

Supplemental information

**Genome reduction and relaxed selection
is associated with the transition to symbiosis
in the basidiomycete genus *Podaxis***

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Supplemental Information:

Figures S1 to S10

Tables S1, S2, S3 and S8

Fig. S1. Maximum Likelihood phylogeny based on 10,000 ultrafast bootstrap replicates of ITS sequences from (Conlon *et al.*, 2016) with the addition of sequences India, LINN Cape, PREM Cape, Madagascar, Machattie, Kuranda, Moombra and all “AQ” sequences generated by extracting DNA from herbarium spores. Specimens used in this study are highlighted in red and type specimens have their species name next to the herbarium number. Related to Figures 1A, 1B and 2A

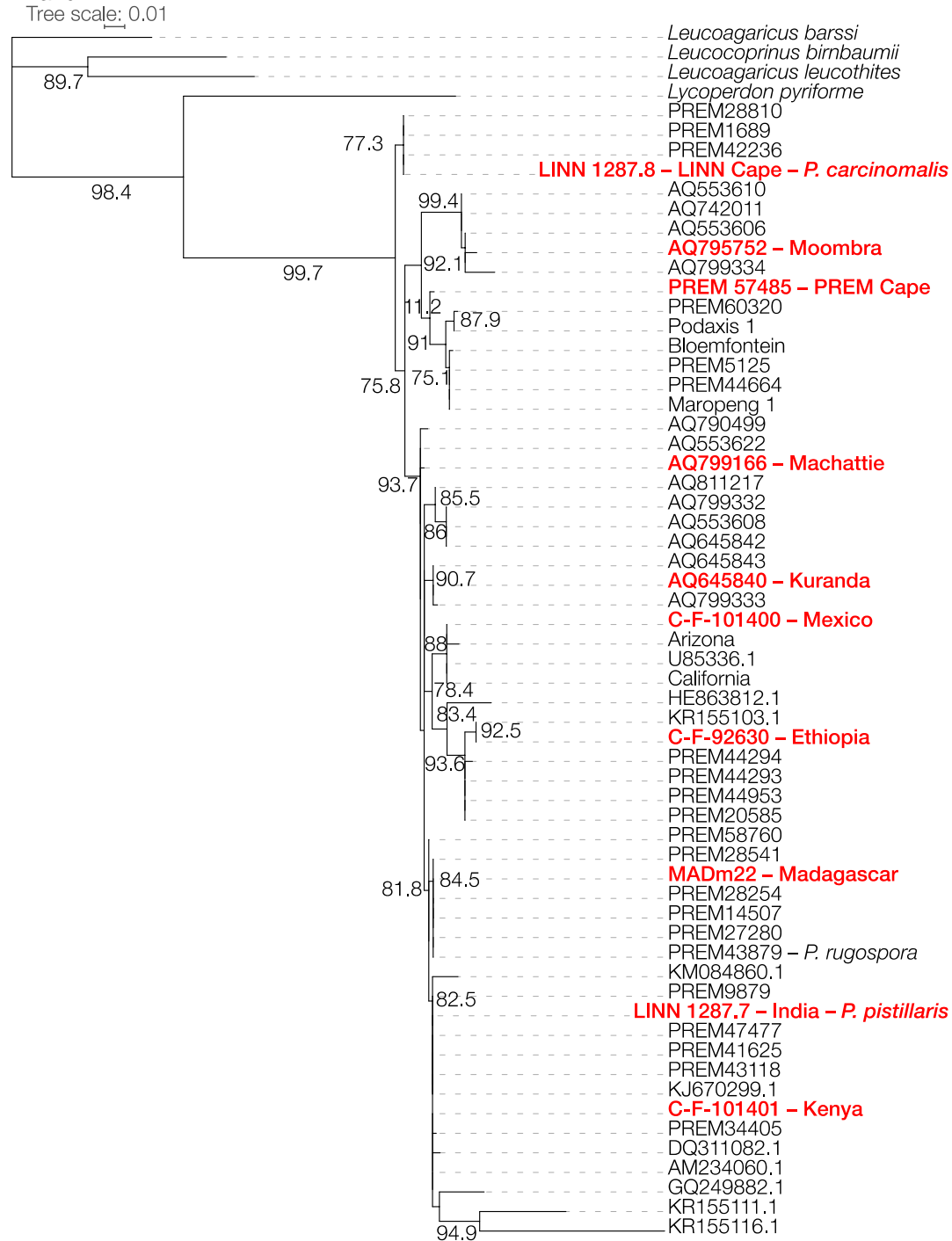


Fig. S2. Mean spore brightness, measured from images in (Conlon *et al.*, 2016) using ImageJ (Schneider *et al.*, 2012) for free-living, facultative and specialist *Podaxis* from photos taken at the PREM herbarium. Violins represent the individual data points (Table S8). Related to Figure 1A.

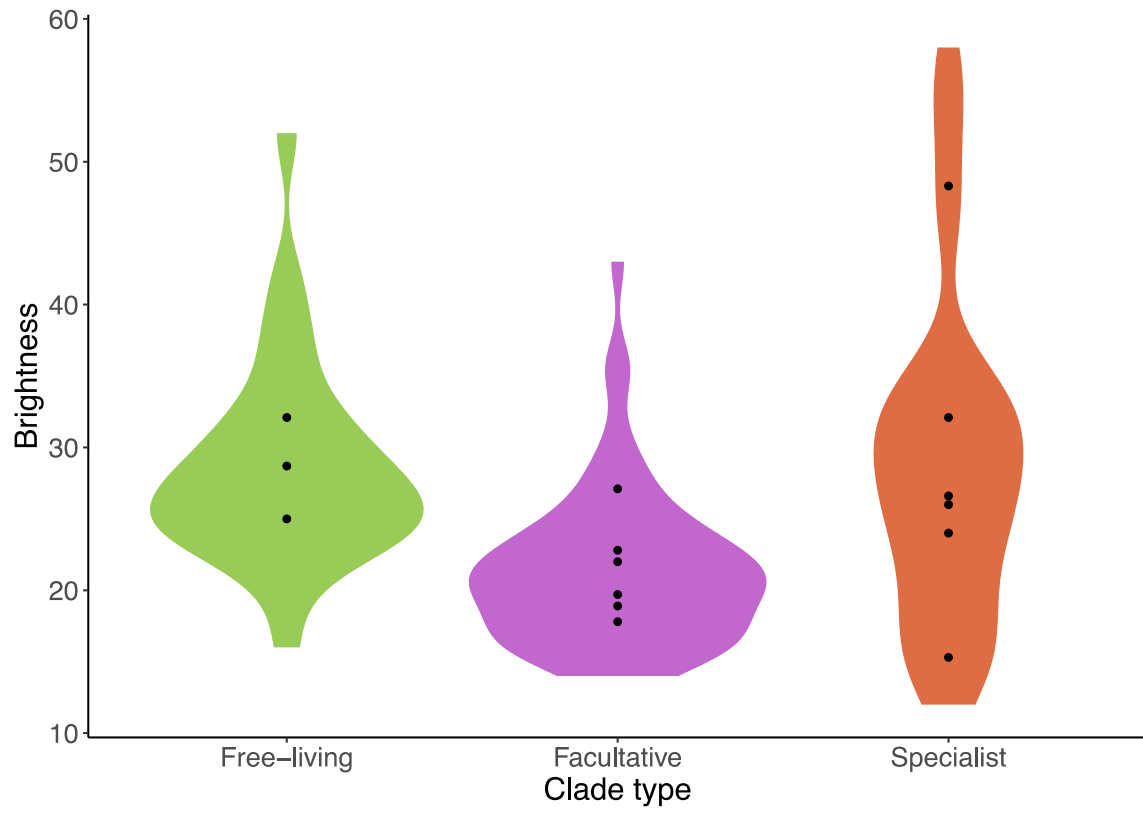


Fig. S3. Alignment of mitochondrial genome assemblies (Table S3) using Mauve (Darling *et al.*, 2004) with locally collinear blocks highlighted in the same colour. From top-to-bottom (right-to-left) the strains are: Moombra, LINN Cape, PREM Cape, Kuranda, Kenya, India, Madagascar, Ethiopia, Machattie and Mexico. Related to Figure 1E.

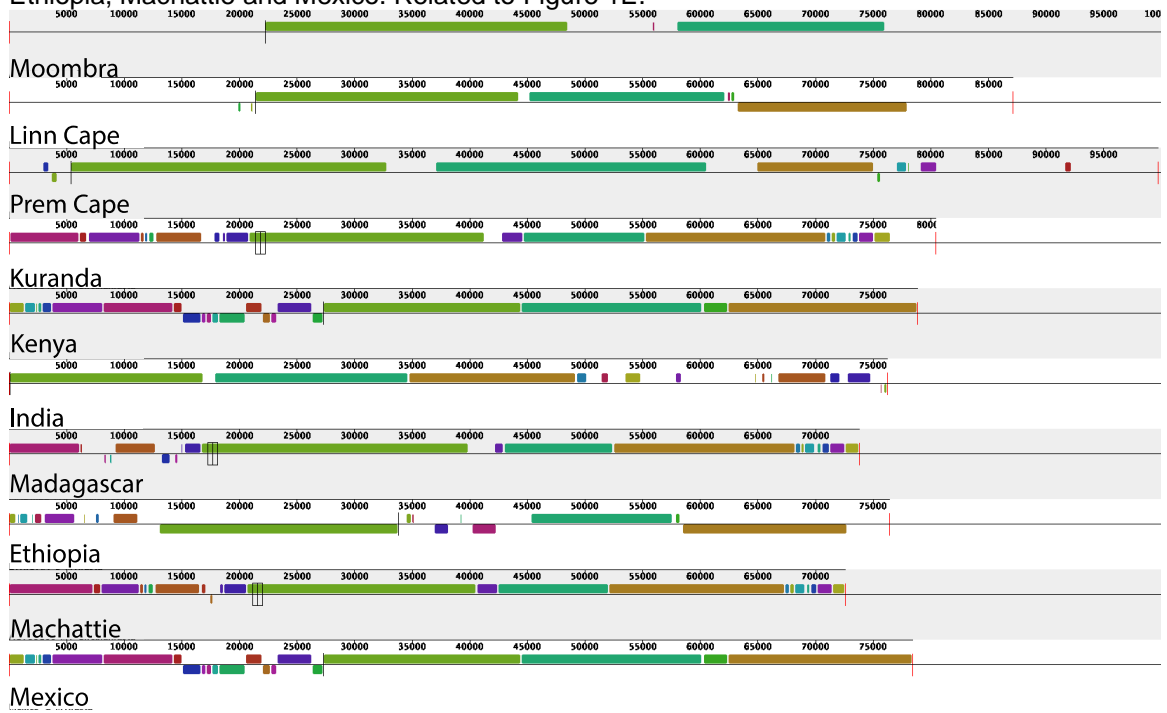


Fig. S4. Whole genome phylogeny using single copy ortholog sequences. Related to Figure 2A.

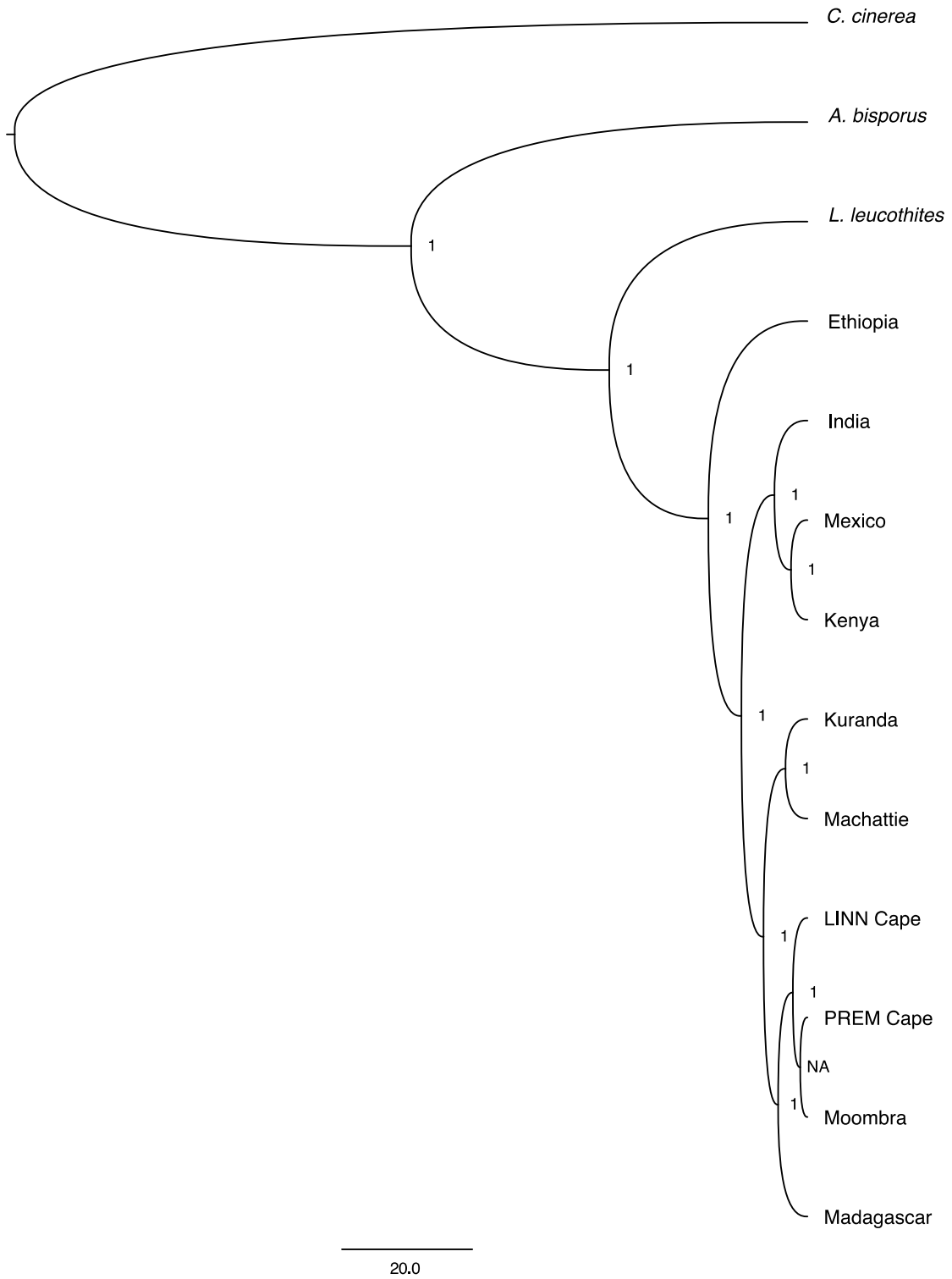
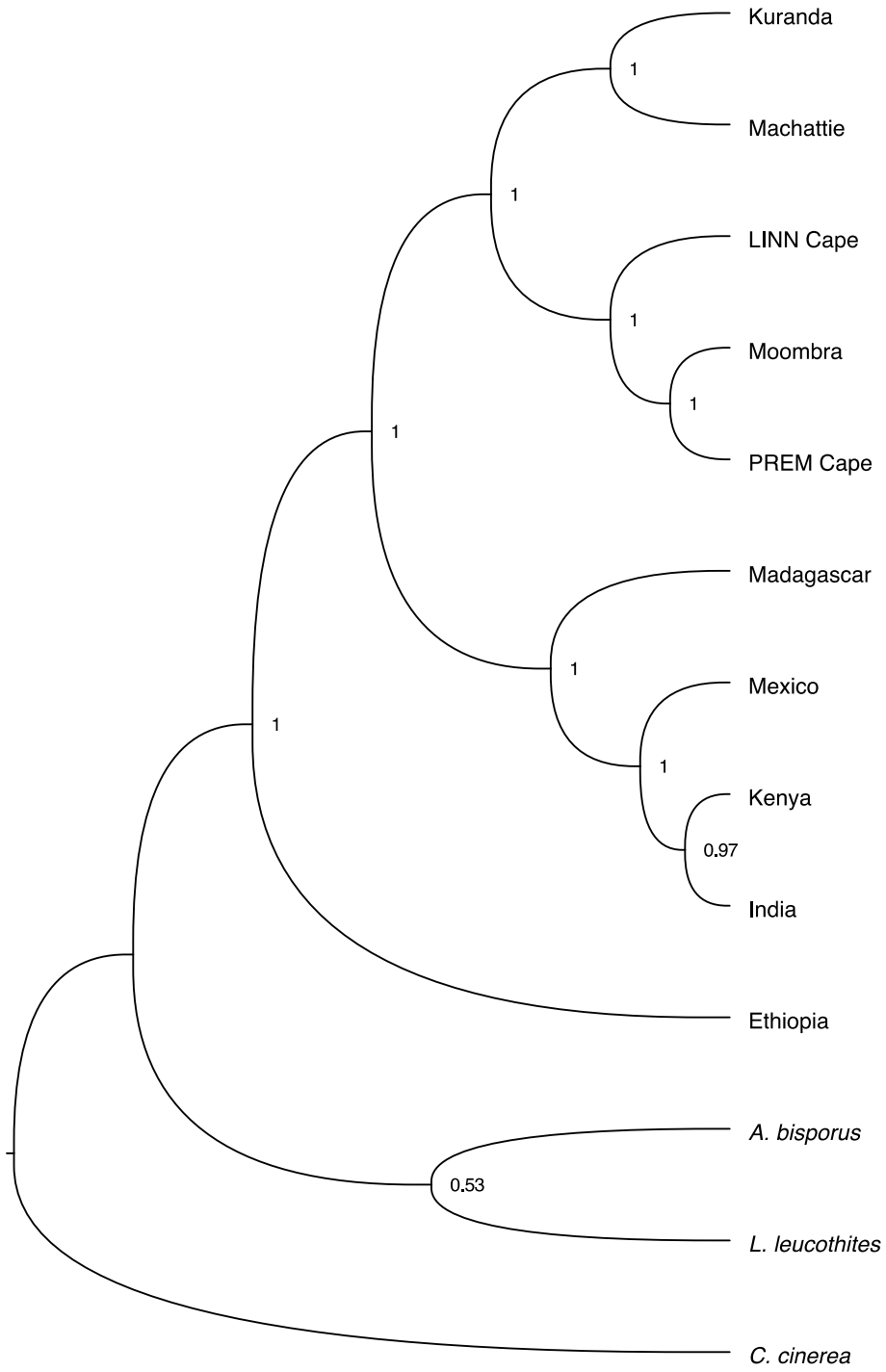


Fig. S5. Whole genome phylogeny using intron sequences from single copy orthologs in Fig. S4. Related to Figure 2A.



20.0

Fig. S6. Phylogenetically-weighted PCA of predicted Carbohydrate-Active enZyme (CAZyme) numbers (Table S5). Related to Figure 3A-D

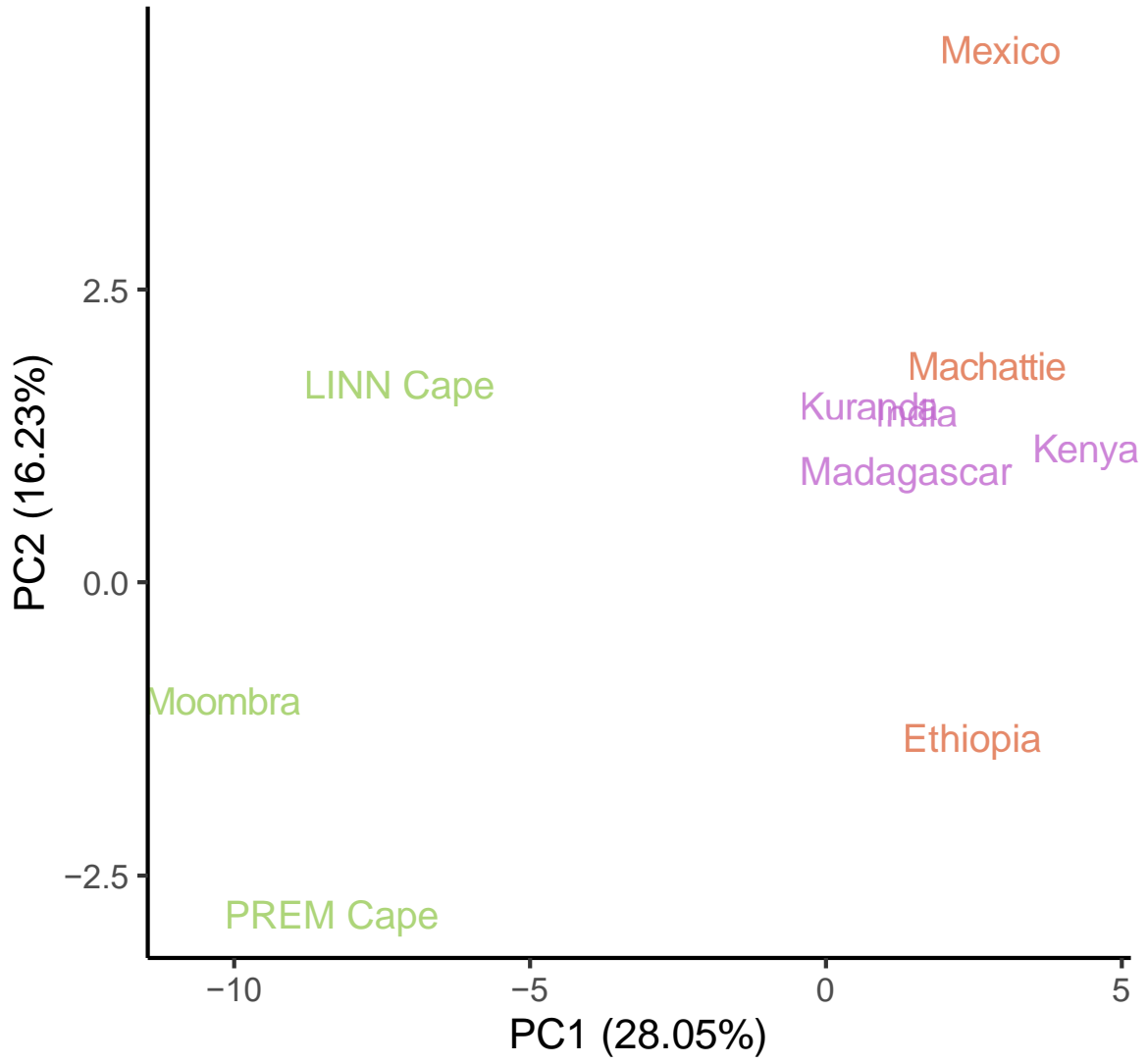


Fig. S9. Phylogenetic analysis of PKSs showed a high degree of similarity across *Podaxis* life histories. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 41 amino acid sequences. Evolutionary history was inferred by using the Maximum Likelihood method and Le_Gascuel_2008 model (Le and Gascuel, 2008). The tree with the highest log likelihood (-13049.13) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Amino acid sequences from the predictive framework are in Key Resources. Related to Figure 4.

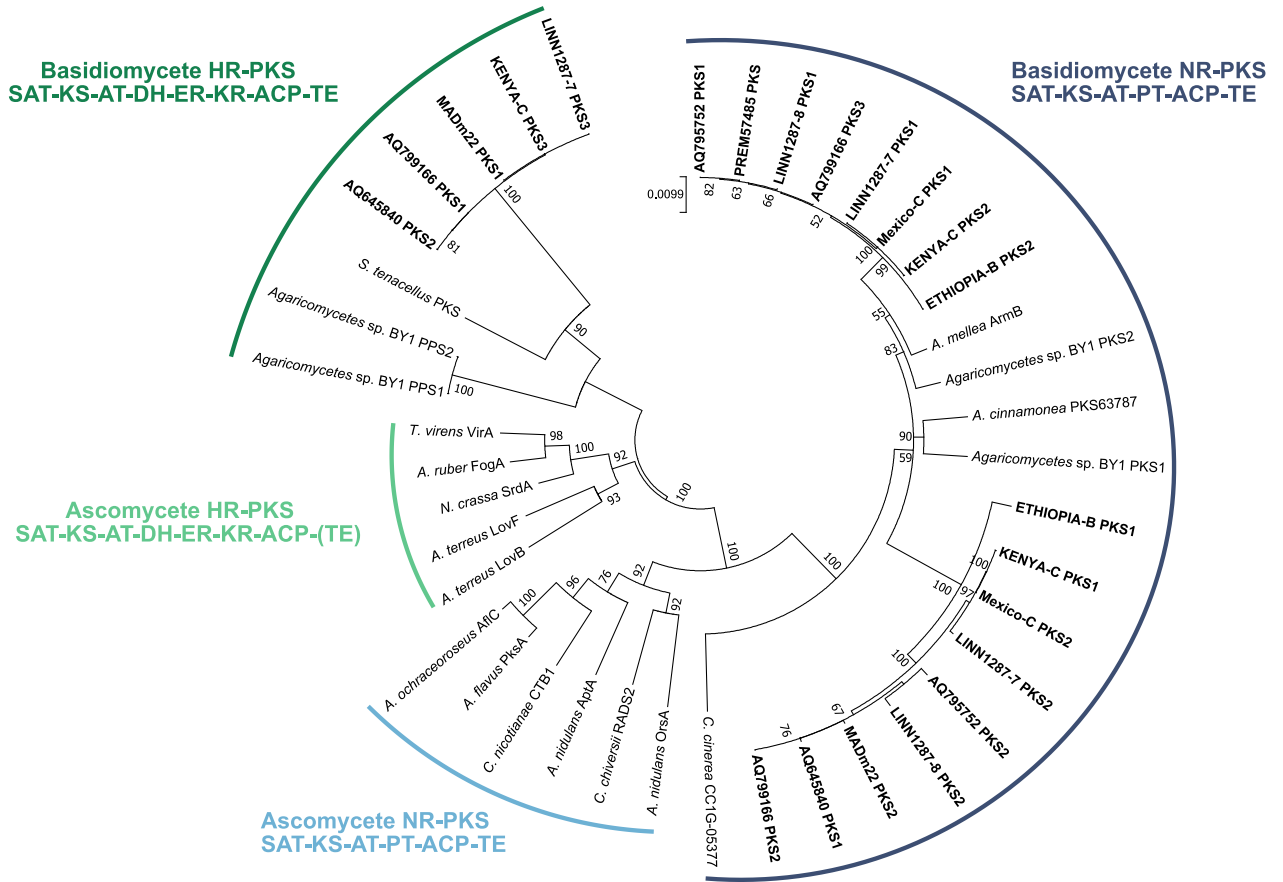


Fig. S10. The number of *Podaxis* specimens collected by month as evident from the notes in the PREM deposits. Summer rains typically occur from October to April in South Africa with a dry season from April-October. Related to Figure 1A.

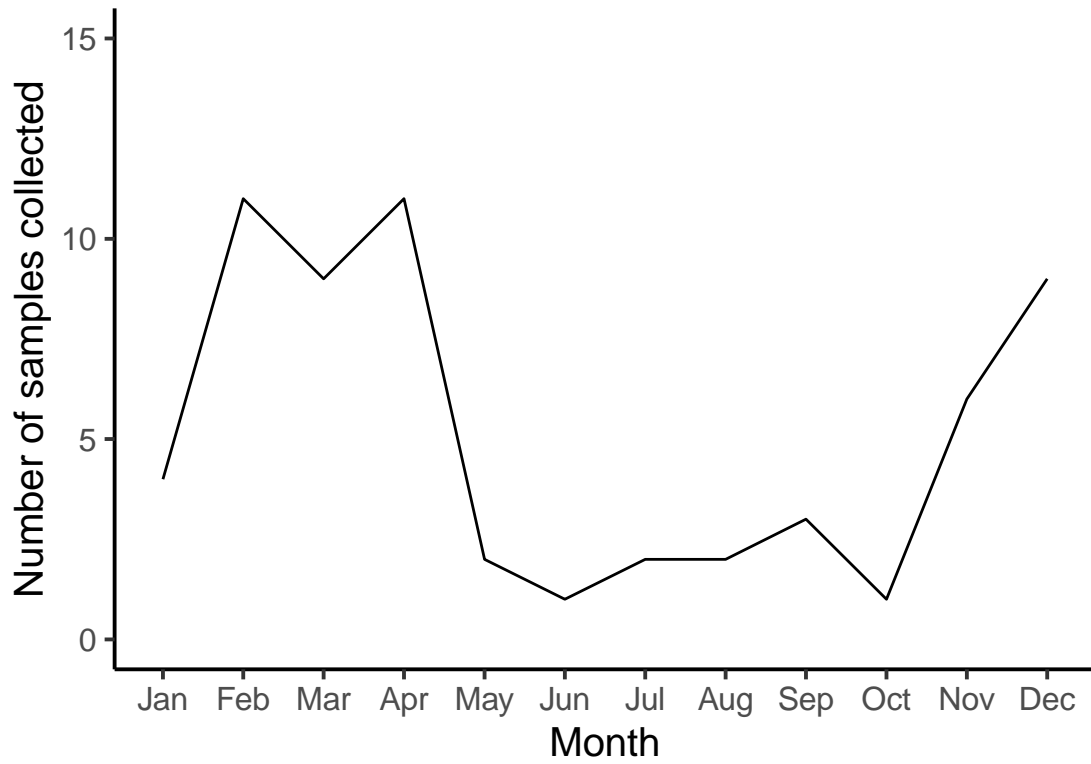


Table S1. *Podaxis* specimens from which spores were successfully germinated during this study along with their herbarium number, collection location, collection date and type status. Related to Figure 1A.

Herbarium	Specimen	Country	Location	Collection date	Species type	Genome sequenced
AQ	553608	Australia	Queensland	22-Feb-2002		
AQ	553622	Australia	Queensland	22-Feb-2002		
AQ	645840	Australia	Queensland	16-Mar-2001		X
AQ	645843	Australia	Queensland	20-Dec-1993		
AQ	795752	Australia	Queensland	28-Feb-2003		X
AQ	799166	Australia	Queensland	14-Jan-2001		X
AQ	799332	Australia	Queensland	30-Mar-2007		
AQ	799334	Australia	Queensland	29-Mar-2007		
C-F	92630	Ethiopia		09-Dec-1998		X
C-F	101400	Mexico	Sonora	05-Jun-1957		X
C-F	101401	Kenya	Tsavo National Park	02-Jan-1976		X
LINN	1287.7	India		1771	<i>Podaxis carcinomalis</i>	X
LINN	1287.8	South Africa	Cape of Good Hope	1781	<i>Podaxis pistillaris</i>	X
PREM	44075	South Africa	Johannesburg	Dec-1942		
PREM	44294	Namibia	Namib Desert Park	28-Apr-1969		
PREM	47477	South Africa	Northern Cape	19-Apr-1984		
PREM	47484	Namibia	Otjozondjupa	18-Apr-1984		
PREM	54389	South Africa	Nylsvlei Nature Reserve	Dec-1995		
PREM	57485	South Africa	Western Cape	03-Dec-2002		X
PREM	58760	South Africa	North-West Province	Apr-2004		
PREM	60320	South Africa	Free State Province	Dec-2004		
PREM	N833	South Africa	Gauteng	16-Mar-1931		
PRUM	3655	South Africa				
PRUM	3913	South Africa	Mpumalanga	29-Mar-1994		
PRUM	4335	South Africa	Gemsbok National Park	Apr-1997		
	MADm22	Madagascar		2012		X

Table S2. Sequencing, assembly and annotation statistics for the 10 *Podaxis* genomes. Related to Figure 1C-D.

Herbarium code	Strain	Lifestyle	Sequencing type	Total length (Mbp)	N50 (Kbp)	L50	N75 (Kbp)	L75
AQ 799166	Machattie	Free-Living	DNBSeq	38.60	66.0	140	19.5	399
MADm22	Madagascar	Facultative	BGISeq	33.28	61.7	145	27.3	346
LINN 1287.7	India	Facultative	BGISeq	35.48	103.2	100	55.1	220
C-F-92630	Ethiopia	Free-Living	PacBio, BGISeq	38.55	74.8	134	32.7	333
C-F-101400	Mexico	Free-Living	DNBSeq	37.63	106.6	95	54.0	220
C-F-101401	Kenya	Facultative	PacBio, BGISeq	37.94	65.4	151	28.5	367
AQ 645840	Kuranda	Facultative	DNBSeq	37.61	135.8	70	68.5	167
LINN 1287.8	LINN Cape	Specialist	BGISeq	31.56	139.2	67	68.4	146
PREM 57485	PREM Cape	Specialist	PacBio, BGISeq	32.64	129.8	74	58.2	166
AQ 795752	Moombra	Specialist	DNBSeq	31.66	70.7	112	29.6	276

Herbarium code	Strain	Estimated heterozygosity	Estimated Sequencing error rate	GC%	BUSCO genome completeness	Annotated Genes	CDS length per gene (Kbp)	Annotated introns per gene	Intron length per gene (bp)
AQ 799166	Machattie	0.014	0.006	48.4	91.60%	10332	1.439	5.19	70.55
MADm22	Madagascar	0.012	0.004	49.0	97.80%	9117	1.539	5.78	69.59
LINN 1287.7	India	0.001	0.001	48.8	97.96%	9517	1.543	5.71	70.13
C-F-92630	Ethiopia	0.007	0.003	48.3	98.09%	10184	1.501	5.52	70.25
C-F-101400	Mexico	0.002	0.002	48.5	94.88%	10231	1.486	5.27	70.74
C-F-101401	Kenya	0.011	0.003	48.6	96.95%	10275	1.495	5.41	70.66
AQ 645840	Kuranda	0.002	0.002	48.4	90.31%	9980	1.503	5.28	71.66
LINN 1287.8	LINN Cape	0.001	0.001	48.8	97.11%	8295	1.560	5.83	69.65
PREM 57485	PREM Cape	0.001	0.001	48.2	98.55%	8503	1.549	5.69	70.03
AQ 795752	Moombra	0.006	0.005	48.2	94.88%	8105	1.501	5.57	69.89

Table S3. Mitochondrial assembly and annotation statistics for the 10 *Podaxis* genomes. Related to Figure 1E.

Herbarium code	Strain	Mitochondrial genome length (Kbp)	Circular assembly	Genes annotated	tRNAs annotated
AQ 799166	Machattie	72.60	Y	12	22
MADm22	MADm22	73.80	Y	12	22
LINN 1287.7	India	76.23	Y	12	22
C-F-92630	Ethiopia	76.40	N	12	22
C-F-101400	Mexico	78.45	N	12	22
C-F-101401	Kenya	78.86	N	12	22
AQ 645840	Kuranda	80.42	Y	12	22
LINN 1287.8	LINN Cape	87.12	N	14	22
PREM 57485	PREM Cape	99.71	Y	11	22
AQ 795752	Moombra	100.06	N	4	16

Table S8. Spore brightness measurements for PREM *Podaxis* specimens from Conlon *et al.* (Conlon *et al.*, 2016) using ImageJ (Schneider *et al.*, 2012). ITS clades were assigned based on Conlon *et al.* (Conlon *et al.*, 2016). Only specimens for which a clade and life history could be assigned were included in Fig. S2 and statistical analyses. Related to Figure 1A and Figure S2.

Sample	ITS Clade	Life history	Brightness									
			1	2	3	4	5	6	7	8	9	10
1689	A	Specialist	35	28	35	34	32	34	28	34	29	32
7362	B	Specialist	21	24	27	30	24	32	25	30	27	26
23672	B	Specialist	36	54	51	58	48	46	46	55	33	56
44075	B	Specialist	32	18	24	24	29	32	16	26	32	27
44664	B	Specialist	16	16	13	14	18	19	14	15	16	12
49048	B	Specialist	22	15	20	19	29	31	23	32	22	27
20585	C	Free-living	25	27	28	37	32	39	30	42	28	33
44293	C	Free-living	27	24	31	24	26	24	25	16	25	28
44294	C	Free-living	52	20	22	29	24	35	24	31	23	27
9789	D	Facultative	16	17	21	16	19	21	17	17	21	24
54405	D	Facultative	15	15	18	16	24	17	16	28	14	15
41625	D	Facultative	26	43	31	35	36	20	21	19	18	22
14507	F	Facultative	20	21	18	19	21	19	22	16	26	15
28254	F	Facultative	23	24	23	17	21	20	23	24	25	20
28641	F	Facultative	19	22	16	21	23	26	28	21	23	29
2119			39	41	33	28	30	31	32	55	31	37
14484			40	23	21	21	18	18	25	24	20	22
14682			15	17	17	18	17	28	21	15	14	21
18109			14	16	12	16	17	16	21	17	19	20
29955			22	25	24	26	25	32	25	28	25	20
30714			24	21	35	26	18	23	22	23	26	20
36117			15	14	15	21	22	22	22	15	27	14
44240			18	17	14	20	20	17	19	23	24	24
54389			11	11	15	17	17	13	14	13	20	15
58760			12	13	10	11	11	11	18	14	17	11