

Supplementary Materials: Hybridization in the Fringed Orchids: An Analysis of Species Boundaries in The Face of Gene Flow

Simone Evans ^{1,2,3} , Dennis Whigham ¹ , Ida Hartvig ^{1,4,*} , and Melissa McCormick ^{1,*} 

1. Supplementary Figures

1

	# flowers measured (# pops)	Lip width (LW)	Lip length (LL)	Lip with fringe (LWF)	Longest fringe (LF)	Spur length (SL)	Flower width (FW)	Inflorescence width (IW)
<i>P. ciliaris</i>	23(4)	11.5±2.8	14.0±1.9	12.3±1.9	7.0±1.7	25.5±3.5	13.0±1.8	58.0±11.0
<i>P. x bicolor</i>	12(2)	8.3±1.8	12.1±1.9	10.2±1.8	5.4±1.5	26.6±4.1	11.4±1.4	57.3±10.0
<i>P. blephariglottis</i>	40(6)	5.8±1.4	10.8±1.9	8.9±2.0	3.2±1.3	24.8±4.0	12.8±1.7	50.0±8.0
<i>P. x canbyi</i>	10(2)	7.1±2.2	7.1±1.5	6.7±0.8	4.0±0.9	10.2±4.4	9.4±1.8	31.6±3.1
<i>P. cristata</i>	26(4)	6.2±2.0	6.5±1.5	6.4±1.5	3.9±1.3	6.4±1.1	7.4±1.3	25.6±3.6

Figure S1. PCA of floral morphology for genetically-identified Average ± Standard deviation for each species and hybrid. We measured two flower for every orchid, so we measured 222 flowers from 111 orchid samples.

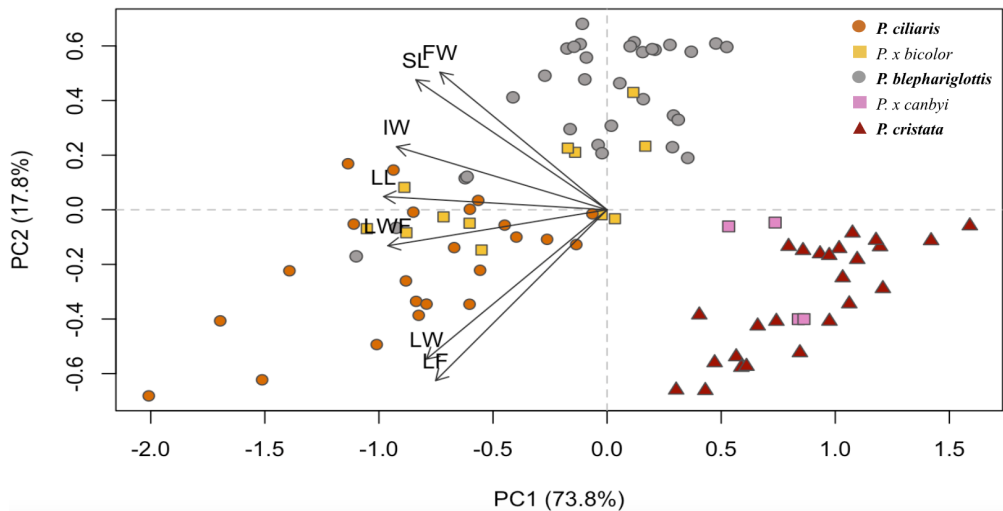


Figure S2. Principal Component Analysis (PCA) reduces the dimensionality of flower morphology measurements, including flower width (FW), spur length (SL), inflorescence width (IW), lip length (LL), length of the lip with fringes (LWF), lip width (LW), longest fringe (LF). Arrows represent the correlation of variables with the two main dimensions identified.

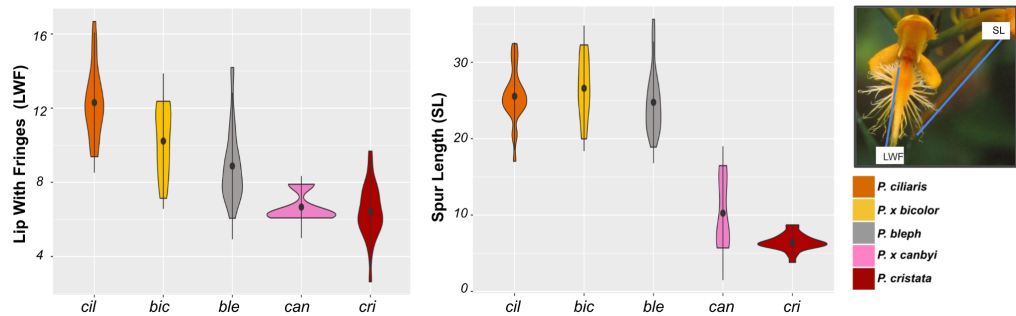


Figure S3. Violin plot of floral morphology. Variation in two representative morphological traits. Dots reflect the mean value and the vertical bars display ±1SD.

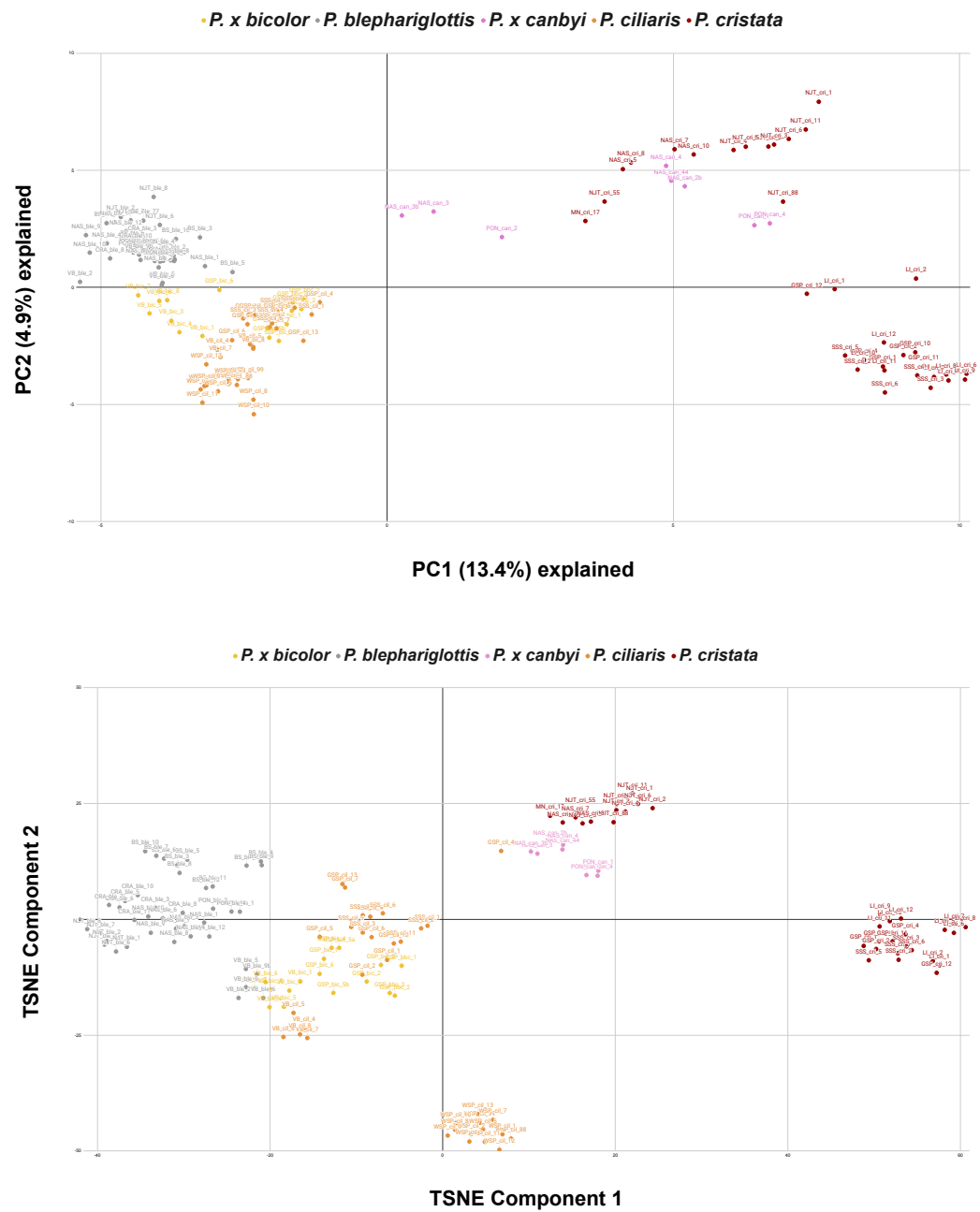


Figure S4. Labeled PCA and tSNE plots of SNP datasets. Sample identifier contains a site acronym, species acronym (cil = *P. ciliaris*, bic & bbc = *P. x bicolor*, ble = *P. blephariglottis*, can = *P. x canbyi*, cri = *P. cristata*), and a sample number.

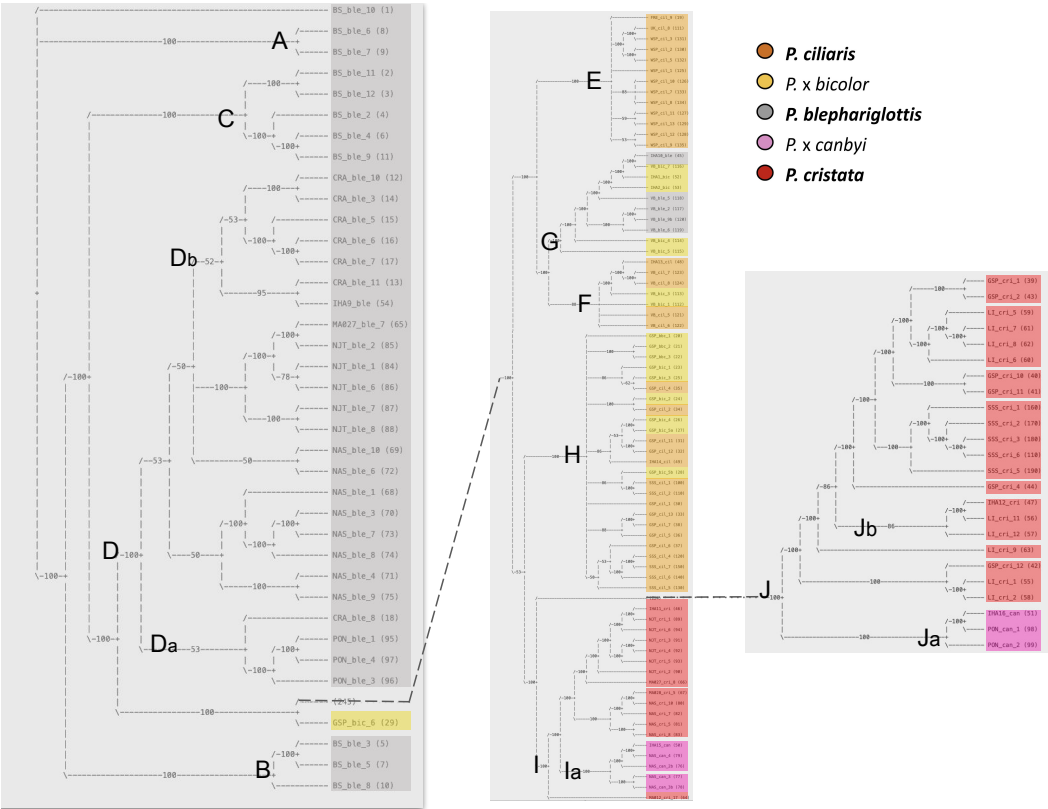
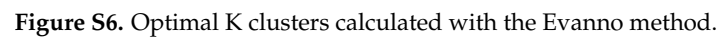


Figure S5. Bayesian posterior probabilities provide robust support for the branching patterns of the phylogenies. Clades containing parent species from sites with a single species (A,B,C,E,Jb) have a posterior probability of 100, aside from Da(52) and Jb(86).



Acronym	County	State	Site Details	<i>P. bleph</i>	<i>P. ciliaris</i>	<i>P. x bicolor</i>	<i>P. cristata</i>	<i>P. x canbyi</i>
NAS	Worcester	MD	Sympatric <i>P. cristata</i> and <i>P. blephariglottis</i> .	Morphology (n = 8) Genomic (n = 10)	NA	NA	Morphology (n = NA) Genomic (n = 6)	Morphology (n = 6) Genomic (n = 5)
NJT	Worcester	MD	Sympatric <i>P. cristata</i> and <i>P. blephariglottis</i> .	Morphology (n = 6) Genomic (n = 5)	NA	NA	Morphology (n = 8) Genomic (n = 7)	NA
VB	Hazleton	PA	Sympatric <i>P. ciliaris</i> and <i>P. blephariglottis</i> .	Morphology (n = 8) Genomic (n = 5)	Morphology (n = 6) Genomic (n = 5)	Morphology (n = 6) Genomic (n = 7)	NA	NA
GSP	Brunswick	NC	Sympatric <i>P. ciliaris</i> and <i>P. cristata</i>	NA	Morphology (n = 3) Genomic (n = 9)	Morphology (n = 6) Genomic (n = 10)	Morphology (n = NA) Genomic (n = 6)	NA
SSS	Brunswick	NC	Sympatric <i>P. ciliaris</i> and <i>P. cristata</i>	NA	Morphology (n = 6) Genomic (n = 7)	NA	Morphology (n = 6) Genomic (n = 5)	NA
WSP	Frederick	MD	Allopatric <i>P. ciliaris</i>	NA	Morphology (n = 8) Genomic (n = 13)	NA	NA	NA
LI	Brunswick	NC	Allopatric <i>P. cristata</i>	NA	NA	NA	Morphology (n = 6) Genomic (n = 10)	NA
BS	Columbus	NC	Allopatric <i>P. blephariglottis</i>	Morphology (n = 6) Genomic (n = 11)	NA	NA	NA	NA
PON	Chatsworth	NJ	Sympatric <i>P. blephariglottis</i> and <i>P. cristata</i>	Morphology (n = 4) Genomic (n = 3)	NA	NA	Morphology (n = 6) Genomic (n = NA)	Morphology (n = 4) Genomic (n = 3)
CRA	Chatsworth	NJ	Allopatric <i>P. blephariglottis</i>	Morphology (n = 8) Genomic (n = 7)	NA	NA	NA	NA

Figure S8. Summary of orchids sampled at each site for morphological and genomic analyses.