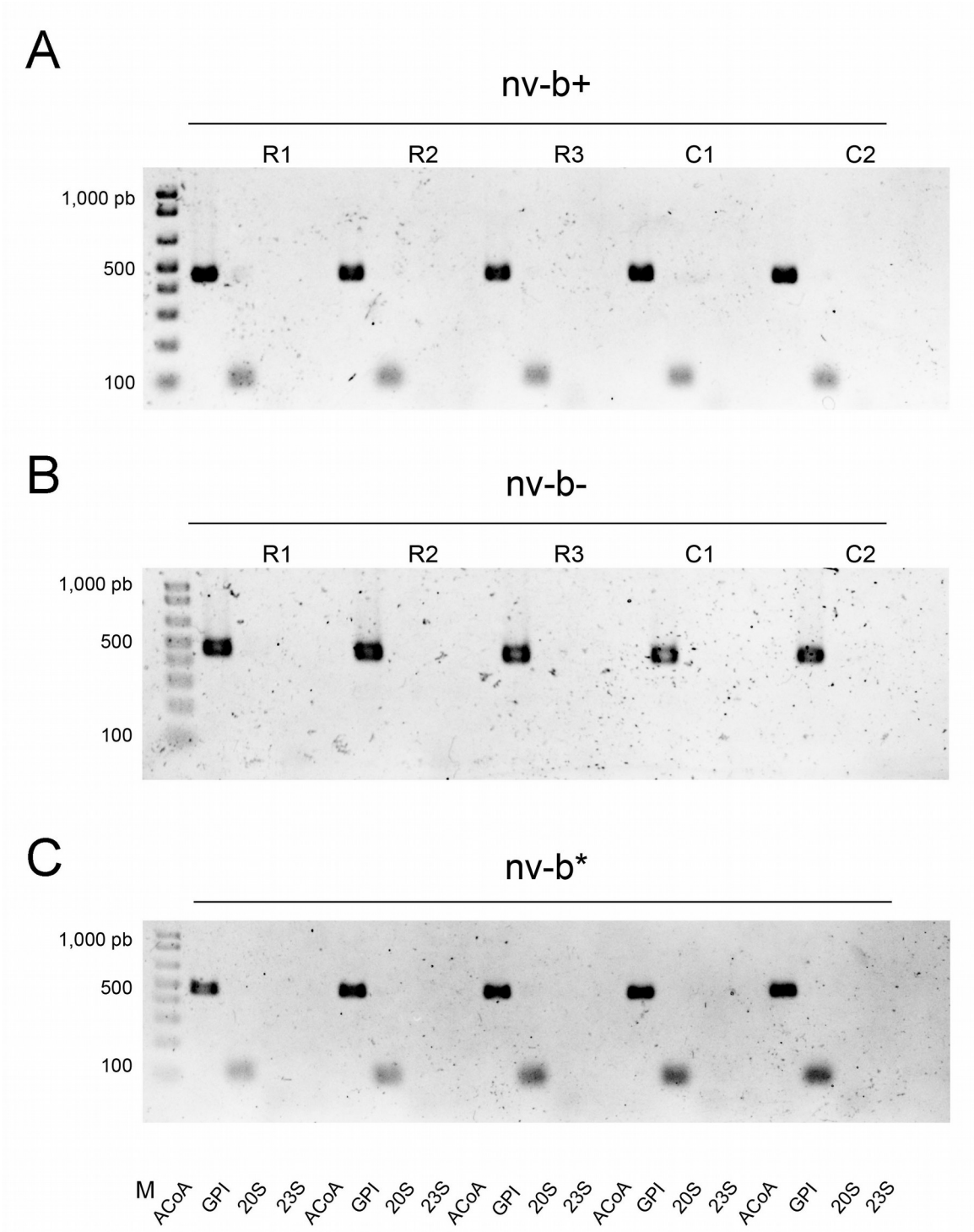

Figure S4. Genotypic characterization of ATCC 52814 narnaviruses-free strains. **A** Strains nv-b+ generated by employing ribavirin (R1, R2, R3) or cycloheximide (C1, C2) as antiviral. **B** Strains nv-b- obtained using ciprofloxacin after antiviral treatment. **C** Strains nv-b* derived from the co-cultivation of strains nv-b- with *Mycetohabitans* sp. B7 (bacteria-reinfected).

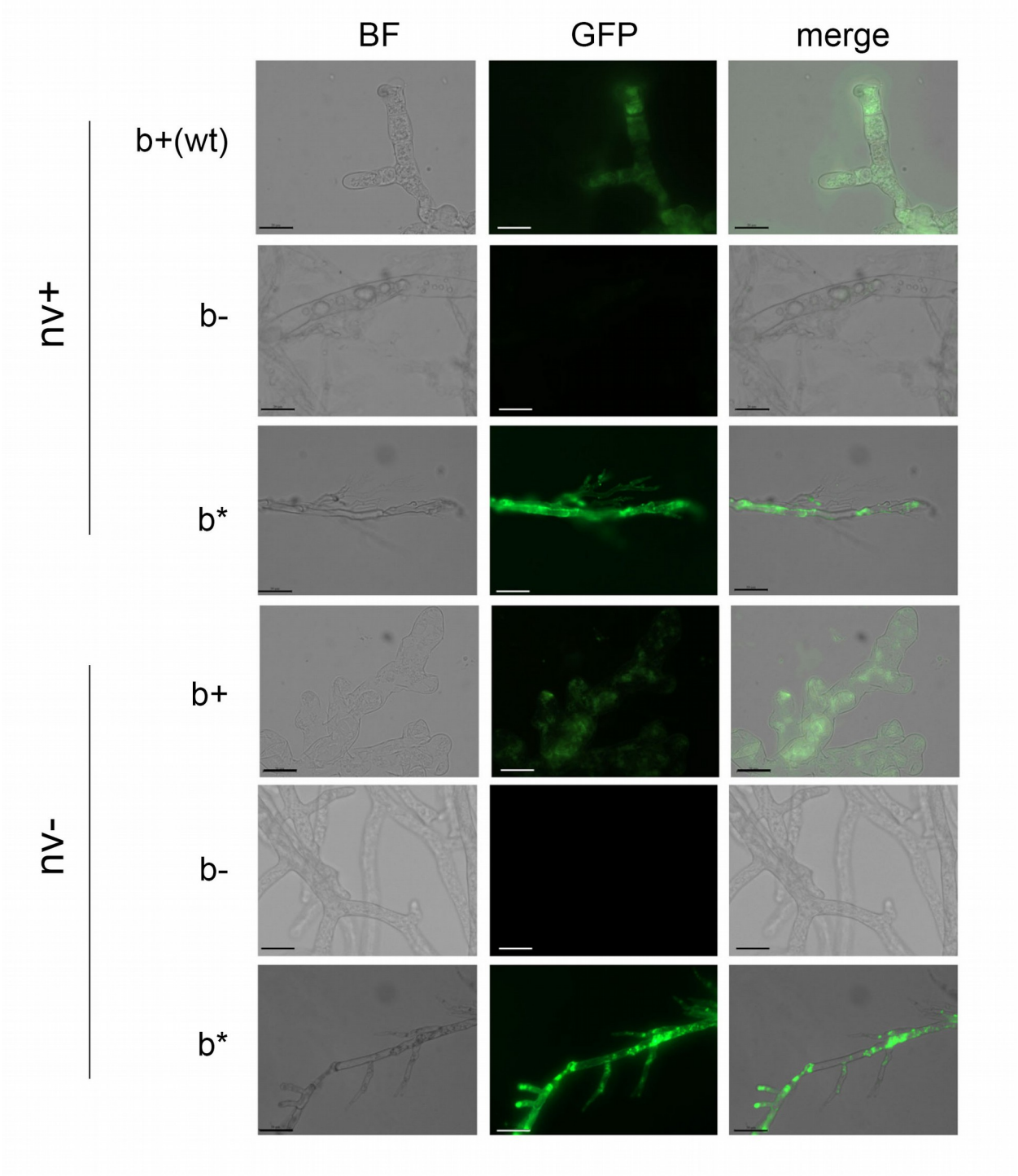

Figure S5. Microscopy of ATCC 52814 narnaviruses-free (nv-), bacteria-free (b-) and bacteria-reinfected (b*) strains employing Syto9 to detect symbiotic *Mycetohabitans* sp. B7 within *R. microsporus*. Fungal mycelium was stained with SYTO 9 (Invitrogen, Molecular Probes) and observed using a Leica DM6000B fluorescence microscope and the GFP filter. Scale bars = 20 µm.

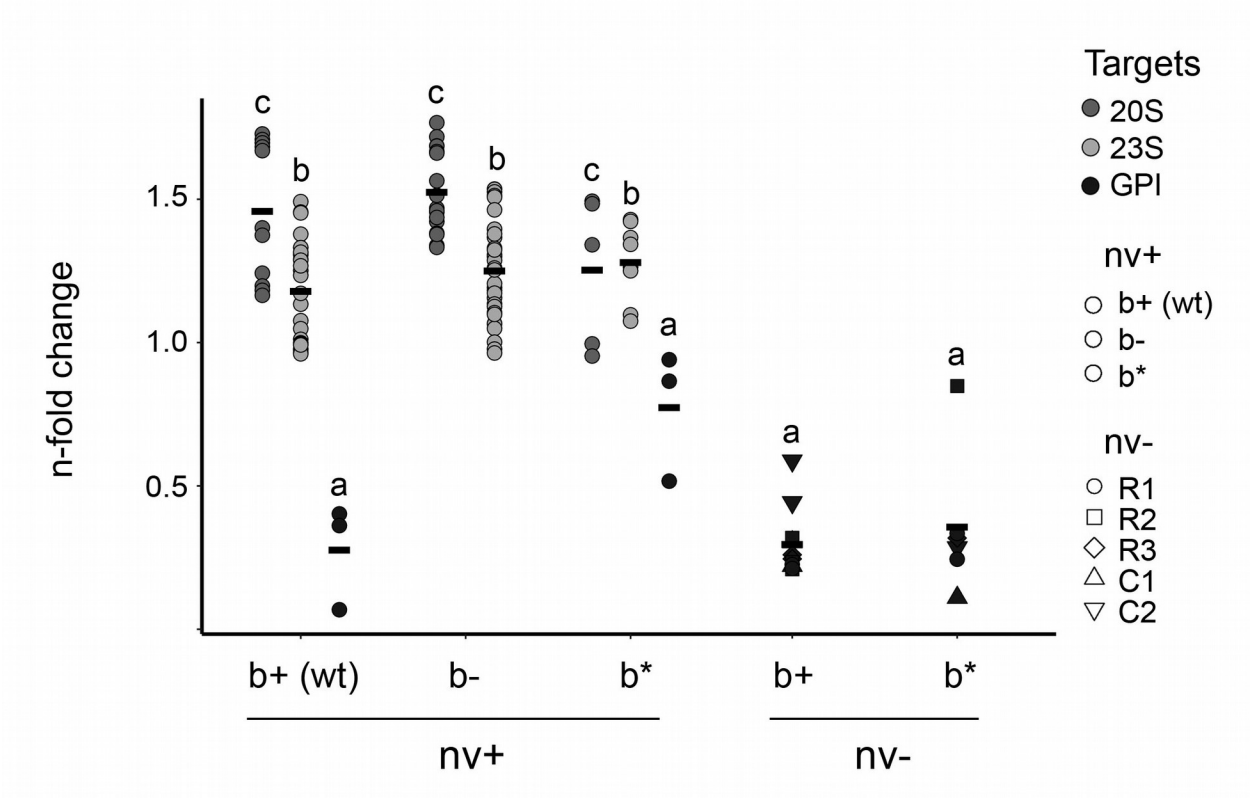

Figure S6. Relative quantification of symbionts in *R. microsporus*. Symbionts relative abundance (n-fold change) was calculated in reference to the host using the bacterial GPI gene, the RmNV-20S and RmNV-23S genome, and the AcoA fungal gene. Different letters above dots denote statistical differences between strains by one-way ANOVA followed by Tukey post-hoc test ($p < 0.05$), n= 3 to 65.

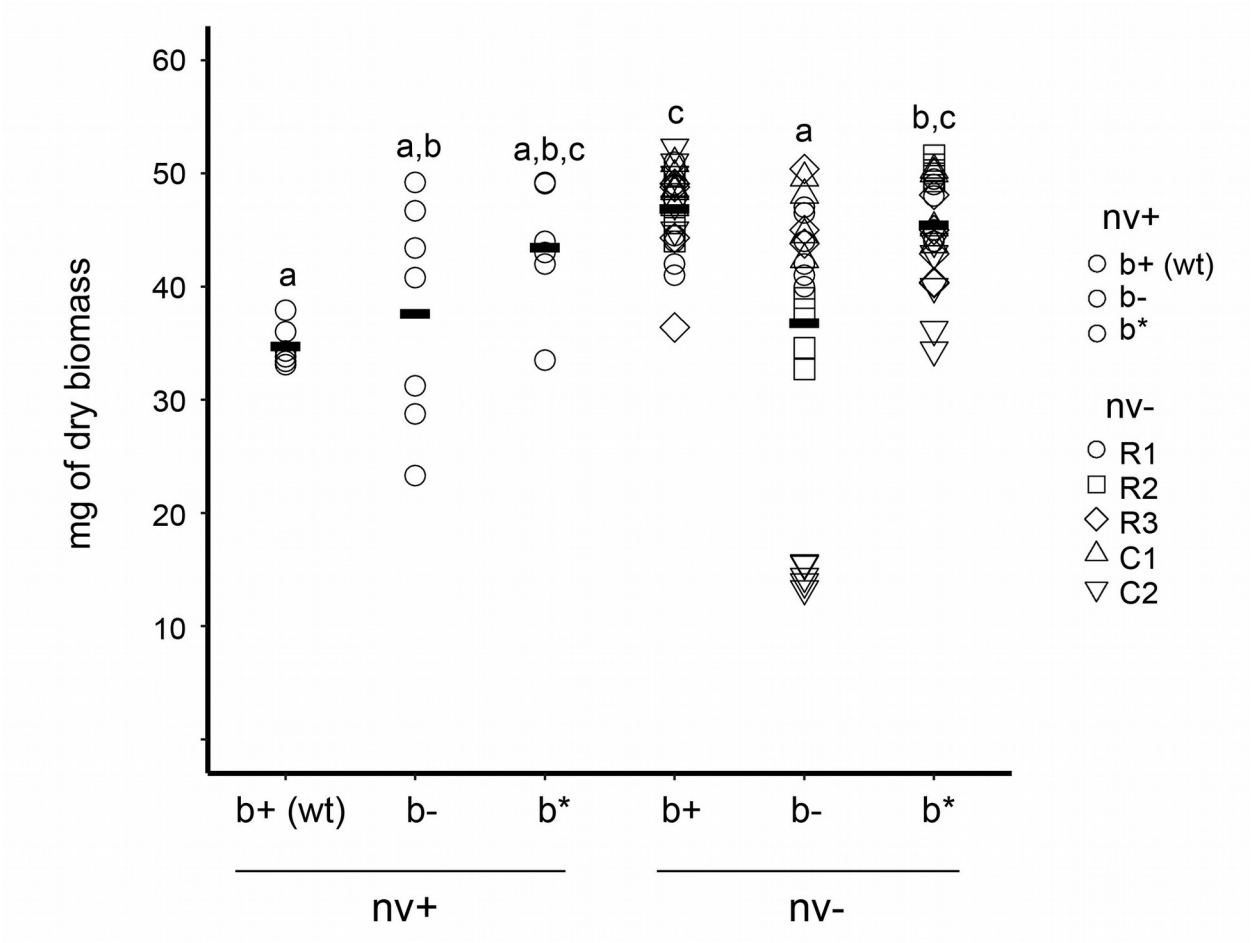

Figure S7. Fungal dry biomass generated by all ATCC 52814 strains used in this study. Fungal strains were grown on half-strength PDA for six days at 30°C. Different letters denote statistical differences between strains by ANOVA followed by Post Hoc Tukey test ($p < 0.05$), n = 4-20. No significant differences were observed across groups of strains due to narnaviruses RmNV-20S and RmNV-23S ($F_{1,91} = 1.81$, $p = 0.182$).