Appendix S2: Supplemental Methods *Targeted PCR Amplification*

"Universal" primers for plant systematics were used for amplification and sequencing of all gene regions (sequences and references can be found in Appendix S3). All gene regions were amplified using a two-round PCR strategy in overlapping ∼400-600 bp amplicons to merge across the 300 bp paired-end reads generated with Illumina MiSeq sequencing, so some gene regions (*atpB*, *matK*, *ndhF*, and *trnTLF*) were amplified in multiple segments. Following Uribe-Convers *et al.* (2016), each target-specific primer sequence contained a conserved sequence tag that was added to the 5' end at the time of oligonucleotide synthesis (CS1 for forward primers and CS2 for reverse primers). The purpose of the added CS1 and CS2 tails is to provide an annealing site for the second pair of primers. After an initial round of PCR using the CS-tagged, target specific primers (PCR1), a second round of PCR was used to add 8 bp sample-specific barcodes and high-throughput sequencing adapters to both the 5' and 3' ends of each PCR amplicon (PCR2). From 3' to 5', the PCR2 primers included the reverse complement of the conserved sequence tags, sample-specific 8 bp barcodes, and either Illumina P5 (CS1-tagged forward primers) or P7 (CS2-tagged reverse primers) sequencing adapters. Sequences for the CS1 and CS2 conserved sequence tags, barcodes, and sequencing adapters were taken from Uribe-Convers *et al.* (2016). Following PCR2, the resulting amplicons all dual-barcoded samples were pooled together and sequenced on an Illumina MiSeq platform using 300 bp paired end reads. PCR conditions were as follows: PCR1 – 25 µL reactions included 2.5 µL of 10x PCR buffer, 3 µL of 25 µM $MgCl_2$, 0.30 µL of 20 mg/ml BSA, 1 µL of 10 µM dNTP mix, 0.125 µL 10 µM CS1-tagged target specific forward primer, 0.125 µL 10 µM CS2-tagged target specific reverse primer, 0.125 µL of 5000 U/ml Taq DNA polymerase, 1 µL template DNA, and PCR-grade H_2O to volume; PCR1 cycling conditions - 95°C for 2 min. followed by 20 cycles of 95°C for 2 min., 50-60°C for 1 min. (depending

on T_m of target specific primers), 68°C for 1 min., followed by a final extension of 68°C for 10 min.; PCR2 – 20 µL reactions included 2 µL of 10x PCR buffer, 3.6 µL of 25 mM $MgCl_2$, 0.60 µL of 20 mg/ml BSA, 0.40 µL of 10 mM dNTP mix, 0.75 µL of 2 µM barcoded primer mix, 0.125 µL of 5000 U/ml Taq DNA polymerase, 1 µL of PCR1 product as template, and PCR-grade H_2O to volume; PCR2 cycling conditions - 95°C for 1 min. followed by 15 cycles of 95°C for 30 sec., 60°C for 30 sec., 68°C for 1 min., followed by a final extension of 68°C for 5 min.

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References

Uribe-Convers S., Settles M.L., and Tank D.C. 2016. A Phylogenomic Approach Based on PCR Target Enrichment and High Throughput Sequencing: Resolving the Diversity within the South American Species of Bartsia L. (Orobanchaceae). PLoS ONE 11:e0148203.