

Supplementary Data S1 – Final alignment of the ITS 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 JN999374 – JN999377 refer to Kuzmina et al. (2012); DQ438093, DQ438086, DQ222949, DQ441597, DQ222947 – refer to Degtjareva et al. (2006); AB198361, AB198343, AB198349 – refer to Kondo & Shimai (2006); LN887946, LN887945, LN887944, LN887943, LN887942 – De Castro et al. (2016). R, K, Y – refer to IUPAC ambiguity codes; - – refers to insertion/deletion, or in the case of GenBank records JN999374 – JN999377 to missing characters. Suggested incongruences are highlighted in “yellow” and heterozygous position “448” in “turquoise”.

Degtjareva G., Casper J., Hellwig F. & Sokoloff D. (2004): Seed morphology in the genus *Pinguicula* (Lentibulariaceae) and its relation to taxonomy and phylogeny. – *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 125: 431–452.

Kondo K. & Shimai H. (2006): Phylogenetic analysis of the Northern *Pinguicula* (Lentibulariaceae) based on Internal Transcribed Spacer (ITS) sequence. – *Acta Phytotaxonomica et Geobotanica* 57: 155–164.

Kuzmina M. L., Johnson K. L., Barron H. R. & Hebert P. D. N. (2012): Identification of the vascular plants of Churchill, Manitoba, using a DNA barcode library. – *BMC Ecology* 12: 25.

De Castro O., Innangi M., Di Maio A., Menale B., Bacchetta G., Pires M., Noble V., Gestri G., Conti F. & Peruzzi L. (2016): Disentangling Phylogenetic Relationships in a Hotspot of Diversity: The Butterworts (*Pinguicula* L., Lentibulariaceae) Endemic to Italy. – *PLoS One* 28: 11: e0167610.

During comparison of the *ITS* records from GeneBank database, we discovered several discrepancies. The compared *ITS* sequences are from two phylogenetic studies of the genus *Pinguicula*: Degtjareva et al. (2006), Kondo & Shimai (2006), and one DNA barcoding study (Kuzmina et al. 2012). We are convinced that some nucleotide positions in sequences with accession numbers DQ441597–*P. vulgaris* subsp. *bohemica*, DQ438086–*P. vulgaris* are not correct (see the alignment below). The observed discrepancies, most probably, are stemming from low quality of sequencing reactions, lower quality of nucleotide calls at the end of reads and/or not correct/absent sequence editing. We believe that our sequences for all the sequenced individuals are more realistic, because of following facts. 1) We found no variability in the *ITS* sequences for the six sequenced *P. vulgaris* subsp. *bohemica* individuals. Moreover, three individuals originated from the same population “Baronský rybník – BR” as samples used by Degtjareva et al. (2006)–DQ441597; and Kondo & Shimai (2006)–AB198343. 2) We detected only two *ITS*-ribotypes in all the sequenced *P. vulgaris* subsp. *vulgaris* and *P. vulgaris* var. *bicolor* (Table 6). Even samples of *P. vulgaris* from Canada (Kuzmina et al. 2012) seem to possess the same two ribotypes, detected within the sequenced samples (although only partial sequences are available). 3) We sequenced more than one individual per taxon, and thus we were able to compare the pattern of ambiguous sites in our high-quality electrophoretograms in between reads from both sides and among all the generated sequences at once. Thus, we were able to finely align and resolve problematic sites within sequences.

[illegible]

[illegible]

[illegible]

VUL_SK_1253_WF
 VUL_DB_1696-2 GGACCCAAACGGCGTGCG
 VUL_CH_667-3 GGACCCAAACGGCGTGCG
 VUL_UK_686-9 GGACCCAAACGGCGTGCG
 VUL_FR_706-1 GGACCCAAACGGCGTGCG
 VUL_FR_713-2 GGACCCAAACGGCGTGCG
 VUL_CZ_1_b GGACCCAAACGGCGTGCG
 VUL_CZ_1_c GGACCCAAACGGCGTGCG
 VUL_CZ_2_a GGACCCAAACGGCGTGCG
 VUL_CZ_2_b GGACCCAAACGGCGTGCG
 VUL_NOR_1_a GGACCCAAACGGCGTGCG
 VUL_NOR_1_d GGACCCAAACGGCGTGCG
 VUL_NOR_2_a GGACCCAAACGGCGTGCG
 VUL_NOR_2_d GGACCCAAACGGCGTGCG
 VUL_IR_1338_2 GGACCCAAACGGCGTGCG
 VUL_FR_709_8 GGACCCAAACGGCGTGCG
 VUL_SK_a GGACCCAAACGGCGTGCG
 VUL_SK_c GGACCCAAACGGCGTGCG
 VUL_SWE_1_a GGACCCAAACGGCGTGCG
 VUL_SWE_1_c GGACCCAAACGGCGTGCG
 VUL_SWE_2_a GGACCCAAACGGCGTGCG
 VUL_SWE_2_d GGACCCAAACGGCGTGCG
 VUL_SWE_3_c GGACCCAAACGGCGTGCG
 VUL_SWE_3_d GGACCCAAACGGCGTGCG
 VUL_PL_ZAK_2 GGACCCAAACGGCGTGCG
 VUL_PL_ZAK_6 GGACCCAAACGGCGTGCG
 VUL_AB198361 GGACCCAAACGGCGTGCG
 VUL_LN887945 GGACCCAAACGGCGTGCG
 VUL_LN887946 GGACCCAAACGGCGTGCG
 VUL_JN999375 GGACCCAAACGGCGTGCG
 VUL_JN999376 GGACCCAAACGGCGTGCG
 VUL_JN999374 GGACCCAAACGGCGTGCG
 VUL_JN999377 GGACCCAAACGGCGTGCG
 VUL_DQ222949 GGACCCAAACGGCGTGCG
 VUL_DQ438093 GGACCCAAACGGCGTGCG
 VUL_DQ438086 GGACCCAAACGGCGTGCG
 BIC_CZ_b GGACCCAAACGGCGTGCG
 BIC_CZ_c GGACCCAAACGGCGTGCG
 BIC_CZ_h GGACCCAAACGGCGTGCG
 BIC_SK_1_a GGACCCAAACGGCGTGCG
 BIC_SK_1_b GGACCCAAACGGCGTGCG
 BIC_SK_2_a GGACCCAAACGGCGTGCG
 BIC_SK_2_c GGACCCAAACGGCGTGCG
 BIC_SK_3_a GGACCCAAACGGCGTGCG
 BIC_SK_3_c GGACCCAAACGGCGTGCG
 DOS_1 GGACCCAAACGGCGTGCG
 POL_1 GGACCCAAACGGCGTGCG
 POL_3 GGACCCAAACGGCGTGCG
 POL_5 GGACCCAAACGGCGTGCG
 BOH_BR_a GGACCCAAACGGCGTGCG
 BOH_BR_b GGACCCAAACGGCGTGCG
 BOH_BR_e GGACCCAAACGGCGTGCG
 BOH_SH_a GGACCCAAACGGCGTGCG
 BOH_SH_d GGACCCAAACGGCGTGCG
 BOH_SH_i GGACCCAAACGGCGTGCG
 BOH_AB198343 GGACCCAAACGGCGTGCG
 BOH_DQ441597 GGACCCAAACGGCGTGCG
 ERN-LN887943 GGACCCAAACGGCGTGCG
 ERN_2 GGACCCAAACGGCGTGCG
 ERN_692-4 GGACCCAAACGGCGTGCG
 VES-LN887944 GGACCCAAACGGCGTGCG
 VES_1 GGACCCAAACGGCGTGCG
 VES_2 GGACCCAAACGGCGTGCG
 ANZ_LN887942 GGACCCAAACGGCGTGCG
 ANZ_1 GGACCCAAACGGCGTGCG
 ANZ_2 GGACCCAAACGGCGTGCG