

Supplementary Material

Investigating the genetic diversity, population differentiation and population dynamics of *Cycas segmentifida* (Cycadaceae) endemic to Southwest China by multiple molecular markers

Xiuyan Feng^{1,2}, Jian Liu^{1,2}, Yu-Chung Chiang^{3*}, and Xun Gong^{1*}

* Correspondence: Corresponding Authors: Xun Gong: gongxun@mail.kib.ac.cn;

Yu-Chung Chiang : yuchung@mail.nsysu.edu.tw.

1 Supplementary Figures and Tables

1.1 Supplementary Figures

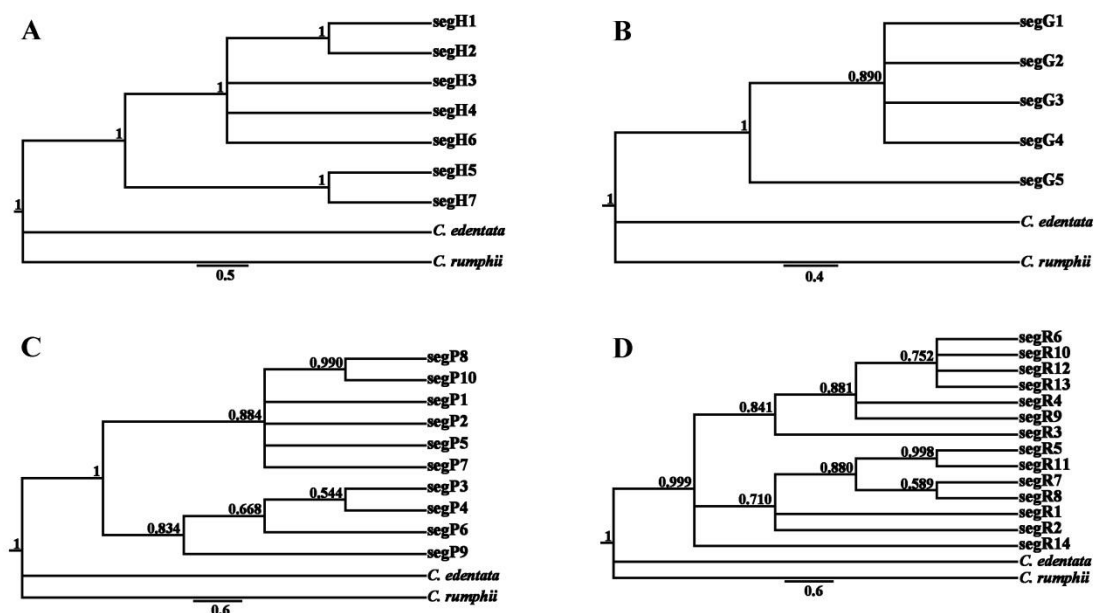


Figure S1. Bayesian tree based on the haplotypes of cpDNA (A), *GTP* (B), *PHYP* (C) and *PPRC* (D). *Cycas edentata* and *Cycas rumphii* were used as outgroups. The number on each branch indicates the posterior probability (PP).

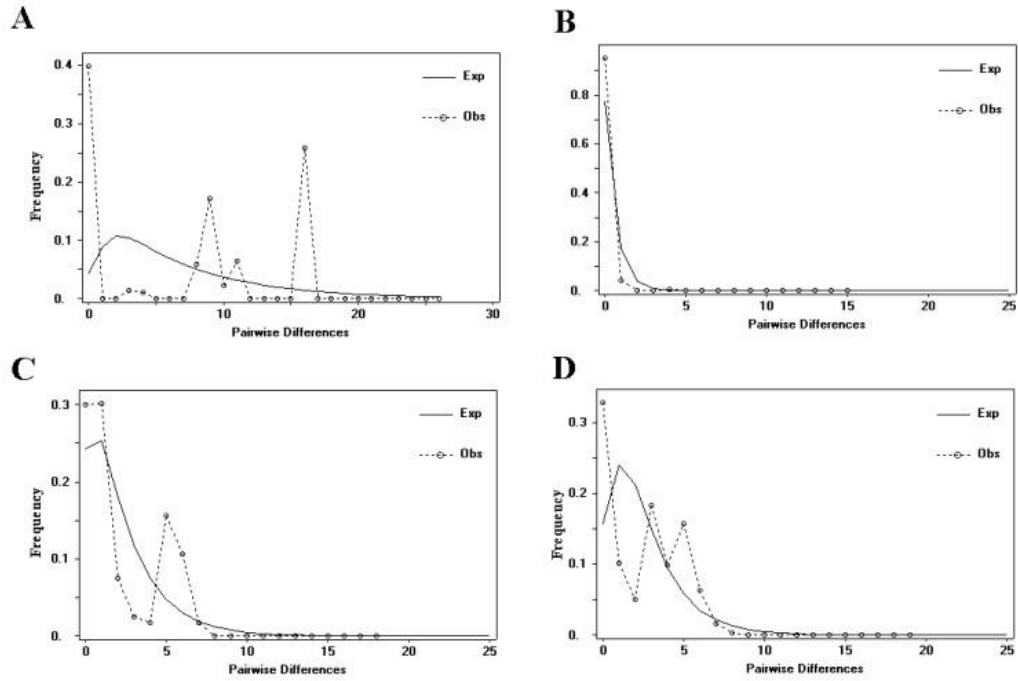


Figure S2. Mismatch distribution of cpDNA (A) and the nuclear genes *GTP* (B), *PHYP* (C) and *PPRC* (D) haplotypes based on pairwise sequence differences against the frequency of occurrence for *C. segmentifida*.

1.2 Supplementary Tables

Table S1. Details of sample locations, sample sizes surveyed for DNA sequences and microsatellites of 14 populations of *C. segmentifida*.

Population Code	Population/habitat (sand or karst)	Latitude N°	Longitude E°	Altitude m	Individuals for DNA sequences/microsatellites
BY	Bianya, Longlin, Guangxi; sand	24.754	105.468	560	10/20
JZ	Jiuzhou, Tianlin; Guangxi; sand	24.657	105.780	490	10/10
LK	Lekuan, Wangmo, Guizhou; sand	25.304	106.363	650	10/20
LKA	Lekang, Wangmo, Guizhou; sand	25.064	106.226	420	10/20
BM	Bamei, Guangnan, Yunnan; sand	24.418	104.897	960	10/20
NZ	Nazuo, Xilin, Guangxi; sand	24.148	105.393	700	10/20
BD	Badu, Tianlin, Guangxi; sand	24.327	105.827	300	10/15
BA	Boai, Funing, Yunnan; sand	23.936	106.090	300	10/20
YX	Yangxu, Baise, Guangxi; sand	23.982	106.485	350	7/7
LLB	Luolou, Lingyun, Guangxi; karst	24.367	106.810	760	10/12
SL	Shali; Lingyun, Guangxi; karst	24.241	106.811	490	10/14
PH	Pohong, Tianyang, Guangxi; karst	23.652	106.736	570	10/20
PHG	Gumei, Pohong, Tianyang, Guangxi; karst	23.605	106.643	660	10/20
BB	Bubing, Tiandong, Guangxi; karst	23.586	107.072	150	10/20
Total	14				137/238

Table S2. Haplotype diversity (*Hd*) and nucleotide diversity (*Pi*) estimated from cpDNA and nuclear genes as well as recombination in nuclear genes and the composition of haplotypes in *C. segmentifida*.

Population code	cpDNA (10)			GTP (20/0)			PHYP (20/1)			PPRC (20/2)		
	Haplotypes (No.)	<i>Hd</i>	<i>Pi</i> x 10 ³	Haplotypes(No.) / Recombination(No.)	<i>Hd</i>	<i>Pi</i> x 10 ³	Haplotypes(No.) / Recombination(No.)	<i>Hd</i>	<i>Pi</i> x 10 ³	Haplotypes(No.) / Recombination(No.)	<i>Hd</i>	<i>Pi</i> x 10 ³
BY	segH1(10)	0	0	segG1(20)	0	0	segP1(9) segP2(11)	0.521	0.56	segR1(15) segR2(5)	0.395	0.55
JZ	segH1(10)	0	0	segG1(20)	0	0	segP1(7) segP2(10) segP3(3)	0.637	2.30	segR2(19) segR3(1)	0.100	0.84
LK	segH1(10)	0	0	segG1(20)	0	0	segP1(15) segP4(5)	0.395	2.12	segR2(20)	0	0
LKA	segH1(10)	0	0	segG1(20)	0	0	segP1(4) segP4(16)	0.337	1.81	segR2(20)	0	0
BM	segH1(10)	0	0	segG1(20)	0	0	segP1(5) segP2(15)	0.395	0.42	segR2(20)	0	0
NZ	segH2(10)	0	0	segG1(20)	0	0	segP1(2) segP2(11) segP5(7)	0.595	1.08	segR1(4) segR2(15) segR5(1)	0.416	1.17
BD	segH1(10)	0	0	segG1(20)	0	0	segP1(12) segP2(3) segP4(3) segP5(2)	0.616	1.94	segR2(20)	0	0
BA	segH1(10)	0	0	segG1(20)	0	0	segP1(15) segP5(5)	0.395	0.42	segR2(7) segR4(13)	0.479	2.00
YX	segH3(7)	0	0	segG1(13) segG2(1)	0.143	0.25	segP1(9) segP2(3) segP6(2)	0.560	1.52	segR2(4) segR4(4) segR5(2) segR6(3) segR7(1)	0.824	4.44
LLB	segH4(10)	0	0	segG1(20)	0	0	segP1(13) segP2(1) segP4(2) segP5(4)	0.553	1.49	segR4(1) segR5(11) segR7(8)	0.563	2.71
SL	segH5(10)	0	0	segG1(20)	0	0	segP1(13) segP5(1) segP7(6)	0.511	1.06	segR4(10) segR5(9) segR6(1)	0.574	4.49
PH	segH6(10)	0	0	segG1(19) segG3(1)	0.100	0.18	segP1(8) segP2(3) segP4(6) segP7(2) segP8(1)	0.753	3.18	segR2(6) segR4(3) segR5(4) segR6(5) segR7(1) segR8(1)	0.821	4.97
PHG	segH5(10)	0	0	segG1(19) segG2(1)	0.100	0.18	segP1(11) segP2(2) segP4(2) segP5(2) segP9(3)	0.679	2.31	segR2(5) segR4(3) segR5(2) segR6(3) segR7(1) segR9-segR14(1)	0.911	4.79
BB	segH5(6) segH7(4)	0.533	1.7	segG1(16) segG3(1) segG4(2) segG5(1)	0.363	1.23	segP1(7) segP4(8) segP8(4) segP10(1)	0.711	3.25	segR2(5) segR4(8) segR5(1) segR6(5) segR12(1)	0.747	3.09
Total	7	0.602	2.29	5	0.05	0.13	10	0.700	2.31	14	0.673	3.42

Table S3. Genetic diversity, differentiation parameters based on the combined cpDNA sequences and nuclear genes in *C. segmentifida*.

Marker	H_s	H_T	G_{ST}	N_{ST}
cpDNA	0.038	0.745	0.949	0.996
<i>GTP</i>	0.050	0.053	0.040	0.008
<i>PHYP</i>	0.547	0.709	0.229	0.282
<i>PPRC</i>	0.416	0.696	0.402	0.415

Table S4. Parameters of neutrality tests and mismatch analysis based on cpDNA and nuclear genes of *C. segmentifida*.

Marker	Tajima' D	Fu and Li' D^*	Fu and Li' F^*	Fu' F_s	SSD	raggedness
cpDNA	2.45969 [*]	1.77508 ^{**}	2.44516 ^{**}	17.413 ^{***}	0.00217 [*]	0.02063 [*]
<i>GTP</i>	-1.91209 [*]	-2.81556 [*]	-2.98755 ^{**}	-6.974 ^{***}	0.00088	0.14636
<i>PHYP</i>	0.24935	0.68737	0.62868	0.763	0.09708	0.26289
<i>PPRC</i>	-0.12793	-1.47618	-1.14551	-0.791	0.05944	0.23616

Note: ^{*}, $P < 0.05$, significant difference; ^{**}, $P < 0.01$, most significant difference; ^{***}, $P < 0.001$, most significant difference.

Table S6. *P*-value of Hardy-Weinberg equilibrium test for 14 populations of *C. segmentifida*.

Population	Cha02	Cha08	Cy-TaiEST-SSR11	E001	E004	Cpz26	HL08	CY232	Cha-estssr01	Cha-estssr02	Cha-estssr04	Cha05	All loci
BY	0.004 ^{**}	0.006 ^{**}	-	1.000 ^{ns}	0.324 ^{ns}	0.006 ^{**}	-	1.000 ^{ns}	0.000 ^{***}	0.049 [*]	0.021 [*]	-	0.000 ^{***}
JZ	0.019 [*]	0.000 ^{***}	-	1.000 ^{ns}	0.152 ^{ns}	0.184 ^{ns}	-	1.000 ^{ns}	0.484 ^{ns}	0.101 ^{ns}	0.462 ^{ns}	1.000 ^{ns}	0.005 ^{**}
LK	0.054 ^{ns}	-	-	1.000 ^{ns}	0.036 [*]	0.707 ^{ns}	-	-	0.000 ^{***}	0.001 ^{**}	1.000 ^{ns}	-	0.000 ^{***}
LKA	0.202 ^{ns}	1.000 ^{ns}	-	0.591 ^{ns}	0.069 [*]	0.468 ^{ns}	-	-	0.000 ^{***}	0.021 [*]	0.913 ^{ns}	0.026 [*]	0.000 ^{***}
BM	0.880 ^{ns}	1.000 ^{ns}	-	0.277 ^{ns}	0.112 ^{ns}	1.000 ^{ns}	-	-	0.043 [*]	1.000 ^{ns}	0.800 ^{ns}	1.000 ^{ns}	0.733 ^{ns}
NZ	0.001 ^{**}	0.000 ^{***}	-	1.000 ^{ns}	0.056 ^{ns}	-	1.000 ^{ns}	0.174 ^{ns}	0.028 [*]	0.002 ^{**}	0.017 [*]	1.000 ^{ns}	0.000 ^{***}
BD	0.265 ^{ns}	0.000 ^{***}	-	0.652 ^{ns}	0.151 ^{ns}	0.528 ^{ns}	1.000 ^{ns}	0.628 ^{ns}	0.017 [*]	0.775 ^{ns}	0.970 ^{ns}	-	0.000 ^{***}
BA	1.000 ^{ns}	0.000 ^{***}	-	-	0.522 ^{ns}	1.000 ^{ns}	0.350 ^{ns}	1.000 ^{ns}	0.000 ^{**}	0.257 ^{ns}	0.983 ^{ns}	-	0.000 ^{***}
YX	0.007 ^{**}	0.009 ^{**}	-	-	0.100 ^{ns}	1.000 ^{ns}	1.000 ^{ns}	0.441 ^{ns}	0.009 ^{**}	1.000 ^{ns}	0.359 ^{ns}	-	0.005 ^{**}
LLB	0.123 ^{ns}	0.976 ^{ns}	-	0.529 ^{ns}	1.000 ^{ns}	1.000 ^{ns}	1.000 ^{ns}	1.000 ^{ns}	0.027 [*]	1.000 ^{ns}	0.894 ^{ns}	-	0.880 ^{ns}
SL	1.000 ^{ns}	0.561 ^{ns}	-	-	0.138 ^{ns}	0.238 ^{ns}	1.000 ^{ns}	0.150 ^{ns}	0.000 ^{***}	0.348 ^{ns}	0.308 ^{ns}	-	0.000 ^{***}
PH	0.124 ^{ns}	0.000 ^{***}	1.000 ^{ns}	0.145 ^{ns}	0.705 ^{ns}	0.754 ^{ns}	0.613 ^{ns}	0.468 ^{ns}	0.000 ^{***}	1.000 ^{ns}	0.698 ^{ns}	-	0.000 ^{***}
PHG	0.040 [*]	0.009 ^{**}	1.000 ^{ns}	0.543 ^{ns}	0.176 ^{ns}	0.657 ^{ns}	0.728 ^{ns}	1.000 ^{ns}	0.000 ^{***}	1.000 ^{ns}	0.239 ^{ns}	-	0.000 ^{***}
BB	0.763 ^{ns}	0.903 ^{ns}	1.000 ^{ns}	0.242 ^{ns}	0.241 ^{ns}	1.000 ^{ns}	0.732 ^{ns}	1.000 ^{ns}	0.000 ^{***}	0.480 ^{ns}	0.996 ^{ns}	-	0.000 ^{***}
All pop.	0.000 ^{***}	0.000 ^{***}	1.000 ^{ns}	0.913 ^{ns}	0.009 ^{**}	0.718 ^{ns}	0.972 ^{ns}	0.969 ^{ns}	0.000 ^{***}	0.005 ^{**}	0.551 ^{ns}	0.502 ^{ns}	0.000 ^{***}

Note: -, Monomorphic; ns, non-significance; *, $P < 0.05$, significant difference; **, $P < 0.01$, most significant difference; ***, $P < 0.001$, most significant difference.