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".

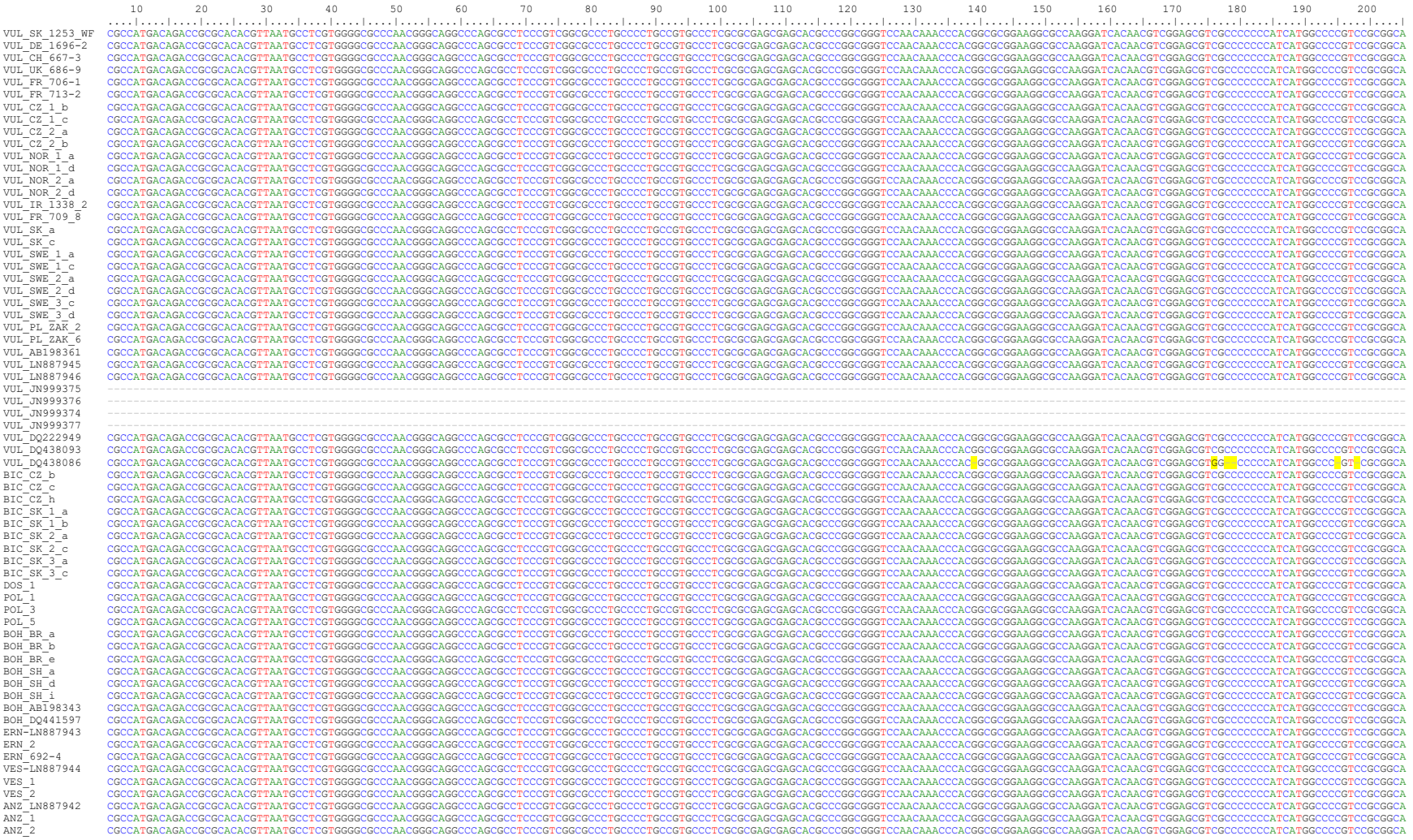
Degtjareva G., Casper J., Hellwig F. & Sokoloff D. (2004): Seed morphology in the genus *Pinguicula* (Lentibulariaceae) and its relation to taxonomy and phylogeny. – Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 125: 431–452.

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.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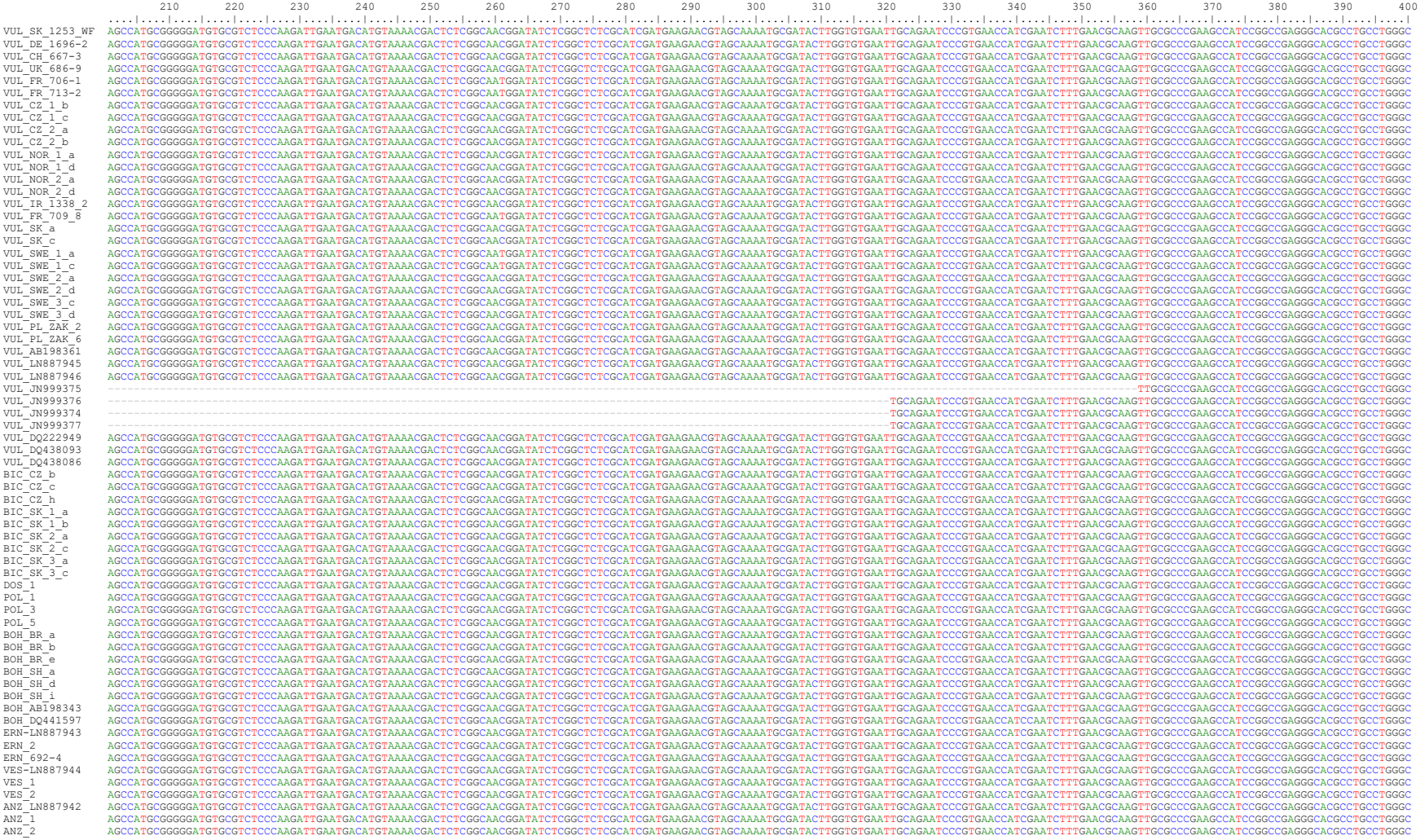
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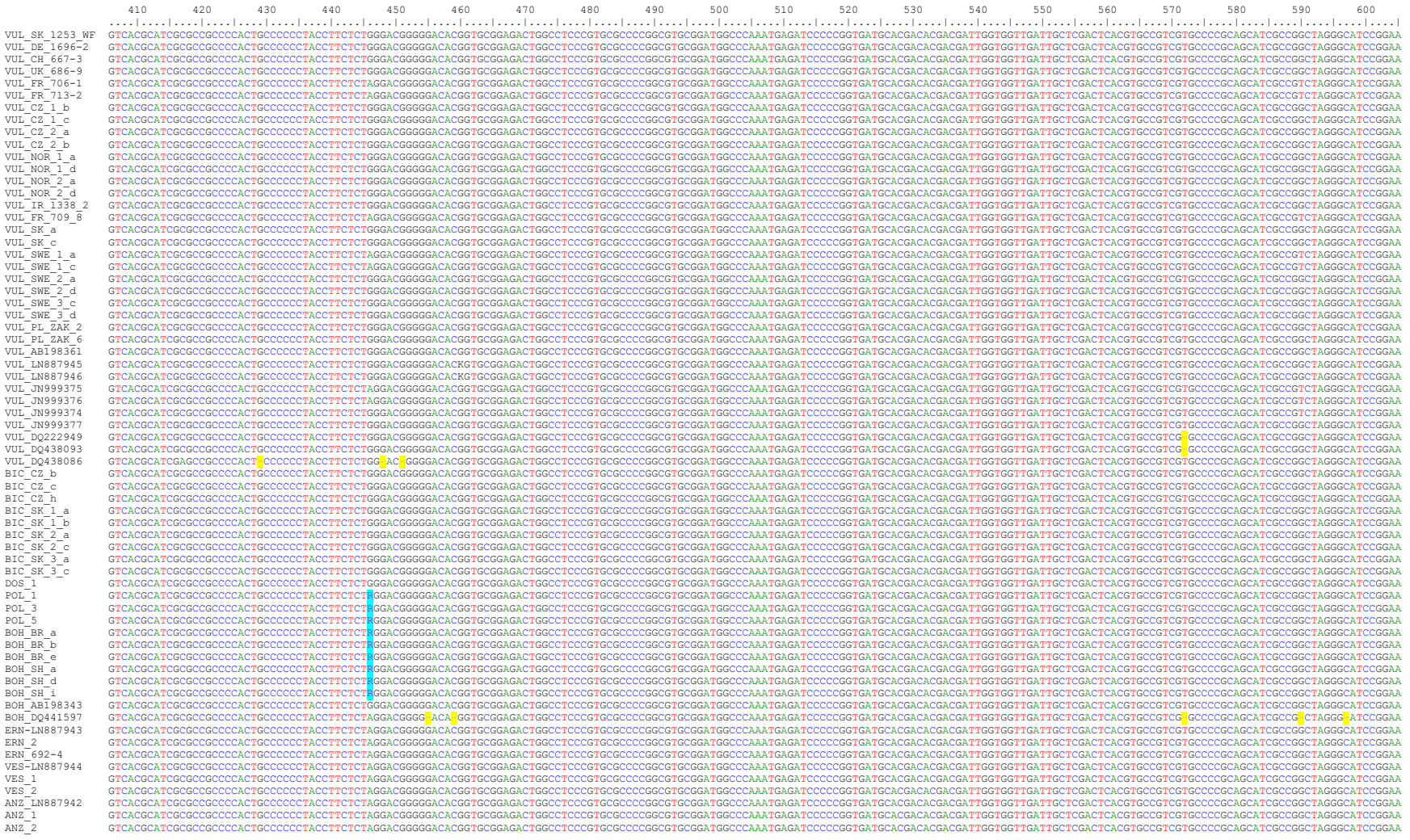
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