

Supplementary Data S2 – Final alignment of the *trnK-matK* region of the investigated species of *Pinguicula* and GenBank records. ANZ – *P. vulgaris* subsp. *anzalonei*, BIC – *P. vulgaris* var. *bicolor*, BOH – *P. vulgaris* subsp. *bohemica*, DOS – *P. vulgaris* nothosubsp. *dostalii*, ERN – *P. vulgaris* subsp. *ernica*, POL – *P. "polonica"*, VES – *P. vulgaris* subsp. *vestina*, VUL – *P. vulgaris* subsp. *vulgaris*. For population abbreviation see Table 2. GenBank records LC348435 and AF531806 refer to Shimai et al. (2021); - – refers to insertion/deletion, or in the case of GenBank records LC348435 and AF531806 to missing characters. Suggested incongruencies are highlighted in “yellow”.

Shimai H., Setoguchi H., Roberts D. L. & Sun M. (2021) Biogeographical patterns and speciation of the genus *Pinguicula* (Lentibulariaceae) inferred by phylogenetic analyses. – PLoS ONE 16: e0252581.

Based on the alignment of sequences generated for locus *trnK-matK* within our study and those from an outstanding biogeographical study of the whole genus *Pinguicula* by Shimai et al. (2021), we observed several discrepancies (highlighted in yellow in alignment below). We did not observe the four single nucleotide polymorphic sites (SNPs; positions: 57, 95, 138, 456) present in LC348435 (*P. bohemica*) which differentiate this taxon from *P. vulgaris* (AF531806) within our dataset. We sequenced six samples of *P. vulgaris* subsp. *bohemica* from natural sites, thus we are convinced our observations are more realistic. The observed discrepancies may stem from sequence processing, e.g., low-quality parts, hardly readable parts with overlapping peaks.

	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400					
LC348435	TGGAA	GAAA	AT	GC	A	G	T	C	G	T	G	A	T	A	T	A	T	A	T	A	G	T			
Pinguicula bohemica	A	F	5	1	8	0	6	4	3	2	1	0	5	4	3	2	1	0	5	4	3	2	1	G	T
AFF51806	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
ANZ_1	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
ANZ_2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VES_1	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VES_2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_C2_h	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_C2_b	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_C2_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_SK_1_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_SK_1_b	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_SK_2_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_SK_2_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_SK_3_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BIC_SK_3_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
DOS_1	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
DOS_2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
POL_1	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
POL_3	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
POL_5	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
ERN_2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
ERN_692-4	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BOH_SH_i	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BOH_BR_e	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BOH_BR_b	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BOH_BR_e	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BOH_SH_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
BOH_SH_d	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_NOR_2_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_NOR_2_d	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SK_I253_WP	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_IR_1338-2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_DL_1696-2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_CH_667-3	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_UK_686-9	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_FR_706-1	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_FR_709-8	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_FR_713-2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_CZ_1_b	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_CZ_1_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_CZ_2_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_CZ_2_b	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_NOR_1_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_NOR_1_d	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SK_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SK_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SWE_1_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SWE_1_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SWE_2_a	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SWE_2_d	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_SWE_3_c	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_PL_ZAK-2	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T
VUL_PL_ZAK-6	T	G	G	A	C	A	G	T	G	G	C	G	G	C	T	A	T	T	C	G	A	T	A	G	T

