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# Examining the utility of DNA barcodes for the identification of tallgrass prairie flora

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**PREMISE:** The tallgrass prairies of North America are one of the most threatened ecosystems in the world, making efficient species identification essential for understanding and managing diversity. Here, we assess DNA barcoding with high-throughput sequencing as a method for rapid plant species identification.

**METHODS:** Using herbarium collections representing the tallgrass prairie flora of Oak Lake Field Station, South Dakota, USA, we amplified and examined four common nuclear and plastid barcode regions (*ITS*, *matK*, *psbA-trnH*, and *rbcL*), individually and in combination, to test their success in identifying samples to family, genus, and species levels using BLAST searches of three databases of varying size.

**RESULTS:** Concatenated barcodes increased performance, although none were significantly different than single-region barcodes. The plastid region *psbA-trnH* performed significantly more poorly than the others, while barcodes containing *ITS* performed best. Database size significantly affected identification success at all three taxonomic levels. Confident species-level identification ranged from 8–44% for the global database, 13–56% for the regional database, and 21–80% for the sampled species database, depending on the barcode used.

**DISCUSSION:** Barcoding was generally successful in identifying tallgrass prairie genera and families, but was of limited use in species-level identifications. Database size was an important factor in successful plant identification. We discuss future directions and considerations for improving the performance of DNA barcoding in tallgrass prairies.

**KEY WORDS** DNA barcode; grassland; high-throughput sequencing; Northern Great Plains; prairie.

Rapid species identification is vital for understanding the continuing losses in threatened communities (Raven and Miller, 2020). Many monitoring programs have sacrificed identification accuracy due to the time it takes for correct taxa assignment, but in doing so are failing to capture critical information that can be used in further research. Compounding the problem of species identification is the decreasing number of taxonomists in the field (Drew, 2011). Morphological plasticity in populations, cryptic species, and dependency on life stage also hinder efficient species identification (Hebert et al., 2003; Hollingsworth et al., 2016). Although morphology and anatomy are extremely important for species identification, it takes time to assess these plant features and requires an increasingly rare level of expertise. DNA barcoding offers one potential solution, as all that is needed is a small amount of tissue, regardless of developmental stage, to identify the plant.

DNA barcoding uses small segments of DNA to identify species and has been effectively used for species-level identification

in many animal and plant groups (Hebert et al., 2003; CBOL Plant Working Group, 2009), invasive species control (Floyd et al., 2010), forensics (Savolainen and Lundeberg, 1999), and regulatory enforcement (Parveen et al., 2016). Although DNA barcoding is unlikely to replace the field identification of species, it is another tool for when morphological features are not available, whether due to disturbance (e.g., grazing or burning), for the analysis of fecal material (Goldberg et al., 2020), or for the verification of morphology-based identification. Whereas animals have a recognized barcoding region (mitochondrial *COI*), a universal barcode for plants has remained elusive. The most effective methods use a combination of gene regions, such as a selection of plastid (*matK*, *rbcL*, *rpoC1*, *rpoB*, *psbA-trnH*, and *trnL*) and nuclear (internal transcribed spacer [ITS]) regions; however, many of these regions are not universally usable across all plant groups (Kress et al., 2005; Chase et al., 2007; CBOL Plant Working Group, 2009; Hollingsworth et al., 2009). The advent of high-throughput

sequencing (HTS) has opened up even more potential for the application of DNA barcodes. HTS methods result in shorter read lengths (~300 bp) compared with Sanger sequencing, potentially leading to insufficient variation in sequences to correctly classify closely related species and species with unresolved boundaries (Seberg and Petersen, 2009); however, the advantage of HTS is the ability to sequence numerous regions and individuals at once, reducing time and cost inputs.

Temperate grasslands are one of the most threatened ecosystems globally (Hoekstra et al., 2005) and could benefit from DNA barcoding as a tool for the rapid identification of taxa. Within the United States, the grasslands of the Great Plains have seen large reductions in area and are continuing to be lost at a relatively higher rate than the Brazilian Amazon Rainforest (World Wildlife Fund, 2018). The tallgrass prairie ecosystems of the Great Plains have been particularly hard hit, with over 99% of pre-settlement tallgrass prairie having been lost, primarily to row-crop agriculture and non-native species planted for grazing (Wright and Wimberly, 2013; Lark et al., 2015; Wright et al., 2017). The rate of loss is especially severe in the Northern Great Plains, with South Dakota having the highest rate of grassland conversion (Wright and Wimberly, 2013; Larkin et al., 2015; Wright et al., 2017). The morphological identification of plant species in tallgrass prairies can be difficult due to disturbance (e.g., grazing or burning), inadequate developmental stage at the time of sampling (e.g., not yet at or past anthesis), and the existence of closely related species that require a taxonomic key and magnification to distinguish (e.g., species in Poaceae, Cyperaceae, and Asteraceae). These challenges have led to observer discrepancies in identification of 10–30% of species in grassland systems compared with a 2–10% error rate in other habitat types (Morrison, 2016). Accelerating the pace of accurate species identification within this ecosystem has the potential to hasten subsequent studies of ecosystem function and biodiversity.

As morphological identification is not always feasible or accurate, HTS could be an alternative method allowing many samples to be identified in parallel to remove some of the identification error; however, the high number of closely related species in grasslands of the Northern Great Plains, including many with uncertain boundaries, may render HTS methods ineffective as an identification tool. This study aims to examine the effectiveness of using commonly proposed DNA barcodes as a potential service to identify tallgrass prairie species using HTS methods. Previous studies have found moderate success in grasslands (31–85% species resolution) when using Sanger sequencing to obtain barcode sequences (Braukmann et al., 2017). If HTS approaches can provide confident species identifications, DNA barcoding could be

a rapid and cost-effective tool for the identification of regional flora.

## METHODS

### Sampling

Leaf material was removed from 286 herbarium samples (C. A. Taylor Herbarium [SDC], South Dakota State University, Brookings, South Dakota, and Oak Lake Field Station herbarium [OLFS], Astoria, South Dakota) based on the OLFS species inventory list (see Appendix 1 for voucher information). Herbarium tissue was used, rather than fresh samples, in an effort to begin documenting historical specimen genetic data for long-term preservation (as discussed in Raven and Miller, 2020). The OLFS species list consists of 269 species in 63 families, with nearly half of the species in four angiosperm families: Asteraceae, Poaceae, Cyperaceae, and Fabaceae (18%, 13%, 10%, and 8% of the total list, respectively).

We prioritized voucher specimens collected at OLFS, choosing the most recent collections for DNA extraction to reduce the amount of degraded DNA and improve sequence amplification (Adams and Sharma, 2010; Staats et al., 2011). For inventoried species lacking vouchers from the OLFS property, we sampled herbarium vouchers from localities near OLFS.

### DNA extraction, amplification, and sequencing

From each sample, 0.02–0.03 mg of tissue was used for total genomic DNA extraction using a modified 2× cetyltrimethylammonium bromide (CTAB) approach (Doyle and Doyle, 1987). The DNA extractions were then visualized on agarose gel to assess DNA quality and concentration. Four DNA regions were selected for this study due to their prominence as “universal” plant barcodes: nuclear ITS2, and plastid *rbcLa*, *matK*, and *psbA-trnH* (see Table 1 for primer sequences and references; *psbA-trnH* abbreviated in figures and tables as *trnH*). These primers were selected due to their ability to amplify across angiosperm families and produce amplicon lengths compatible with the Illumina MiSeq (San Diego, California, USA) HTS platform limit of 300-bp paired-end reads (CBOL Plant Working Group, 2009; China Plant BOL Group, 2011; Braukmann et al., 2017).

We followed a modified 16S Illumina library construction protocol (Illumina, 2013) and optimized the annealing temperatures for each primer set using the OligoAnalyzer Tool (Owczarzy et al., 2008). This process consisted of an initial amplification of

**TABLE 1.** Selected DNA barcoding regions and their primer pairs compatible for high-throughput sequencing.

| Region                         | Primer               | Primer sequence from 5' end | Amplicon length (bp) | References                                |
|--------------------------------|----------------------|-----------------------------|----------------------|---|
| ITS2 (nuclear)                 | UniPlantF (5')       | TGTGAATTGCARRATYCMG         | 300                  | Moorhouse-Gann et al., 2018               |
| <i>matK</i> (chloroplast)      | UniPlantR (3')       | CCCGHYTGAYYTGRGGTCDC        |                      |   |
|                                | matK-1F (5')         | ACTGTATCGCACTATGTATCA       | 400–600              | Bremer et al., 2002                       |
| <i>rbcLa</i> (chloroplast)     | matK-4R (3')         | GCATCTTTACCCARTAGCGAAG      |                      |   |
|                                | <i>rbcLa</i> -F (5') | ATGTCACCACAAACAGAGACTAAAGC  | 550                  | Kress and Erickson, 2007                  |
| <i>psbA-trnH</i> (chloroplast) | <i>rbcLa</i> -R (3') | GTAAATCAAGTCACCRCG          |                      |   |
|                                | <i>psbA3_f</i> (5')  | GTTATGCATGAACGTAATGCTC      | 500                  | Sang et al., 1997; Tate and Simpson, 2003 |
|                                | trnHf_05 (3')        | CGCGCATGGTGGATTACAATCC      |                      |   |

target regions using site-specific primers (“PCR 1”), with an additional adapter sequence tag added to the 5' end of the synthesized oligonucleotide. These tags acted as a binding site for an additional pair of primers to add an 8-bp index sequence in a second round of PCR (“PCR 2”; see Appendix 2 for sequences), allowing for the identification of samples after the amplicons were pooled. To construct the library, Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the Nextera XT Index Kit v2 (Illumina) were used. All four amplicons for each specimen were pooled after PCR 1, which gave each individual the same index for later identification while still allowing each region to be identified by primer sequence. Bead cleanup was conducted after PCR 2 to remove unwanted reaction components (e.g., fragments shorter than 50 bp) using HighPrep PCR Clean-up System magnetic beads (MAGBIO, Gaithersburg, Maryland, USA) in an IntegenX Apollo 324 automated library preparation system (Thermo Fisher Scientific). All samples were pooled after PCR 2 to a concentration of 4 nM based on the concentration values determined using a Qubit 3 Fluorometer (Thermo Fisher Scientific). The pooled library was then sequenced in one run with the Illumina MiSeq platform using 300-bp paired-end reads.

The data were received through BaseSpace (cloud-based Illumina software; <https://basespace.illumina.com>), pre-demultiplexed to individual sample, and the index sequences were removed. Raw sequence files were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive database (BioProject accession PRJNA649768). Pooled reads for each individual were run through Fluidigm2PURC (Blischak et al., 2018) using default settings, which trims the sequences and combines paired reads. Because the data were pre-demultiplexed using the Illumina software based on the index sequences, we used a custom script to further group sequences by amplicon based on the primer sequences and then remove primers (locus\\_assigner; E.-W. Li, Cornell University, personal communication). Consensus sequences were generated and chimeric sequences removed using purc\_recluster2 in Fluidigm2PURC, with clustering values of 0.92 and 0.93, and the largest consensus cluster was used for downstream analysis. Sequences identified as fungal contaminants were removed. Cleaned sequences were then concatenated in all possible combinations between the four single-locus barcodes in Geneious Prime version 2019.2.3 (<https://www.geneious.com>).

## BLAST

In order to evaluate the performance of individual regions and concatenated regions (both hereafter referred to as “barcodes”), we constructed three different sequence databases: (1) the entirety of GenBank sequence data, representing a broad range of species (accessed 4 October 2020; Clark et al., 2016); (2) a regional database using South Dakota and regional tallgrass prairie species occurrence data from the Great Plains Regional Herbarium Network (<https://ngpherbaria.org/>) and voucherized sequences from GenBank to create a more realistic scenario for the use of DNA barcodes (i.e., no knowledge of species identity *a priori*) (accessed 4 October 2020); and (3) only species sampled from OLFS, creating a “best-case scenario” to reduce the amount of closely related species (accessed 4 October 2020). Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990; Camacho et al., 2009)

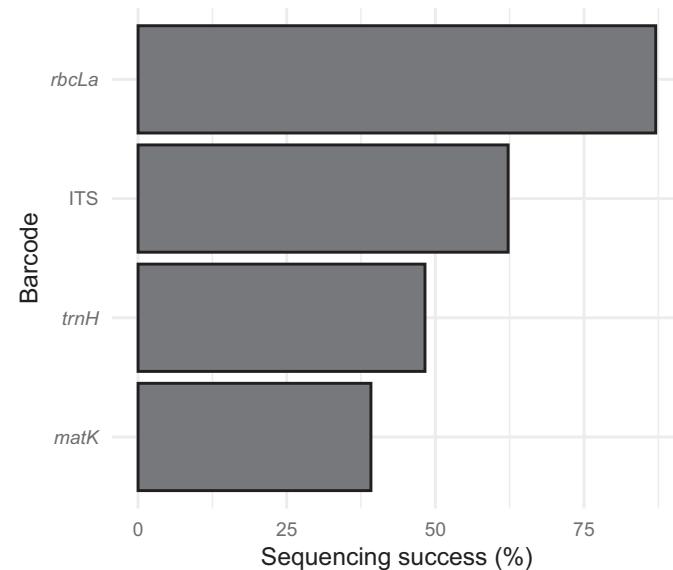
was used to find the sequences that best match the generated DNA barcode sequences in each of the databases.

## BLAST processing

We retrieved each top hit with the highest *E*-value from the BLAST searches against our three databases and compared them against our voucherized specimens. If multiple top hits were returned, they were filtered to use the ones with highest percentage identity and bitscore. “Confident” successful identification was recorded for species that were always correctly identified by the top results, “ambiguous” identification was assigned when results contained both correct and incorrect species, and an “incorrect” identification was assigned when none of the top hits contained the correct species. Success was determined for family-, genus-, and species-level identification. Family names were generated and species names updated using the Catalogue of Life: 2019 Annual Checklist (Roskov et al., 2019) with taxize (Chamberlain and Szocs, 2013; Chamberlain et al., 2020) in R (version 0.9.92; R Core Team, 2018) for both BLAST results and the original OLFS species list. To evaluate the confident identification success between barcodes, we used prop.test in the base R package stats (version 3.5.1) to run a pairwise Pearson's chi-squared test statistic (Holm [1979] correction method) on the mean confident correct identification levels for each barcode ( $\alpha = 0.05$ ).

## RESULTS

After updating the taxonomic names in taxize, we identified 266 distinct species for our 286 samples, as some taxa were combined under the same name and four samples failed to amplify. The *rbcL* region had the highest amplification success, with 249 retrieved sequences, while *matK* amplification was worst, with only 112 sequences retrieved (Fig. 1). We retrieved 178 sequences of ITS2 and



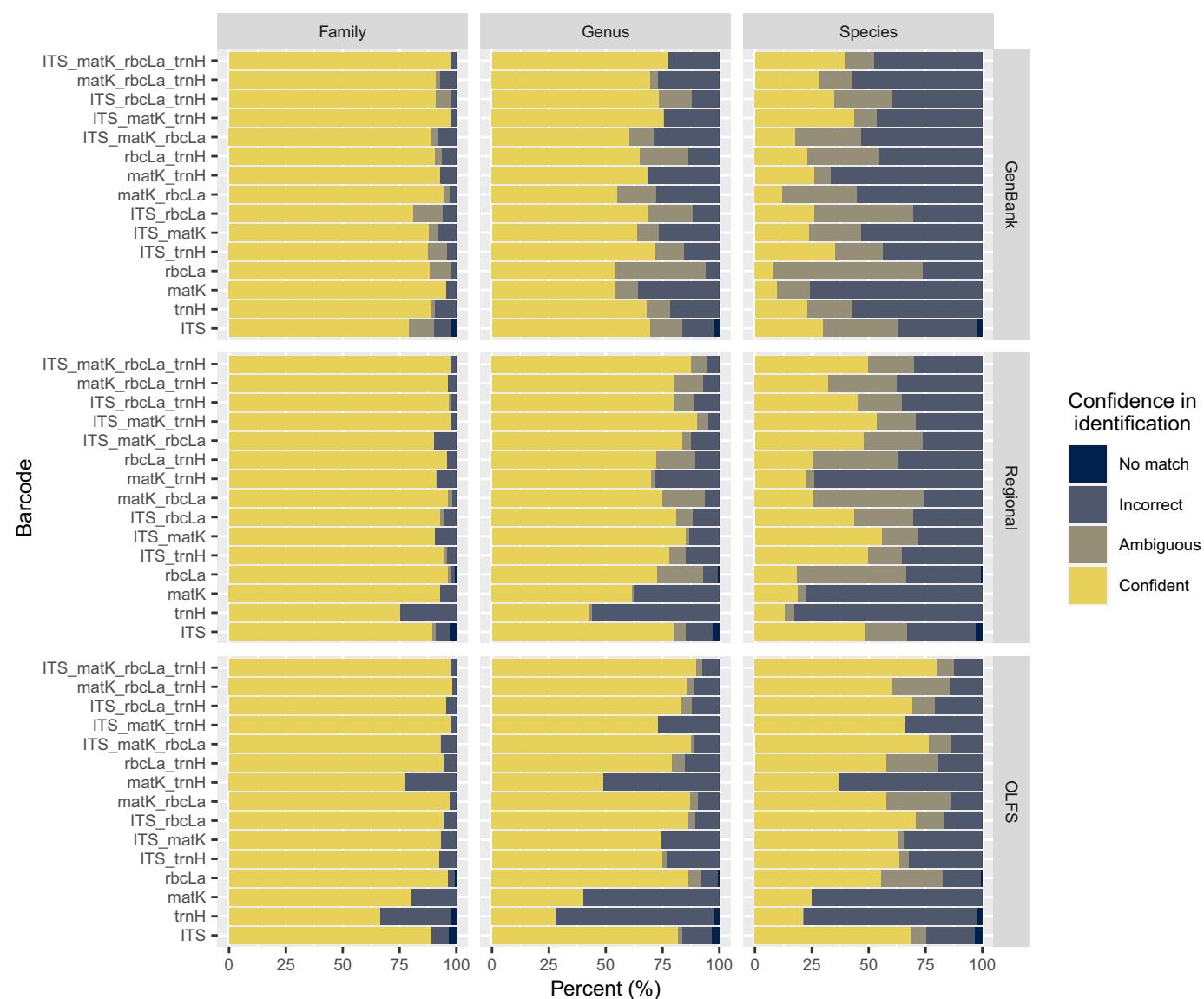
**FIGURE 1.** Sequence retrieval success of amplicons for 286 tallgrass prairie plant herbarium specimens sampled. *psbA-trnH* is listed as *trnH* on the figure.

138 sequences of *psbA-trnH*. Of our 266 taxa, nine species were not represented in GenBank. Additionally, the coverage of the tested regions in GenBank varied, with 253 of the 266 species represented for the *rbcLa* region (97%), 246 species covered by ITS2 (94%), 239 by *matK* (91%), and 123 by *psbA-trnH* (47%). Species not in databases were still included in the results because we were looking for an overall view of barcoding success for the identification of the regional flora in the context of a potential barcoding service for regional stakeholders.

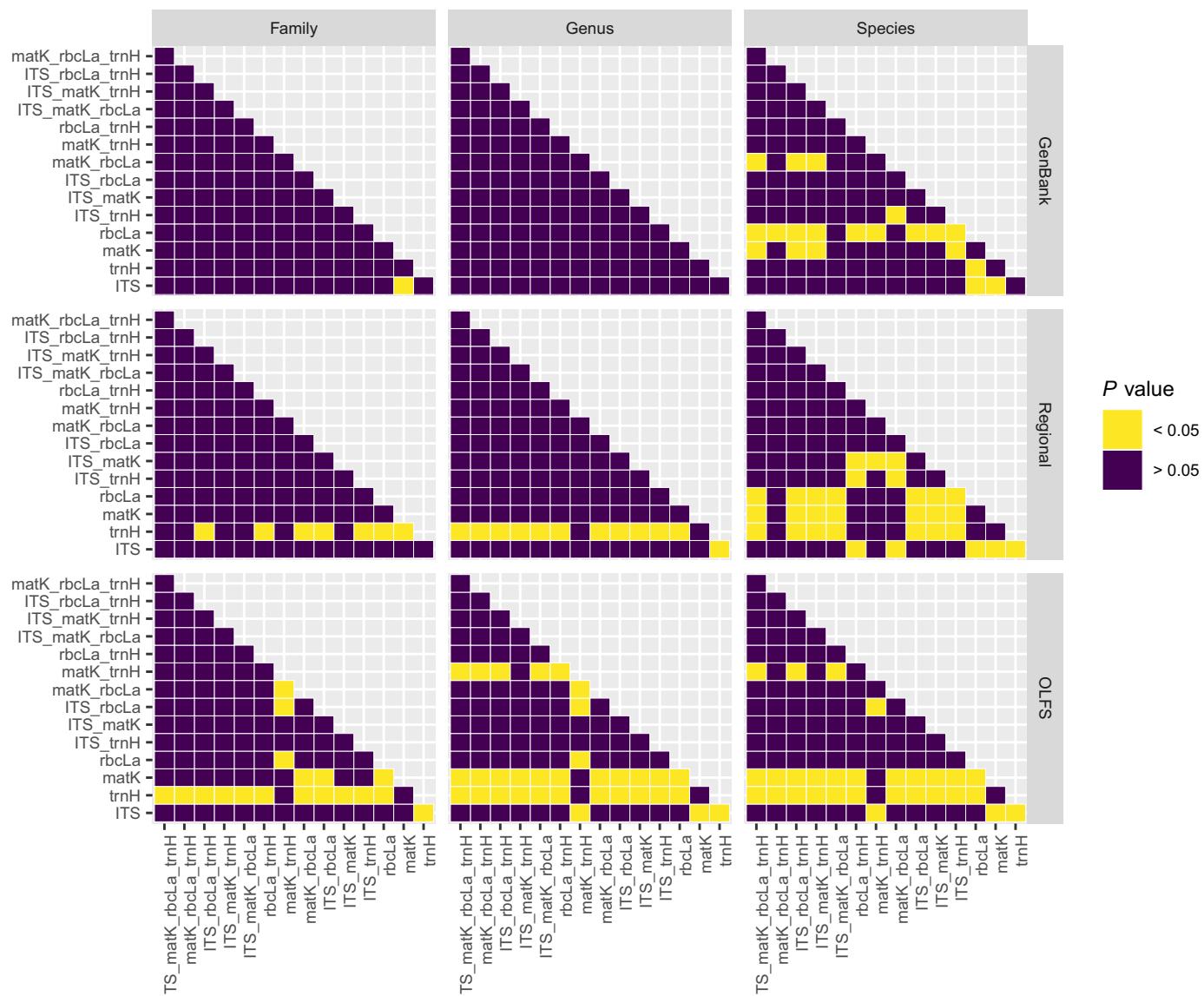
The GenBank database resulted in 79–98% confident and 0–12% ambiguous family identifications (Fig. 2, Appendix 3). Genus identification rates ranged from 54–78% confident and 0–40% ambiguous. Species identification when using GenBank was the lowest of the three databases, with confident identification rates of 8–44% and ambiguous identification rates of 7–65%. No barcodes yielded a significantly different level of confidence in genus or species identification, but *matK* and ITS2 gave significantly

different rates of confidence in family identification (Figs. 2, 3; see Appendix S1 for table of chi-squared test results). The *matK* and *rbcLa* gene regions were the least successful individual regions for confident species-level identification (10% and 8%, respectively) using the GenBank database as a reference, followed by *psbA-trnH* (23%). The ITS2 region had the highest success (30%) of the single-region barcodes. The concatenation of ITS2, *matK*, and *psbA-trnH* proved to yield the most successful confident species discrimination at 44%.

The regional database resulted in identifications with a significantly higher rate of confidence (average 34%) than GenBank (average 23%). Family identification rates ranged from 75–98% confident, while 0–2% were ambiguous (Fig. 2, Appendix 3). Genus-level identification rates ranged from 42–90% confident, while 1–20% were ambiguous. Using the regional database, ITS2 was again the best-performing single-locus barcode at 48% confident species identifications, followed by *matK* and *rbcLa* at 18% and



**FIGURE 2.** Success of identification to family, genus, and species using various barcodes. Barcode names linked with an underscore indicate multi-region barcodes. OLFS, database of species sampled from Oak Lake Field Station.



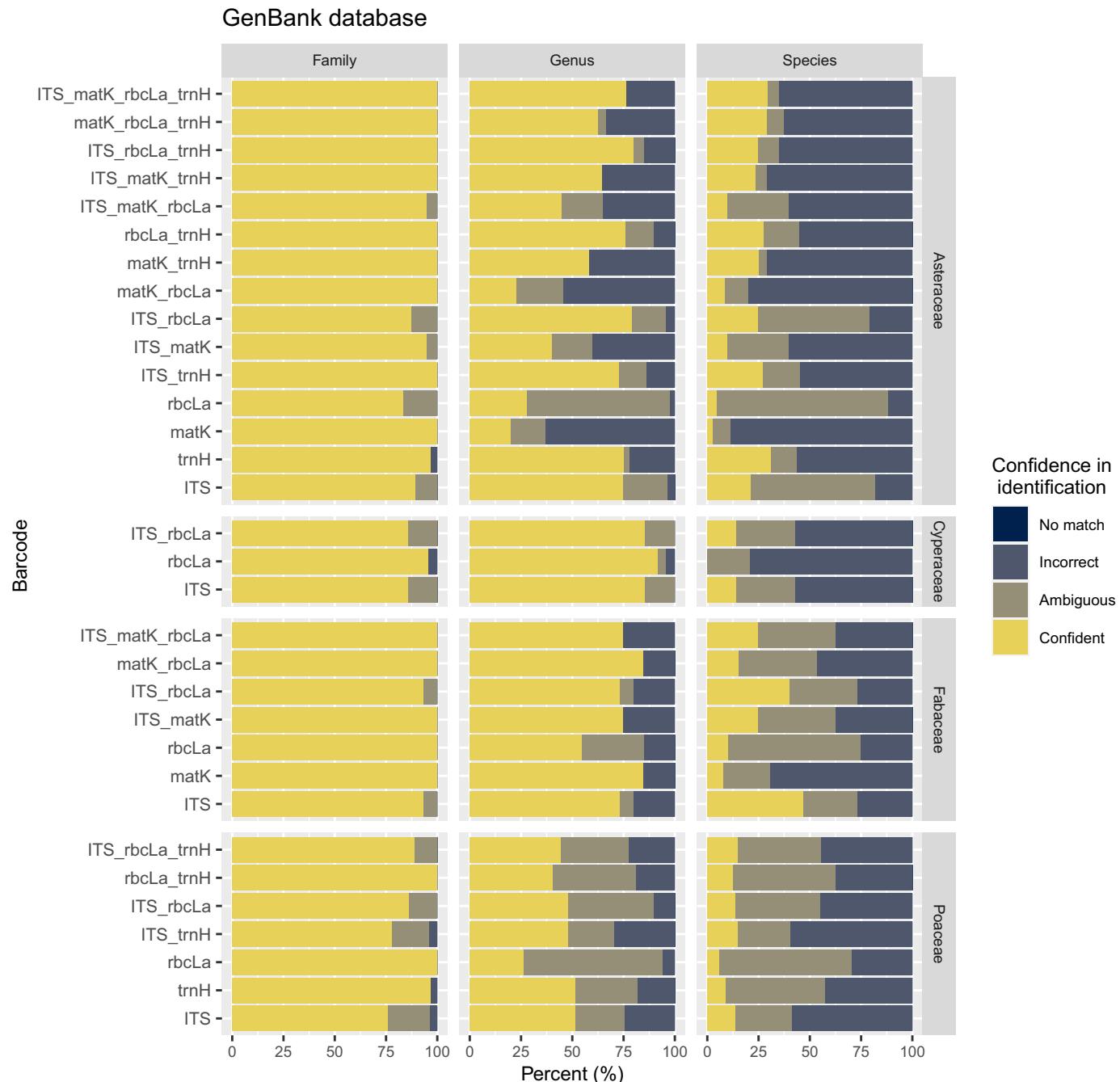
**FIGURE 3.** Pairwise proportion tests indicate significant differences in the rate of confident identification at the species, genus, and family taxonomic levels for the three tested databases. OLFS, database of species sampled from Oak Lake Field Station.

*psbA-trnH* at 13%. The ITS2 and *matK* barcodes performed the best, at 56% each. At the family-, genus-, and species-level identification, *psbA-trnH* performed significantly more poorly than the other barcodes (Figs. 2, 3).

When using the smallest database containing only sampled species (average confident identification = 57%), we found confident family identification rates of 66–98%, while none were ambiguous (Fig. 2, Appendix 3). Genus identifications ranged from 28–90% confident and 0–6% ambiguous. As in the other databases, ITS2 performed the best as a single-locus barcode (69% confident species identifications), followed by *rbcLa* (55%), *matK* (25%), and *psbA-trnH* (21%). Combining all four barcodes resulted in the highest confident species identification (80%). Barcodes *psbA-trnH* and *matK* were significantly poorer for making confident identifications than the other barcodes across the three taxonomic levels (Figs. 2, 3). The use of the OLFS database resulted in significantly

better confident species identifications than GenBank and the regional database.

Breaking down the results by the four most speciose families in our tallgrass prairie community (Asteraceae, Poaceae, Cyperaceae, and Fabaceae, with 49, 35, 25, and 22 specimens, respectively), we found ITS2 and *rbcLa* had the highest levels of sequence retrieval. The *rbcLa* barcode was most successful for identifying the Asteraceae (30/49), Fabaceae (20/22), and Cyperaceae (23/25), while ITS2 and *psbA-trnH* were most useful for the Poaceae (25/35). The barcode that performed best for confident identifications varied for each family; however, concatenated barcodes containing ITS2 generally performed best (Figs. 4–6, Appendix 4). Identification success was low (<25%) for these families when using GenBank (except when using ITS2 for Fabaceae). Reducing the database size improved the taxonomic resolution for all four families.

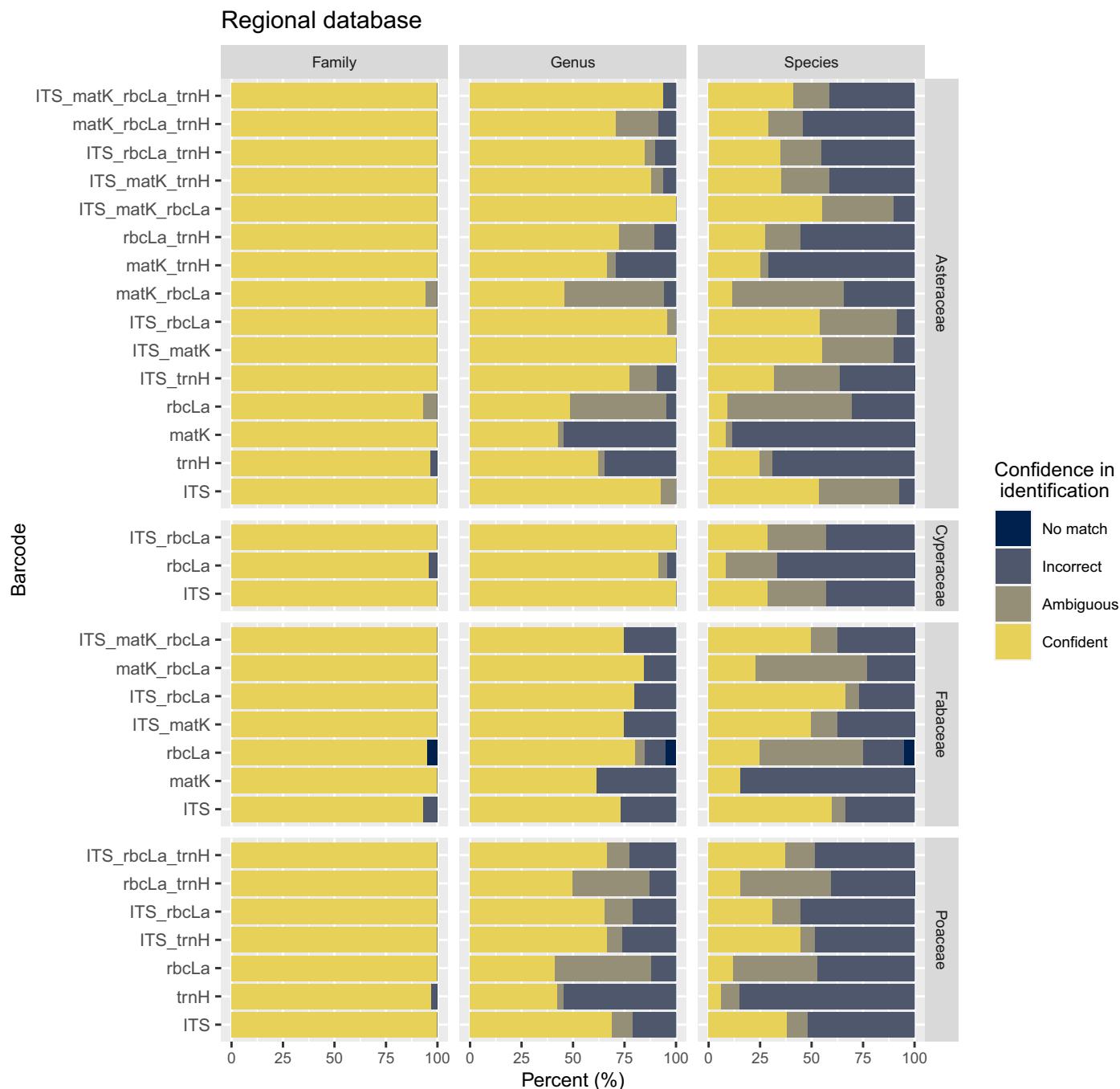


**FIGURE 4.** Barcode success for the four most diverse families when using GenBank as the reference database (see Figs. 5 and 6 for other databases and Appendix 4 for more information). Only barcodes for which more than five sequences were obtained are shown. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## DISCUSSION

Our results indicate DNA barcoding is useful for the identification of taxa at the ranks of genus and family across the tested barcodes but demonstrated only low to moderate success at the species level. We found higher success when using smaller, more focused databases, as was expected due to the presence of more closely related species in larger databases than are found regionally, lowering species resolution, which was also reported by

Parmentier et al. (2013). Reducing database size is especially helpful when identifying closely related species, such as members of the Asteraceae, Poaceae, Fabaceae, and Cyperaceae, as our identifications greatly improved when using smaller databases for our identification of these families (Figs. 4–6). We did see reductions in taxa discrimination at the higher taxonomic levels for some barcodes when using the smaller databases, likely due to removal of closely related species (i.e., congeners). Here, we were particularly interested in the ability of DNA barcodes to differentiate

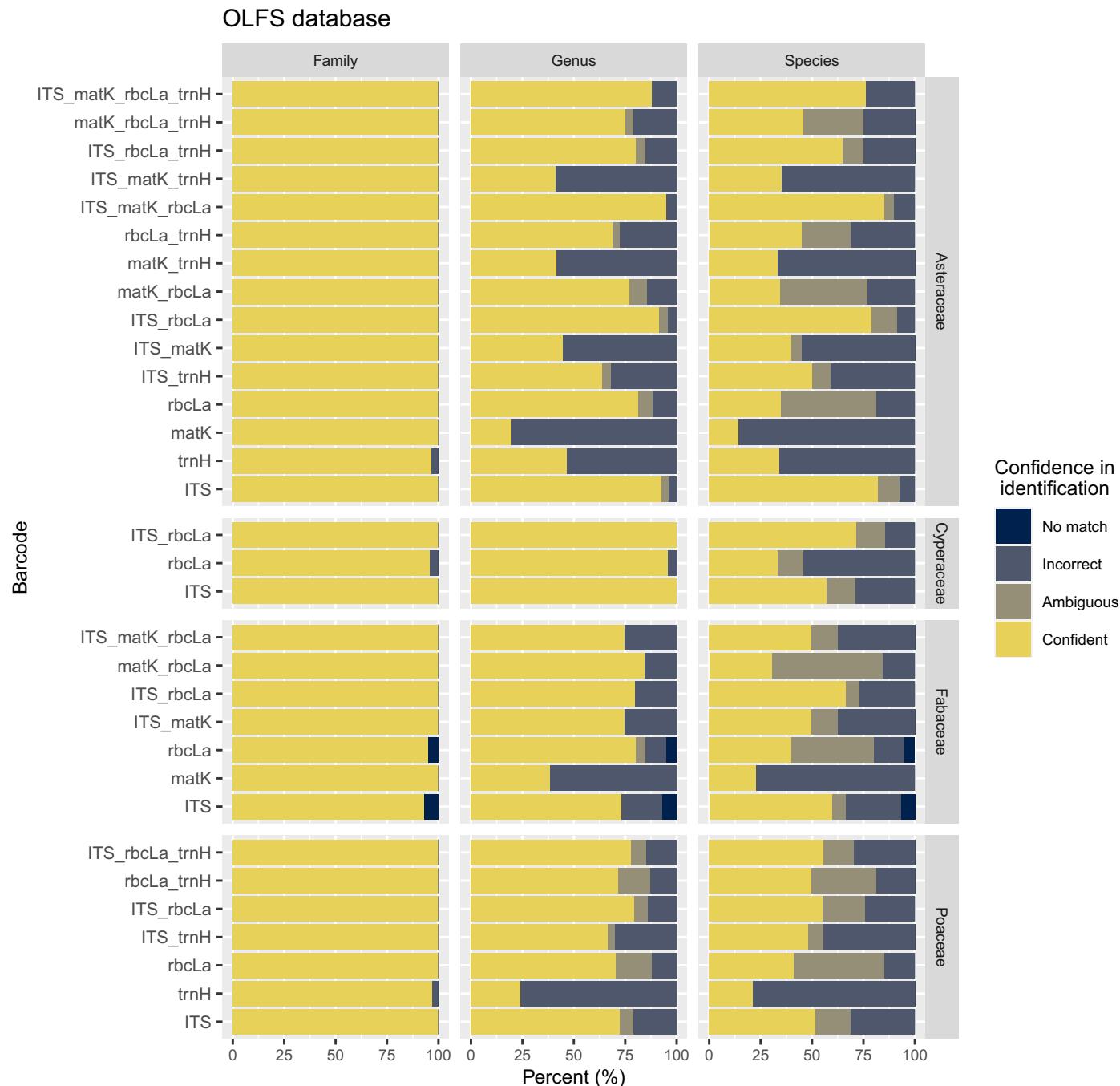


**FIGURE 5.** Barcode success for the four most diverse families when using a regional database as the reference (see Figs. 4 and 6 for other databases and Appendix 4 for more information). Only barcodes for which more than five sequences were obtained are shown. [Color figure can be viewed at wileyonlinelibrary.com]

plant taxa of the tallgrass prairies of the Northern Great Plains as a possible identification service. As such, using a focused database of only sampled species (as shown in our OLFS-specific database) would require *a priori* knowledge of species identity; however, we included it to examine the barcode success rates under a “best-case” scenario. Our findings indicate that reducing a regional database of state-wide and regional plant species to a smaller, more focused database would be beneficial if using DNA barcodes as an

identification service. Using a regional database is preferable over a large-scale database such as GenBank.

Generally, single-region barcodes had lower success at species-level identification than multi-region barcodes, in accordance with previous studies (Chase et al., 2007; CBOL Plant Working Group, 2009; China Plant BOL Group, 2011). We found significant variation in the ability of a barcode to successfully identify individuals at the species, genus, and family levels. The nuclear ITS2 region consistently



**FIGURE 6.** Barcode success for the four most diverse families when using the database of species sampled from Oak Lake Field Station (OLFS) (see Figs. 4 and 5 for other databases and Appendix 4 for more information). Only barcodes for which more than five sequences were obtained are shown.

performed best in terms of confident species identification, both as a single-locus barcode and as a member of the top-performing concatenated barcodes. Plastid regions *matK* and *psbA-trnH* were particularly poor at identifying species when used as single-region barcodes.

The low success rate in species discrimination when using *matK* contrasts with other studies, where *matK* was one of the most successful barcoding regions for plant identification (Lahaye et al., 2008; CBOL Plant Working Group, 2009; Braukmann et al., 2017). A major limitation in using *matK* is the difficulty of finding universal primer pairs (CBOL Plant Working Group, 2009; Hollingsworth

et al., 2011). In contrast, although *rbcL* only has moderate identification success, it amplifies well across taxa, in our study and others, which has led to it being promoted as a good candidate for inclusion in a multi-region barcode (CBOL Plant Working Group, 2009; China Plant BOL Group, 2011; Hollingsworth et al., 2011). The combination of *matK* and *rbcL* has been promoted as one of the most promising universal two-region plant barcodes (CBOL Plant Working Group, 2009). The failure of this two-region barcode for species identification in some systems, particularly for closely related taxa (Seberg and Petersen, 2009; Roy et al., 2010; Parmentier

et al., 2013), has resulted in the recommendation to include a nuclear-encoded ribosomal internal transcribed spacer, ITS2 (Chen et al., 2010; China Plant BOL Group, 2011; Hollingsworth et al., 2011). The universal presence of ITS2 across plant taxa and its short length (~350 bp) make it a promising barcode for use in community assessments and HTS approaches for DNA barcoding. Our results correspond with previous studies that ITS performs well at both amplification and taxon discrimination.

It is possible to increase identification success for tallgrass prairie species. Likely the best methodology for DNA barcoding of these species would be through the creation of a specific probe set suited for this plant community to increase the amplification success of some taxonomic groups, particularly for regions such as *matK* as was done by Heckenauer et al. (2016). A concentrated effort to supplement molecular data from species missing from the database will be beneficial, as will continued effort to collect the genetic information of regional species. Creating a smaller database based on verified vouchered specimens, as we have started to do through our sequencing efforts in this project, will result in fewer potential identification errors than occur when using GenBank (although misidentification levels appear to be low in GenBank, as noted by Leray et al. [2019]). Additionally, HTS makes it more feasible to increase the number of sequenced regions to offset the lower identification success created by using shorter reads, leading to the proposal of whole plastid genome sequencing for species discrimination (Parks et al., 2009; Nock et al., 2011; Steele and Pires, 2011; Kane et al., 2012). Nuclear probe sets such as Angiosperms353, which targets 353 nuclear single protein-coding regions, might be promising, as these amplified regions are variable at shallow taxonomic levels (Johnson et al., 2018; Larridon et al., 2020). The increased number of regions returned with HTS also allows for the inclusion of chloroplast regions, in addition to nuclear, potentially increasing the resolution of species identification. This could be particularly helpful for species with ambiguous species boundaries or historical hybridization events, as chloroplast genomes are generally inherited maternally and nuclear genomes are inherited biparentally (Rieseberg and Soltis, 1991; Soltis and Kuzoff, 1995).

Our results indicate there is potential for the use of DNA barcoding to identify tallgrass prairie plant species of the Northern Great Plains using HTS methods, particularly at the family and genus levels; however, species-level identification with these barcoding regions could be limiting, depending on the resolution needed. The optimization of primers for prairie species and the addition of missing species in a regional database are promising future directions that will likely increase successful identification at these shallow taxonomic scales.

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## AUTHOR CONTRIBUTION

M.L. conceived of the study. S.A.H. performed sample preparation, analyzed the data, and wrote the manuscript with assistance from M.L. All authors approved the final manuscript.

## DATA AVAILABILITY

The raw Illumina output files have been uploaded to the National Center for Biotechnology Information Sequence Read Archive database (BioProject accession PRJNA649768).

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**APPENDIX S1.** *P* values from pairwise chi-squared tests between each barcode's confident correct species identification. Barcodes were compared within each database.

## LITERATURE CITED

- Adams, R. P., and L. N. Sharma. 2010. DNA from herbarium specimens: I. Correlation of DNA size with specimen age. *Phytologia* 92: 346–353.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- Blischak, P. D., M. Latvis, D. F. Morales-Briones, J. C. Johnson, V. S. Di Stilio, A. D. Wolfe, and D. C. Tank. 2018. Fluidigm2PURC: Automated processing and haplotype inference for double-barcoded PCR amplicons. *Applications in Plant Sciences* 6(6): e01156.
- Braukmann, T. W., M. L. Kuzmina, J. Sills, E. V. Zakharov, and P. D. Hebert. 2017. Testing the efficacy of DNA barcodes for identifying the vascular plants of Canada. *PLoS ONE* 12: e0169515.
- Bremer, B., K. Bremer, N. Heidari, P. Erixon, R. G. Olmstead, A. A. Anderberg, M. Källersjö, and E. Barkhordarian. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24: 274–301.
- Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T. L. Madden. 2009. BLAST+: Architecture and applications. *BMC Bioinformatics* 10: 421.
- CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, USA* 106: 12794–12797.
- Chamberlain, S., and E. Szocs. 2013. taxize: Taxonomic search and retrieval in R [version 2]. *F1000Research* 2: 191.
- Chamberlain, S., E. Szocs, Z. Foster, Z. Arendsee, C. Boettiger, K. Ram, I. Bartomeus, et al. 2020. taxize: Taxonomic information from around the web. Website <https://github.com/ropensci/taxize> [accessed 12 November 2020].
- Chase, M. W., R. S. Cowan, P. M. Hollingsworth, C. van den Berg, S. Madrinan, G. Petersen, O. Seberg, et al. 2007. A proposal for a standardised protocol to barcode all land plants. *Taxon* 56: 295–299.
- Chen, S., H. Yao, J. Han, C. Liu, J. Song, L. Shi, Y. Zhu, et al. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE* 5: e8613.
- China Plant BOL Group. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences, USA* 108: 19641–19646.

- Clark, K., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers. 2016. GenBank. *Nucleic Acids Research* 44: D67–D72.
- Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Drew, L. W. 2011. Are we losing the science of taxonomy?: As need grows, numbers and training are failing to keep up. *BioScience* 61: 942–946.
- Floyd, R., J. Lima, L. Humble, and R. Hanner. 2010. Common goals: Policy implications of DNA barcoding as a protocol for identification of arthropod pests. *Biological Invasions* 12: 2947–2954.
- Goldberg, A., C. Conway, D. Tank, K. Andrews, D. Gour, and L. Waits. 2020. Diet of a rare herbivore based on DNA metabarcoding of feces: Selection, seasonality, and survival. *Ecology and Evolution* 10: 7627–7643.
- Hebert, P. D., A. Cywinski, S. L. Ball, and J. R. deWaard. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B, Biological Sciences* 270: 313–321.
- Heckenhauer, J., M. H. J. Barfuss, and R. Samuel. 2016. Universal multiplexable *matK* primers for DNA barcoding of angiosperms. *Applications in Plant Sciences* 4(6): 1500137.
- Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2005. Confronting a biome crisis: Global disparities of habitat loss and protection. *Ecology Letters* 8: 23–29.
- Hollingsworth, M. L., A. Clark, L. L. Forrest, J. Richardson, R. T. Pennington, D. G. Long, R. Cowan, et al. 2009. Selecting barcoding loci for plants: Evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. *Molecular Ecology Resources* 9: 439–457.
- Hollingsworth, P. M., S. W. Graham, and D. P. Little. 2011. Choosing and using a plant DNA barcode. *PLoS ONE* 6: e19254.
- Hollingsworth, P. M., D. Z. Li, M. van der Bank, and A. D. Twyford. 2016. Telling plant species apart with DNA: From barcodes to genomes. *Philosophical Transactions of the Royal Society B, Biological Sciences* 371: 20150338.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6: 65–70.
- Illumina. 2013. 16S metagenomic sequencing library preparation. Available at: [https://support.illumina.com/documents/documentation/chemistry\\_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf](https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) [accessed 25 November 2020].
- Johnson, M. G., L. Pokorny, S. Dodsworth, L. R. Botigué, R. S. Cowan, A. Devault, W. L. Eiserhardt, et al. 2018. A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. *Systematic Biology* 68: 594–606.
- Kane, N., S. Sveinsson, H. Dempewolf, J. Y. Yang, D. Zhang, J. M. M. Engels, and Q. Cronk. 2012. Ultra-barcoding in cacao (*Theobroma* spp.; Malvaceae) using whole chloroplast genomes and nuclear ribosomal DNA. *American Journal of Botany* 99: 320–329.
- Kress, W. J., and D. L. Erickson. 2007. A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS ONE* 2: e508.
- Kress, W. J., K. J. Wurdack, E. A. Zimmer, L. A. Weigt, and D. H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences, USA* 102: 8369–8374.
- Lahaye, R., M. van der Bank, D. Bogarin, J. Warner, F. Pupulin, G. Gigot, O. Maurin, et al. 2008. DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences, USA* 105: 2923.
- Lark, T. J., M. J. Salmon, and H. K. Gibbs. 2015. Cropland expansion outpaces agricultural and biofuel policies in the United States. *Environmental Research Letters* 10: 044003.
- Larkin, D. J., A. L. Hipp, J. Kattge, W. Prescott, R. K. Tonietto, S. K. Jacobi, and M. L. Bowles. 2015. Phylogenetic measures of plant communities show long-term change and impacts of fire management in tallgrass prairie remnants. *Journal of Applied Ecology* 52: 1638–1648.
- Larridon, I., T. Villaverde, A. R. Zuntini, L. Pokorny, G. E. Brewer, N. Epitawalage, I. Fairlie, et al. 2020. Tackling rapid radiations with targeted sequencing. *Frontiers in Plant Science* 10: 1655.
- Leray, M., N. Knowlton, S.-L. Ho, B. N. Nguyen, and R. J. Machida. 2019. GenBank is a reliable resource for 21st century biodiversity research. *Proceedings of the National Academy of Sciences, USA* 116: 22651.
- Moorhouse-Gann, R. J., J. C. Dunn, N. de Vere, M. Goder, N. Cole, H. Hipperson, and W. O. C. Symondson. 2018. New universal ITS2 primers for high-resolution herbivory analyses using DNA metabarcoding in both tropical and temperate zones. *Scientific Reports* 8: 8542.
- Morrison, L. W. 2016. Observer error in vegetation surveys: A review. *Journal of Plant Ecology* 9: 367–379.
- Nock, C. J., D. L. E. Waters, M. A. Edwards, S. G. Bowen, N. Rice, G. M. Cordeiro, and R. J. Henry. 2011. Chloroplast genome sequences from total DNA for plant identification. *Plant Biotechnology Journal* 9: 328–333.
- Owczarzy, R., A. V. Tataurov, Y. Wu, J. A. Manthey, K. A. McQuisten, H. G. Almabazi, K. F. Pedersen, et al. 2008. IDT SciTools: A suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Research* 36: W163–W169.
- Parks, M., R. Cronn, and A. Liston. 2009. Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology* 7: 84.
- Parmentier, I., J. Dumainil, M. Kuzmina, M. Philippe, D. W. Thomas, D. Kenfack, G. B. Chuyong, et al. 2013. How effective are DNA barcodes in the identification of African rainforest trees? *PLoS ONE* 8: e54921.
- Parveen, I., S. Gafner, N. Techén, S. J. Murch, and I. A. Khan. 2016. DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: Strengths and limitations. *Planta Medica* 82: 1225–1235.
- R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website <https://www.R-project.org/> [accessed 12 November 2020].
- Raven, P. H., and S. E. Miller. 2020. Here today, gone tomorrow. *Science* 370: 149.
- Rieseberg, L. H., and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.
- Roskov, Y., G. Ower, T. Orrell, D. Nicolson, N. Bailly, P. M. Kirk, T. Bourgoin, et al. 2019. Species 2000 & ITIS Catalogue of Life, 2019 annual checklist. Species 2000, Naturalis, Leiden, the Netherlands. Website <http://www.catalogueoflife.org/annual-checklist/2019/> [accessed 12 November 2020].
- Roy, S., A. Tyagi, V. Shukla, A. Kumar, U. M. Singh, L. B. Chaudhary, B. Datt, et al. 2010. Universal plant DNA barcode loci may not work in complex groups: A case study with Indian *Berberis* species. *PLoS ONE* 5: e13674.
- Sang, T., D. Crawford, and T. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.
- Savolainen, P., and J. Lundeberg. 1999. Forensic evidence based on mtDNA from dog and wolf hairs. *Journal of Forensic Science* 44: 77–81.
- Seberg, O., and G. Petersen. 2009. How many loci does it take to DNA barcode a crocus? *PLoS ONE* 4: e4598.
- Soltis, D. E., and R. K. Kuzoff. 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). *Evolution* 49: 727–742.
- Staats, M., A. Cuenca, J. E. Richardson, R. Vrielink-van Ginkel, G. Petersen, O. Seberg, and F. T. Bakker. 2011. DNA damage in plant herbarium tissue. *PLoS ONE* 6: e28448.
- Steele, P. R., and J. C. Pires. 2011. Biodiversity assessment: State-of-the-art techniques in phylogenomics and species identification. *American Journal of Botany* 98: 415–425.
- Tate, J., and B. Simpson. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* 28: 723.
- World Wildlife Fund. 2018. The Plowprint Report: 2018. Website <https://www.worldwildlife.org/projects/plowprint-report> [accessed 12 November 2020].
- Wright, C. K., and M. C. Wimberly. 2013. Recent land use change in the Western Corn Belt threatens grasslands and wetlands. *Proceedings of the National Academy of Sciences, USA* 110: 4134–4139.
- Wright, C. K., B. Larson, T. J. Lark, and H. K. Gibbs. 2017. Recent grassland losses are concentrated around US ethanol refineries. *Environmental Research Letters* 12: 044001.

**APPENDIX 1.** Herbarium voucher information of sampled tallgrass prairie plants.

| Family         | Species                            | Collection       | Herbarium | BioSample accession |
|----------------|------------------------------------|------------------|-----------|---------------------|
| Amaryllidaceae | <i>Allium stellatum</i>            | Mixon s.n.       | OLFS      | SAMN15679782        |
| Anardiaceae    | <i>Rhus glabra</i>                 | Larson 6925      | OLFS      | SAMN15679923        |
| Anardiaceae    | <i>Toxicodendron rydbergii</i>     | Larson 11716     | OLFS      | SAMN15679861        |
| Apiaceae       | <i>Cicuta bulbifera</i>            | Beauzay 323      | OLFS      | SAMN15679813        |
| Apiaceae       | <i>Cicuta maculata</i>             | Beauzay 332      | SDC       | SAMN15679650        |
| Apiaceae       | <i>Cryptotaenia canadensis</i>     | Ode 84-102       | OLFS      | SAMN15679770        |
| Apiaceae       | <i>Osmorhiza longistylis</i>       | Stahnke s.n.     | SDC       | SAMN15679651        |
| Apiaceae       | <i>Osmorhiza longistylis</i>       | Larson 8724      | OLFS      | SAMN15679888        |
| Apiaceae       | <i>Sium suave</i>                  | Beauzay 322      | SDC       | SAMN15679727        |
| Apiaceae       | <i>Zizia aptera</i>                | Troelstrup s.n.  | SDC       | SAMN15679726        |
| Apiaceae       | <i>Zizia aurea</i>                 | Jensen s.n.      | SDC       | SAMN15679725        |
| Apocynaceae    | <i>Apocynum cannabinum</i>         | King 94          | OLFS      | SAMN15679798        |
| Apocynaceae    | <i>Apocynum cannabinum</i>         | Stahnke s.n.     | SDC       | SAMN15679724        |
| Apocynaceae    | <i>Apocynum cannabinum</i>         | Jensen s.n.      | SDC       | SAMN15679723        |
| Apocynaceae    | <i>Asclepias incarnata</i>         | Stahnke s.n.     | SDC       | SAMN15679722        |
| Apocynaceae    | <i>Asclepias incarnata</i>         | Beauzay 327      | SDC       | SAMN15679721        |
| Apocynaceae    | <i>Asclepias syriaca</i>           | Mixon s.n.       | OLFS      | SAMN15679797        |
| Apocynaceae    | <i>Asclepias verticillata</i>      | Jensen s.n.      | SDC       | SAMN15679720        |
| Apocynaceae    | <i>Asclepias verticillata</i>      | Troelstrup s.n.  | SDC       | SAMN15679719        |
| Araceae        | <i>Lemna trisulca</i>              | Larson 9016      | OLFS      | SAMN15679835        |
| Araceae        | <i>Lemna turionifera</i>           | Fredrickson s.n. | OLFS      | SAMN15679834        |
| Asparagaceae   | <i>Maianthemum stellatum</i>       | Larson 8750      | OLFS      | SAMN15679873        |
| Asteraceae     | <i>Achillea millefolium</i>        | Beauzay 296      | OLFS      | SAMN15679781        |
| Asteraceae     | <i>Agoseris glauca</i>             | Pooler 173       | OLFS      | SAMN15679779        |
| Asteraceae     | <i>Ambrosia artemisiifolia</i>     | Larson 9917      | OLFS      | SAMN15679778        |
| Asteraceae     | <i>Ambrosia psilostachya</i>       | Larson 9922      | OLFS      | SAMN15679777        |
| Asteraceae     | <i>Artemisia frigida</i>           | Roemmich 114     | OLFS      | SAMN15679776        |
| Asteraceae     | <i>Artemisia ludoviciana</i>       | Taylor 7506      | OLFS      | SAMN15679774        |
| Asteraceae     | <i>Artemisia ludoviciana</i>       | Buckert s.n.     | SDC       | SAMN15679775        |
| Asteraceae     | <i>Bidens frondosa</i>             | Sargent s.n.     | OLFS      | SAMN15679795        |
| Asteraceae     | <i>Carduus nutans</i>              | Johnson 419      | OLFS      | SAMN15679773        |
| Asteraceae     | <i>Cirsium arvense</i>             | Mixon s.n.       | OLFS      | SAMN15679772        |
| Asteraceae     | <i>Cronquistianthus bulliferus</i> | Bauer 042        | OLFS      | FAILED              |
| Asteraceae     | <i>Echinacea angustifolia</i>      | Jensen s.n.      | SDC       | SAMN15679715        |
| Asteraceae     | <i>Echinacea angustifolia</i>      | Jensen s.n.      | SDC       | SAMN15679716        |
| Asteraceae     | <i>Erigeron canadensis</i>         | Law 105          | OLFS      | SAMN15679771        |
| Asteraceae     | <i>Erigeron philadelphicus</i>     | Larson 8980      | OLFS      | SAMN15679791        |
| Asteraceae     | <i>Erigeron strigosus</i>          | Taylor s.n.      | OLFS      | SAMN15679790        |
| Asteraceae     | <i>Eupatorium perfoliatum</i>      | Anders 24        | OLFS      | SAMN15679759        |
| Asteraceae     | <i>Euthamia graminifolia</i>       | Ode s.n.         | OLFS      | SAMN15679758        |
| Asteraceae     | <i>Eutrochium maculatum</i>        | Beauzay 326      | SDC       | SAMN15679714        |
| Asteraceae     | <i>Grindelia squarrosa</i>         | Mixon s.n.       | OLFS      | SAMN15679789        |
| Asteraceae     | <i>Helenium autumnale</i>          | Stahnke s.n.     | SDC       | SAMN15679713        |
| Asteraceae     | <i>Helianthus grosseserratus</i>   | Larson s.n.      | OLFS      | SAMN15679787        |
| Asteraceae     | <i>Helianthus maximiliani</i>      | Jensen s.n.      | SDC       | SAMN15679712        |
| Asteraceae     | <i>Helianthus maximiliani</i>      | Law 73           | OLFS      | SAMN15679786        |
| Asteraceae     | <i>Helianthus nuttallii</i>        | Larson 11732     | OLFS      | SAMN15679788        |
| Asteraceae     | <i>Heliospilos helianthoides</i>   | Jensen s.n.      | SDC       | SAMN15679711        |
| Asteraceae     | <i>Liatris aspera</i>              | Stahnke s.n.     | SDC       | SAMN15679709        |
| Asteraceae     | <i>Liatris punctata</i>            | Mixon s.n.       | OLFS      | SAMN15679785        |
| Asteraceae     | <i>Lygodesmia juncea</i>           | Troelstrup s.n.  | SDC       | SAMN15679708        |
| Asteraceae     | <i>Matricaria discoidea</i>        | Anderson 09      | OLFS      | SAMN15679898        |
| Asteraceae     | <i>Packera paupercula</i>          | Larson 8920      | OLFS      | SAMN15679906        |
| Asteraceae     | <i>Packera plattensis</i>          | Jensen s.n.      | SDC       | SAMN15679705        |
| Asteraceae     | <i>Packera pseudaura</i>           | Larson 9967      | OLFS      | FAILED              |
| Asteraceae     | <i>Ratibida columnifera</i>        | Stahnke s.n.     | SDC       | SAMN15679707        |
| Asteraceae     | <i>Ratibida columnifera</i>        | Jensen s.n.      | SDC       | SAMN15679706        |
| Asteraceae     | <i>Ratibida columnifera</i>        | Mixon s.n.       | OLFS      | SAMN15679925        |
| Asteraceae     | <i>Rudbeckia laciniata</i>         | Larson 6912      | OLFS      | SAMN15679924        |
| Asteraceae     | <i>Silphium perfoliatum</i>        | Mixon s.n.       | OLFS      | SAMN15679876        |
| Asteraceae     | <i>Solidago canadensis</i>         | Jensen s.n.      | SDC       | SAMN15679704        |
| Asteraceae     | <i>Solidago gigantea</i>           | Beauzay 337      | SDC       | SAMN15679703        |

(Continues)

**APPENDIX 1.** Continued

| Family           | Species                            | Collection        | Herbarium | BioSample accession |
|------------------|------------------------------------|-------------------|-----------|---------------------|
| Asteraceae       | <i>Solidago missouriensis</i>      | Bortem 102        | OLFS      | SAMN15679875        |
| Asteraceae       | <i>Solidago mollis</i>             | Stahnke s.n.      | SDC       | SAMN15679702        |
| Asteraceae       | <i>Solidago rigida</i>             | Larson 9926       | SDC       | SAMN15679701        |
| Asteraceae       | <i>Symphyotrichum ericoides</i>    | Bortnem 110       | SDC       | SAMN15679718        |
| Asteraceae       | <i>Symphyotrichum laeve</i>        | Stahnke s.n.      | SDC       | SAMN15679717        |
| Asteraceae       | <i>Taraxacum officinale</i>        | Monteith s.n.     | OLFS      | SAMN15679863        |
| Asteraceae       | <i>Tragopogon dubius</i>           | Jensen s.n.       | SDC       | SAMN15679700        |
| Asteraceae       | <i>Vernonia fasciculata</i>        | Larson 11538      | OLFS      | SAMN15679848        |
| Asteraceae       | <i>Vernonia fasciculata</i>        | Jensen s.n.       | SDC       | SAMN15679699        |
| Balsaminaceae    | <i>Impatiens capensis</i>          | Beauzay 335       | SDC       | SAMN15679676        |
| Boraginaceae     | <i>Lithospermum canescens</i>      | Stahnke s.n.      | SDC       | SAMN15679678        |
| Boraginaceae     | <i>Lithospermum canescens</i>      | Troelstrup s.n.   | SDC       | SAMN15679679        |
| Boraginaceae     | <i>Lithospermum incisum</i>        | Jensen s.n.       | SDC       | SAMN15679680        |
| Boraginaceae     | <i>Lithospermum incisum</i>        | Stahnke s.n.      | SDC       | SAMN15679677        |
| Boraginaceae     | <i>Lithospermum onosmodium</i>     | Beauzay 282       | OLFS      | SAMN15679889        |
| Brassicaceae     | <i>Capsella bursa-pastoris</i>     | Taylor 11799      | OLFS      | SAMN15679833        |
| Brassicaceae     | <i>Cardamine bulbosa</i>           | Steinauer s.n.    | OLFS      | SAMN15679832        |
| Brassicaceae     | <i>Erysimum cheiranthoides</i>     | Larson 11335      | OLFS      | SAMN15679760        |
| Brassicaceae     | <i>Lepidium densiflorum</i>        | Law 93            | OLFS      | SAMN15679836        |
| Brassicaceae     | <i>Rorippa palustris</i>           | McLead s.n.       | OLFS      | SAMN15679918        |
| Campanulaceae    | <i>Lobelia siphilitica</i>         | Beauzay 330       | OLFS      | SAMN15679698        |
| Campanulaceae    | <i>Lobelia siphilitica</i>         | Beauzay 330       | SDC       | SAMN15679904        |
| Campanulaceae    | <i>Lobelia spicata</i>             | Larson s.n.       | OLFS      | SAMN15679903        |
| Cannabaceae      | <i>Celtis occidentalis</i>         | Ode 12-27         | OLFS      | SAMN15679815        |
| Caprifoliaceae   | <i>Lonicera tatarica</i>           | Stahnke s.n.      | SDC       | SAMN15679696        |
| Caprifoliaceae   | <i>Symphoricarpos occidentalis</i> | Stahnke s.n.      | SDC       | SAMN15679697        |
| Caryophyllaceae  | <i>Callitrichia brutia</i>         | Larson s.n.       | OLFS      | SAMN15679865        |
| Caryophyllaceae  | <i>Stellaria media</i>             | Bortem 2          | OLFS      | SAMN15679866        |
| Celastraceae     | <i>Celastrus scandens</i>          | Larson 11227      | OLFS      | SAMN15679816        |
| Ceratophyllaceae | <i>Ceratophyllum demersum</i>      | Larson 6963       | OLFS      | SAMN15679814        |
| Comandraceae     | <i>Comandra umbellata</i>          | Jensen s.n.       | SDC       | SAMN15679684        |
| Commelinaceae    | <i>Tradescantia bracteata</i>      | Roberts 72-5-28:6 | OLFS      | SAMN15679860        |
| Convolvulaceae   | <i>Calystegia macounii</i>         | Larson 11358      | OLFS      | SAMN15679793        |
| Convolvulaceae   | <i>Calystegia sepium</i>           | Pooler 84996      | OLFS      | SAMN15679792        |
| Convolvulaceae   | <i>Convolvulus sepium</i>          | Jensen s.n.       | SDC       | SAMN15679695        |
| Cornaceae        | <i>Cornus sericea</i>              | Larson 11240      | OLFS      | SAMN15679812        |
| Cupressaceae     | <i>Juniperus virginiana</i>        | Taylor s.n.       | OLFS      | FAILED              |
| Cyperaceae       | <i>Carex aquatilis</i>             | Ode 82-11         | OLFS      | SAMN15679830        |
| Cyperaceae       | <i>Carex blanda</i>                | Larson 11215      | OLFS      | SAMN15679829        |
| Cyperaceae       | <i>Carex brevior</i>               | Larson 11132      | OLFS      | SAMN15679828        |
| Cyperaceae       | <i>Carex cristatella</i>           | Larson 11330      | SDC       | SAMN15679694        |
| Cyperaceae       | <i>Carex emoryi</i>                | Larson 6393       | OLFS      | SAMN15679827        |
| Cyperaceae       | <i>Carex granularis</i>            | Larson 11346      | OLFS      | SAMN15679826        |
| Cyperaceae       | <i>Carex gravida</i>               | Larson 6395       | OLFS      | SAMN15679825        |
| Cyperaceae       | <i>Carex hystericina</i>           | Larson 11337      | SDC       | SAMN15679693        |
| Cyperaceae       | <i>Carex meadii</i>                | Ode 83-57         | OLFS      | SAMN15679824        |
| Cyperaceae       | <i>Carex molesta</i>               | Larson 9365       | OLFS      | SAMN15679822        |
| Cyperaceae       | <i>Carex pellita</i>               | Larson 9270       | OLFS      | SAMN15679823        |
| Cyperaceae       | <i>Carex praegracilis</i>          | Peterson s.n.     | SDC       | SAMN15679692        |
| Cyperaceae       | <i>Carex sartwellii</i>            | Larson s.n.       | OLFS      | SAMN15679821        |
| Cyperaceae       | <i>Carex sprengelii</i>            | Larson 11216      | OLFS      | SAMN15679820        |
| Cyperaceae       | <i>Carex stricta</i>               | Larson 11329      | SDC       | SAMN15679690        |
| Cyperaceae       | <i>Carex tenera</i>                | Larson 6396       | OLFS      | SAMN15679819        |
| Cyperaceae       | <i>Carex tetanica</i>              | Larson s.n.       | OLFS      | SAMN15679818        |
| Cyperaceae       | <i>Carex utriculata</i>            | Larson 9165       | OLFS      | SAMN15679817        |
| Cyperaceae       | <i>Carex vulpinoidea</i>           | Larson 11345      | SDC       | SAMN15679691        |
| Cyperaceae       | <i>Cyperus odoratus</i>            | Beauzay 341       | SDC       | SAMN15679689        |
| Cyperaceae       | <i>Eleocharis erythropoda</i>      | Larson 11639      | OLFS      | SAMN15679766        |
| Cyperaceae       | <i>Eleocharis palustris</i>        | Sletten 169       | OLFS      | SAMN15679765        |
| Cyperaceae       | <i>Eriophorum angustifolium</i>    | Larson 11340      | OLFS      | SAMN15679761        |
| Cyperaceae       | <i>Scirpus microcarpus</i>         | Larson s.n.       | OLFS      | SAMN15679910        |
| Cyperaceae       | <i>Scirpus pallidus</i>            | Beauzay 257       | OLFS      | SAMN15679909        |

(Continues)

**APPENDIX 1.** Continued

| Family          | Species                         | Collection      | Herbarium | BioSample accession |
|-----------------|---------------------------------|-----------------|-----------|---------------------|
| Elaeagnaceae    | <i>Elaeagnus angustifolia</i>   | Johnson 420     | OLFS      | SAMN15679767        |
| Equisetaceae    | <i>Equisetum arvense</i>        | Jensen s.n.     | SDC       | SAMN15679688        |
| Fabaceae        | <i>Amorpha canescens</i>        | Jensen s.n.     | SDC       | SAMN15679675        |
| Fabaceae        | <i>Amorpha fruticosa</i>        | Beauzay 317     | SDC       | SAMN15679674        |
| Fabaceae        | <i>Amphicarpa bracteata</i>     | Larson 6680     | OLFS      | SAMN15679811        |
| Fabaceae        | <i>Astragalus crassicarpus</i>  | Pooler 186      | OLFS      | SAMN15679796        |
| Fabaceae        | <i>Caragana arborescens</i>     | Fairlee 54      | OLFS      | SAMN15679831        |
| Fabaceae        | <i>Dalea candida</i>            | Stahnke s.n.    | SDC       | SAMN15679667        |
| Fabaceae        | <i>Dalea purpurea</i>           | Stahnke s.n.    | SDC       | SAMN15679666        |
| Fabaceae        | <i>Gleditsia triacanthos</i>    | Purinton s.n.   | OLFS      | SAMN15679841        |
| Fabaceae        | <i>Lathyrus palustris</i>       | Pengra P-16-17  | OLFS      | SAMN15679838        |
| Fabaceae        | <i>Lathyrus polymorphus</i>     | Troelstrup s.n. | SDC       | SAMN15679665        |
| Fabaceae        | <i>Lathyrus venosus</i>         | Mixon s.n.      | OLFS      | SAMN15679837        |
| Fabaceae        | <i>Medicago lupulina</i>        | Anderson 17     | OLFS      | SAMN15679897        |
| Fabaceae        | <i>Medicago sativa</i>          | Larson 11421    | OLFS      | SAMN15679896        |
| Fabaceae        | <i>Melilotus officinalis</i>    | Johnson s.n.    | SDC       | SAMN15679673        |
| Fabaceae        | <i>Melilotus officinalis</i>    | Johnson s.n.    | SDC       | SAMN15679672        |
| Fabaceae        | <i>Melilotus officinalis</i>    | Larson 9041     | OLFS      | SAMN15679895        |
| Fabaceae        | <i>Psoralea argophylla</i>      | Stahnke s.n.    | SDC       | SAMN15679671        |
| Fabaceae        | <i>Psoralea esculenta</i>       | Jensen s.n.     | SDC       | SAMN15679670        |
| Fabaceae        | <i>Trifolium pratense</i>       | Jensen s.n.     | SDC       | SAMN15679669        |
| Fabaceae        | <i>Trifolium pratense</i>       | Beauzay 276     | OLFS      | SAMN15679859        |
| Fabaceae        | <i>Vicia americana</i>          | Jensen s.n.     | SDC       | SAMN15679668        |
| Fabaceae        | <i>Dalea purpurea</i>           | Jensen s.n.     | SDC       | SAMN15679664        |
| Fagaceae        | <i>Quercus macrocarpa</i>       | Stahnke s.n.    | OLFS      | SAMN15679927        |
| Gentianaceae    | <i>Gentiana andrewsii</i>       | Troelstrup s.n. | SDC       | SAMN15679687        |
| Gentianaceae    | <i>Gentiana puberulenta</i>     | Roemmich 98     | OLFS      | SAMN15679842        |
| Grossulariaceae | <i>Ribes americanum</i>         | Larson 8885     | OLFS      | SAMN15679922        |
| Grossulariaceae | <i>Ribes americanum</i>         | Pooler 84045    | OLFS      | SAMN15679921        |
| Hydrophyllaceae | <i>Hydrophyllum virginianum</i> | Taylor 7540     | OLFS      | SAMN15679840        |
| Iridaceae       | <i>Sisyrinchium campestre</i>   | Larson 8733     | OLFS      | SAMN15679874        |
| Juncaceae       | <i>Juncus dudleyi</i>           | Bettross 51     | OLFS      | SAMN15679839        |
| Juncaceae       | <i>Juncus nodosus</i>           | Larson 11334    | SDC       | SAMN15679657        |
| Lamiaceae       | <i>Lycopus americanus</i>       | Beauzay 346     | SDC       | SAMN15679663        |
| Lamiaceae       | <i>Lycopus asper</i>            | Beauzay 342     | SDC       | SAMN15679662        |
| Lamiaceae       | <i>Lycopus uniflorus</i>        | Larson 11426    | OLFS      | SAMN15679901        |
| Lamiaceae       | <i>Mentha arvensis</i>          | Troelstrup s.n. | SDC       | SAMN15679660        |
| Lamiaceae       | <i>Monarda fistulosa</i>        | Stahnke s.n.    | SDC       | SAMN15679661        |
| Lamiaceae       | <i>Scutellaria lateriflora</i>  | Roemmich 259    | OLFS      | SAMN15679907        |
| Lamiaceae       | <i>Stachys palustris</i>        | Stahnke s.n.    | SDC       | SAMN15679659        |
| Lamiaceae       | <i>Stachys palustris</i>        | Larson 11508    | OLFS      | SAMN15679867        |
| Liliaceae       | <i>Lilium philadelphicum</i>    | Jensen s.n.     | SDC       | SAMN15679658        |
| Mazocraeidae    | <i>Brickellia eupatorioides</i> | Stahnke s.n.    | SDC       | SAMN15679710        |
| Melanthiaceae   | <i>Anticlea elegans</i>         | Mixon s.n.      | OLFS      | SAMN15679843        |
| Nyctaginaceae   | <i>Mirabilis nyctaginea</i>     | Bortem 23       | OLFS      | SAMN15679894        |
| Oleaceae        | <i>Fraxinus pennsylvanica</i>   | Ode s.n.        | OLFS      | SAMN15679757        |
| Onagraceae      | <i>Epilobium leptophyllum</i>   | Millar 37       | OLFS      | SAMN15679764        |
| Onagraceae      | <i>Oenothera biennis</i>        | Beauzay 334     | SDC       | SAMN15679686        |
| Onagraceae      | <i>Oenothera serrulata</i>      | Larson 11247    | OLFS      | SAMN15679794        |
| Orchidaceae     | <i>Cypripedium candidum</i>     | Leorschke 1531  | OLFS      | SAMN15679769        |
| Orchidaceae     | <i>Liparis loeselii</i>         | Larson 11327    | OLFS      | SAMN15679905        |
| Orchidaceae     | <i>Platanthera aquilonis</i>    | Larson 11328    | OLFS      | SAMN15679878        |
| Orchidaceae     | <i>Platanthera hyperborea</i>   | Larson 9140     | OLFS      | SAMN15679877        |
| Orobanchaceae   | <i>Pedicularis lanceolata</i>   | Ode 00-21       | OLFS      | SAMN15679885        |
| Oxalidaceae     | <i>Oxalis corniculata</i>       | Mixon s.n.      | OLFS      | SAMN15679887        |
| Oxalidaceae     | <i>Oxalis violacea</i>          | Larson 11212    | OLFS      | SAMN15679886        |
| Papaveraceae    | <i>Dicentra cucullaria</i>      | Larson 6380     | OLFS      | SAMN15679768        |
| Phrymaceae      | <i>Erythranthe glabrata</i>     | Larson 11325    | SDC       | SAMN15679683        |
| Phrymaceae      | <i>Mimulus ringens</i>          | Beauzay 351     | SDC       | SAMN15679682        |
| Phrymaceae      | <i>Phryma leptostachya</i>      | Ode 84-97       | OLFS      | SAMN15679883        |
| Pinaceae        | <i>Picea glauca</i>             | Taylor s.n.     | OLFS      | SAMN15679881        |
| Pinaceae        | <i>Pinus ponderosa</i>          | Lehman 38       | OLFS      | SAMN15679880        |

(Continues)

**APPENDIX 1.** Continued

| Family         | Species                            | Collection        | Herbarium | BioSample accession |
|----------------|------------------------------------|-------------------|-----------|---------------------|
| Plantaginaceae | <i>Penstemon albidus</i>           | Larson 11234      | OLFS      | SAMN15679884        |
| Plantaginaceae | <i>Plantago major</i>              | Beauzay 303       | OLFS      | SAMN15679879        |
| Plantaginaceae | <i>Veronica anagallis-aquatica</i> | Larson 10785      | OLFS      | SAMN15679851        |
| Plantaginaceae | <i>Veronica peregrina</i>          | Larson 9999       | OLFS      | SAMN15679850        |
| Poaceae        | <i>Agropyron cristatum</i>         | Pooler 84027      | OLFS      | SAMN15679784        |
| Poaceae        | <i>Agrostis stolonifera</i>        | Ode s.n.          | OLFS      | SAMN15679783        |
| Poaceae        | <i>Andropogon gerardii</i>         | Roemmich 81       | OLFS      | SAMN15679810        |
| Poaceae        | <i>Bouteloua curtipendula</i>      | Jensen s.n.       | SDC       | SAMN15679656        |
| Poaceae        | <i>Bromus inermis</i>              | Peterson s.n.     | SDC       | SAMN15679655        |
| Poaceae        | <i>Calamagrostis canadensis</i>    | Sletten 305       | OLFS      | SAMN15679809        |
| Poaceae        | <i>Calamagrostis stricta</i>       | Hansen 852        | OLFS      | SAMN15679808        |
| Poaceae        | <i>Cenchrus americanus</i>         | Beauzay 257       | OLFS      | SAMN15679912        |
| Poaceae        | <i>Echinochloa muricata</i>        | Beauzay 347       | SDC       | SAMN15679653        |
| Poaceae        | <i>Elymus repens</i>               | Kanoute 033       | OLFS      | SAMN15679803        |
| Poaceae        | <i>Elymus villosus</i>             | Genereux s.n.     | OLFS      | SAMN15679804        |
| Poaceae        | <i>Elymus virginicus</i>           | VanSickle 586     | OLFS      | SAMN15679805        |
| Poaceae        | <i>Glyceria grandis</i>            | Sletten 303       | OLFS      | SAMN15679802        |
| Poaceae        | <i>Glyceria striata</i>            | Kjellsen 35       | OLFS      | SAMN15679801        |
| Poaceae        | <i>Hordeum jubatum</i>             | Orth s.n.         | OLFS      | SAMN15679800        |
| Poaceae        | <i>Koeleria macrantha</i>          | Kopp 225          | OLFS      | SAMN15679799        |
| Poaceae        | <i>Muhlenbergia cuspidata</i>      | Kanoute 092       | OLFS      | SAMN15679893        |
| Poaceae        | <i>Nassella viridula</i>           | Lehmon 179        | OLFS      | SAMN15679864        |
| Poaceae        | <i>Panicum acuminatum</i>          | Larson 11060      | OLFS      | SAMN15679807        |
| Poaceae        | <i>Panicum oligosanthes</i>        | Bortnem s.n.      | SDC       | SAMN15679654        |
| Poaceae        | <i>Panicum virgatum</i>            | King 103          | OLFS      | SAMN15679892        |
| Poaceae        | <i>Panicum wilcoxianum</i>         | Larson 10982      | OLFS      | SAMN15679806        |
| Poaceae        | <i>Phalaris arundinacea</i>        | Sletten 136       | OLFS      | FAILED              |
| Poaceae        | <i>Phleum pratense</i>             | Christner s.n.    | OLFS      | SAMN15679891        |
| Poaceae        | <i>Poa palustris</i>               | Ode 84-107        | OLFS      | SAMN15679890        |
| Poaceae        | <i>Poa pratensis</i>               | Larson 6850       | SDC       | SAMN15679652        |
| Poaceae        | <i>Schizachyrium scoparium</i>     | VanSickle 536     | OLFS      | SAMN15679913        |
| Poaceae        | <i>Setaria viridis</i>             | Roemmich 82       | OLFS      | SAMN15679911        |
| Poaceae        | <i>Sorghastrum nutans</i>          | Larson s.n.       | SDC       | SAMN15679728        |
| Poaceae        | <i>Sphenopholis intermedia</i>     | Larson 11341      | OLFS      | SAMN15679730        |
| Poaceae        | <i>Sphenopholis intermedia</i>     | Larson 11341      | SDC       | SAMN15679871        |
| Poaceae        | <i>Sphenopholis obtusata</i>       | Sletten 379       | OLFS      | SAMN15679870        |
| Poaceae        | <i>Sporobolus compositus</i>       | Dirks s.n.        | OLFS      | SAMN15679869        |
| Poaceae        | <i>Sporobolus heterolepis</i>      | Pauly s.n.        | OLFS      | SAMN15679868        |
| Poaceae        | <i>Sporobolus michauxianus</i>     | Beauzay 325       | SDC       | SAMN15679729        |
| Polemoniaceae  | <i>Phlox pilosa</i>                | Troelstrup s.n.   | SDC       | SAMN15679731        |
| Polygonaceae   | <i>Persicaria amphibia</i>         | Beauzay 321       | SDC       | SAMN15679732        |
| Polygonaceae   | <i>Persicaria amphibia</i>         | Ode s.n.          | OLFS      | SAMN15679929        |
| Polygonaceae   | <i>Persicaria lapathifolia</i>     | Beauzay 320       | SDC       | SAMN15679733        |
| Polygonaceae   | <i>Persicaria punctata</i>         | Beauzay 339       | SDC       | SAMN15679734        |
| Polygonaceae   | <i>Polygonum aviculare</i>         | Larson 11565      | OLFS      | SAMN15679930        |
| Polygonaceae   | <i>Polygonatum biflorum</i>        | Pooler 84029      | OLFS      | SAMN15679931        |
| Polygonaceae   | <i>Rumex crispus</i>               | Unkenholz s.n.    | OLFS      | SAMN15679920        |
| Polygonaceae   | <i>Rumex orbiculatus</i>           | Roberts 73-8-19:1 | OLFS      | SAMN15679919        |
| Primulaceae    | <i>Lysimachia ciliata</i>          | Beauzay 352       | OLFS      | SAMN15679900        |
| Primulaceae    | <i>Lysimachia thyrsiflora</i>      | Sletten 273       | OLFS      | SAMN15679899        |
| Ranunculaceae  | <i>Anemonastrum canadense</i>      | Troelstrup s.n.   | SDC       | SAMN15679735        |
| Ranunculaceae  | <i>Anemone cylindrica</i>          | Stahnke s.n.      | SDC       | SAMN15679736        |
| Ranunculaceae  | <i>Aquilegia canadensis</i>        | Jensen s.n.       | SDC       | SAMN15679738        |
| Ranunculaceae  | <i>Caltha palustris</i>            | Troelstrup s.n.   | SDC       | SAMN15679739        |
| Ranunculaceae  | <i>Delphinium carolinianum</i>     | Jensen s.n.       | SDC       | SAMN15679740        |
| Ranunculaceae  | <i>Pulsatilla patens</i>           | Stahnke s.n.      | SDC       | SAMN15679737        |
| Ranunculaceae  | <i>Ranunculus hispida</i>          | Ode 02-7          | OLFS      | SAMN15679926        |
| Ranunculaceae  | <i>Ranunculus macounii</i>         | Jensen s.n.       | SDC       | SAMN15679741        |
| Ranunculaceae  | <i>Thalictrum dasycarpum</i>       | Larson 6651       | OLFS      | SAMN15679862        |
| Rhamnaceae     | <i>Rhamnus cathartica</i>          | Stahnke s.n.      | SDC       | SAMN15679742        |
| Rosaceae       | <i>Agrimonia striata</i>           | Beauzay 349       | SDC       | SAMN15679743        |
| Rosaceae       | <i>Amelanchier alnifolia</i>       | Stahnke s.n.      | SDC       | SAMN15679744        |

(Continues)

**APPENDIX 1.** Continued

| Family           | Species                        | Collection    | Herbarium | BioSample accession |
|------------------|--------------------------------|---------------|-----------|---------------------|
| Rosaceae         | <i>Amelanchier ovalis</i>      | Reese s.n.    | SDC       | SAMN15679745        |
| Rosaceae         | <i>Fragaria virginiana</i>     | Monteith s.n. | OLFS      | SAMN15679763        |
| Rosaceae         | <i>Geum aleppicum</i>          | Larson 11332  | SDC       | SAMN15679746        |
| Rosaceae         | <i>Geum canadense</i>          | Larson 11336  | OLFS      | SAMN15679762        |
| Rosaceae         | <i>Geum rossii</i>             | Jensen s.n.   | SDC       | SAMN15679747        |
| Rosaceae         | <i>Prunus americana</i>        | Stahnke s.n.  | SDC       | SAMN15679748        |
| Rosaceae         | <i>Prunus serotina</i>         | Stahnke s.n.  | SDC       | SAMN15679749        |
| Rosaceae         | <i>Rosa arkansana</i>          | Stahnke s.n.  | SDC       | SAMN15679750        |
| Rosaceae         | <i>Rubus occidentalis</i>      | Larson 10437  | OLFS      | SAMN15679917        |
| Rubiaceae        | <i>Galium aparine</i>          | Anderson 02   | OLFS      | SAMN15679756        |
| Rubiaceae        | <i>Galium boreale</i>          | Jensen s.n.   | SDC       | SAMN15679751        |
| Rubiaceae        | <i>Galium trifidum</i>         | Larson 9213   | OLFS      | SAMN15679754        |
| Rubiaceae        | <i>Galium triflorum</i>        | Larson 6882   | OLFS      | SAMN15679755        |
| Salicaceae       | <i>Populus tremuloides</i>     | Larson 11227  | OLFS      | SAMN15679928        |
| Salicaceae       | <i>Populus ×jackii</i>         | Larson 1131   | SDC       | SAMN15679685        |
| Salicaceae       | <i>Salix alba</i>              | Millar 011    | OLFS      | SAMN15679916        |
| Salicaceae       | <i>Salix amygdaloides</i>      | Larson 7023   | OLFS      | SAMN15679915        |
| Salicaceae       | <i>Salix interior</i>          | Larson 11264  | OLFS      | SAMN15679914        |
| Sapindaceae      | <i>Aesculus glabra</i>         | Taylor s.n.   | OLFS      | SAMN15679780        |
| Scrophulariaceae | <i>Scrophularia lanceolata</i> | Mixon s.n.    | OLFS      | SAMN15679908        |
| Solanaceae       | <i>Lycium barbarum</i>         | Taylor 11723  | OLFS      | SAMN15679902        |
| Solanaceae       | <i>Physalis virginiana</i>     | Roberts s.n.  | OLFS      | SAMN15679882        |
| Typhaceae        | <i>Sparganium eurycarpum</i>   | Sletten 210   | OLFS      | SAMN15679872        |
| Typhaceae        | <i>Typha angustifolia</i>      | Stahnke s.n.  | SDC       | SAMN15679681        |
| Typhaceae        | <i>Typha latifolia</i>         | Larson 9117   | OLFS      | SAMN15679857        |
| Typhaceae        | <i>Typha ×glauca</i>           | Larson 11386  | OLFS      | SAMN15679858        |
| Ulmaceae         | <i>Ulmus americana</i>         | Riley 51      | OLFS      | SAMN15679856        |
| Ulmaceae         | <i>Ulmus pumila</i>            | Larson 11559  | OLFS      | SAMN15679855        |
| Urticaceae       | <i>Urtica dioica</i>           | Mixon s.n.    | OLFS      | SAMN15679854        |
| Verbenaceae      | <i>Verbena bracteata</i>       | Law 103       | OLFS      | SAMN15679853        |
| Verbenaceae      | <i>Verbena hastata</i>         | Beauzay 319   | SDC       | SAMN15679753        |
| Verbenaceae      | <i>Verbena stricta</i>         | Jensen s.n.   | SDC       | SAMN15679752        |
| Verbenaceae      | <i>Verbena stricta</i>         | Pooler 84028  | OLFS      | SAMN15679852        |
| Violaceae        | <i>Viola canadensis</i>        | Larson 11214  | OLFS      | SAMN15679847        |
| Violaceae        | <i>Viola nephrophylla</i>      | Larson 9985   | OLFS      | SAMN15679846        |
| Violaceae        | <i>Viola pedatifida</i>        | Larson 11219  | OLFS      | SAMN15679845        |
| Violaceae        | <i>Viola sororia</i>           | Larson 7019   | OLFS      | SAMN15679844        |
| Vitaceae         | <i>Vitis riparia</i>           | Sletten 175   | OLFS      | SAMN15679849        |

Note: OLFS = Oak Lake Field Station at South Dakota State University; SDC = C. A. Taylor Herbarium at South Dakota State University.

**APPENDIX 2.** The dual-indexing strategy uses two 8-base indices, Index 1 (i7) and Index 2 (i5), which provides unique sequences at the ends of amplicons. The following indices and sequences are from the 24-sample Nextera XT Index Kit (Illumina).

| Index               | Index adapter | Primer sequence |
|---------------------|---------------|-----------------|
| <b>Index 1 (i7)</b> |               |                 |
| N701                |               | TAAGGCGA        |
| N702                |               | CGTACTAG        |
| N703                |               | AGGCAGAA        |
| N704                |               | TCCTGAGC        |
| N705                |               | GGACTCCT        |
| N706                |               | TAGGCATG        |
| N707                |               | CTCTCTAC        |
| N708                |               | CAGAGAGG        |
| N709                |               | GCTACGCT        |
| N710                |               | CGAGGCTG        |
| N711                |               | AAGAGGCA        |
| N712                |               | GTAGAGGA        |
| <b>Index 2 (i5)</b> |               |                 |
| S501                |               | TAGATCGC        |
| S502                |               | CTCTCTAT        |
| S503                |               | TATCCTCT        |
| S504                |               | AGAGTAGA        |
| S505                |               | GTAAGGAG        |
| S506                |               | ACTGCATA        |
| S507                |               | AAGGAGTA        |
| S508                |               | CTAACGCCT       |

**APPENDIX 3.** Identification success for tested barcodes (individual and concatenated regions) of tallgrass prairie plant species, listing the number of retrieved sequences and the number of correctly identified sequences at the family, genus, and species levels for the three tested databases.

| Locus <sup>a</sup> | Taxon level | Database | Sequences | Confident    | Ambiguous    | Incorrect    | No match  |
|--------------------|-------------|----------|-----------|--------------|--------------|--------------|-----------|
| ITS                | Family      | GenBank  | 178       | 141 (79.21%) | 20 (11.24%)  | 13 (7.3%)    | 4 (2.25%) |
| ITS                | Family      | Regional | 178       | 159 (89.33%) | 3 (1.69%)    | 11 (6.18%)   | 5 (2.81%) |
| ITS                | Family      | OLFS     | 178       | 159 (89.33%) | 0            | 13 (7.3%)    | 6 (3.37%) |
| ITS                | Genus       | GenBank  | 178       | 124 (69.66%) | 25 (14.04%)  | 25 (14.04%)  | 4 (2.25%) |
| ITS                | Genus       | Regional | 178       | 142 (79.78%) | 10 (5.62%)   | 21 (11.8%)   | 5 (2.81%) |
| ITS                | Genus       | OLFS     | 178       | 146 (82.02%) | 3 (1.69%)    | 23 (12.92%)  | 6 (3.37%) |
| ITS                | Species     | GenBank  | 178       | 53 (29.78%)  | 59 (33.15%)  | 62 (34.83%)  | 4 (2.25%) |
| ITS                | Species     | Regional | 178       | 86 (48.31%)  | 33 (18.54%)  | 54 (30.34%)  | 5 (2.81%) |
| ITS                | Species     | OLFS     | 178       | 122 (68.54%) | 12 (6.74%)   | 38 (21.35%)  | 6 (3.37%) |
| trnH               | Family      | GenBank  | 138       | 123 (89.13%) | 2 (1.45%)    | 13 (9.42%)   | 0         |
| trnH               | Family      | Regional | 138       | 104 (75.36%) | 0            | 34 (24.64%)  | 0         |
| trnH               | Family      | OLFS     | 138       | 92 (66.67%)  | 0            | 43 (31.16%)  | 3 (2.17%) |
| trnH               | Genus       | GenBank  | 138       | 94 (68.12%)  | 14 (10.14%)  | 30 (21.74%)  | 0         |
| trnH               | Genus       | Regional | 138       | 59 (42.75%)  | 2 (1.45%)    | 77 (55.8%)   | 0         |
| trnH               | Genus       | OLFS     | 138       | 39 (28.26%)  | 0            | 96 (69.57%)  | 3 (2.17%) |
| trnH               | Species     | GenBank  | 138       | 32 (23.19%)  | 27 (19.57%)  | 79 (57.25%)  | 0         |
| trnH               | Species     | Regional | 138       | 18 (13.04%)  | 6 (4.35%)    | 114 (82.61%) | 0         |
| trnH               | Species     | OLFS     | 138       | 29 (21.01%)  | 1 (0.72%)    | 105 (76.09%) | 3 (2.17%) |
| matK               | Family      | GenBank  | 112       | 107 (95.54%) | 0            | 5 (4.46%)    | 0         |
| matK               | Family      | Regional | 112       | 104 (92.86%) | 0            | 8 (7.14%)    | 0         |
| matK               | Family      | OLFS     | 112       | 90 (80.36%)  | 0            | 22 (19.64%)  | 0         |
| matK               | Genus       | GenBank  | 112       | 61 (54.46%)  | 11 (9.82%)   | 40 (35.71%)  | 0         |
| matK               | Genus       | Regional | 112       | 69 (61.61%)  | 1 (0.89%)    | 42 (37.5%)   | 0         |
| matK               | Genus       | OLFS     | 112       | 45 (40.18%)  | 0            | 67 (59.82%)  | 0         |
| matK               | Species     | GenBank  | 112       | 11 (9.82%)   | 16 (14.29%)  | 85 (75.89%)  | 0         |
| matK               | Species     | Regional | 112       | 21 (18.75%)  | 4 (3.57%)    | 87 (77.68%)  | 0         |
| matK               | Species     | OLFS     | 112       | 28 (25.00%)  | 0            | 84 (75.00%)  | 0         |
| rbcLa              | Family      | GenBank  | 249       | 220 (88.35%) | 24 (9.64%)   | 5 (2.01%)    | 0         |
| rbcLa              | Family      | Regional | 249       | 240 (96.39%) | 3 (1.20%)    | 5 (2.01%)    | 1 (0.4%)  |
| rbcLa              | Family      | OLFS     | 249       | 240 (96.39%) | 0            | 8 (3.21%)    | 1 (0.4%)  |
| rbcLa              | Genus       | GenBank  | 249       | 135 (54.22%) | 99 (39.76%)  | 15 (6.02%)   | 0         |
| rbcLa              | Genus       | Regional | 249       | 181 (72.69%) | 50 (20.08%)  | 17 (6.83%)   | 1 (0.4%)  |
| rbcLa              | Genus       | OLFS     | 249       | 215 (86.35%) | 14 (5.62%)   | 19 (7.63%)   | 1 (0.4%)  |
| rbcLa              | Species     | GenBank  | 249       | 20 (8.03%)   | 164 (65.86%) | 65 (26.1%)   | 0         |
| rbcLa              | Species     | Regional | 249       | 46 (18.47%)  | 120 (48.19%) | 82 (32.93%)  | 1 (0.4%)  |
| rbcLa              | Species     | OLFS     | 249       | 138 (55.42%) | 68 (27.31%)  | 42 (16.87%)  | 1 (0.4%)  |
| ITS_trnH           | Family      | GenBank  | 96        | 84 (87.5%)   | 8 (8.33%)    | 4 (4.17%)    | 0         |
| ITS_trnH           | Family      | Regional | 96        | 91 (94.79%)  | 1 (1.04%)    | 4 (4.17%)    | 0         |
| ITS_trnH           | Family      | OLFS     | 96        | 89 (92.71%)  | 0            | 7 (7.29%)    | 0         |
| ITS_trnH           | Genus       | GenBank  | 96        | 69 (71.88%)  | 12 (12.5%)   | 15 (15.62%)  | 0         |
| ITS_trnH           | Genus       | Regional | 96        | 75 (78.12%)  | 7 (7.29%)    | 14 (14.58%)  | 0         |
| ITS_trnH           | Genus       | OLFS     | 96        | 72 (75%)     | 2 (2.08%)    | 22 (22.92%)  | 0         |
| ITS_trnH           | Species     | GenBank  | 96        | 34 (35.42%)  | 20 (20.83%)  | 42 (43.75%)  | 0         |
| ITS_trnH           | Species     | Regional | 96        | 48 (50%)     | 14 (14.58%)  | 34 (35.42%)  | 0         |
| ITS_trnH           | Species     | OLFS     | 96        | 61 (63.54%)  | 4 (4.17%)    | 31 (32.29%)  | 0         |
| ITS_matK           | Family      | GenBank  | 75        | 66 (88%)     | 3 (4%)       | 6 (8%)       | 0         |
| ITS_matK           | Family      | Regional | 75        | 68 (90.67%)  | 0            | 7 (9.33%)    | 0         |
| ITS_matK           | Family      | OLFS     | 75        | 70 (93.33%)  | 0            | 5 (6.67%)    | 0         |
| ITS_matK           | Genus       | GenBank  | 75        | 48 (64%)     | 7 (9.33%)    | 20 (26.67%)  | 0         |
| ITS_matK           | Genus       | Regional | 75        | 64 (85.33%)  | 1 (1.33%)    | 10 (13.33%)  | 0         |
| ITS_matK           | Genus       | OLFS     | 75        | 56 (74.67%)  | 0            | 19 (25.33%)  | 0         |
| ITS_matK           | Species     | GenBank  | 75        | 18 (24%)     | 17 (22.67%)  | 40 (53.33%)  | 0         |
| ITS_matK           | Species     | Regional | 75        | 42 (56%)     | 12 (16%)     | 21 (28%)     | 0         |
| ITS_matK           | Species     | OLFS     | 75        | 47 (62.67%)  | 2 (2.67%)    | 26 (34.67%)  | 0         |
| ITS_rbcLa          | Family      | GenBank  | 164       | 133 (81.1%)  | 21 (12.8%)   | 10 (6.1%)    | 0         |
| ITS_rbcLa          | Family      | Regional | 164       | 152 (92.68%) | 3 (1.83%)    | 9 (5.49%)    | 0         |
| ITS_rbcLa          | Family      | OLFS     | 164       | 155 (94.51%) | 0            | 9 (5.49%)    | 0         |
| ITS_rbcLa          | Genus       | GenBank  | 164       | 113 (68.9%)  | 32 (19.51%)  | 19 (11.59%)  | 0         |
| ITS_rbcLa          | Genus       | Regional | 164       | 133 (81.1%)  | 12 (7.32%)   | 19 (11.59%)  | 0         |
| ITS_rbcLa          | Genus       | OLFS     | 164       | 141 (85.98%) | 6 (3.66%)    | 17 (10.37%)  | 0         |
| ITS_rbcLa          | Species     | GenBank  | 164       | 43 (26.22%)  | 71 (43.29%)  | 50 (30.49%)  | 0         |

(Continues)

**APPENDIX 3.** Continued

| Locus <sup>a</sup> | Taxon level | Database | Sequences | Confident    | Ambiguous   | Incorrect   | No match |
|--------------------|-------------|----------|-----------|--------------|-------------|-------------|----------|
| ITS_rbcLa          | Species     | Regional | 164       | 72 (43.9%)   | 42 (25.61%) | 50 (30.49%) | 0        |
| ITS_rbcLa          | Species     | OLFS     | 164       | 116 (70.73%) | 21 (12.8%)  | 27 (16.46%) | 0        |
| matK_rbcLa         | Family      | GenBank  | 109       | 103 (94.5%)  | 3 (2.75%)   | 3 (2.75%)   | 0        |
| matK_rbcLa         | Family      | Regional | 109       | 105 (96.33%) | 2 (1.83%)   | 2 (1.83%)   | 0        |
| matK_rbcLa         | Family      | OLFS     | 109       | 106 (97.25%) | 0           | 3 (2.75%)   | 0        |
| matK_rbcLa         | Genus       | GenBank  | 109       | 60 (55.05%)  | 19 (17.43%) | 30 (27.52%) | 0        |
| matK_rbcLa         | Genus       | Regional | 109       | 82 (75.23%)  | 20 (18.35%) | 7 (6.42%)   | 0        |
| matK_rbcLa         | Genus       | OLFS     | 109       | 95 (87.16%)  | 4 (3.67%)   | 10 (9.17%)  | 0        |
| matK_rbcLa         | Species     | GenBank  | 109       | 13 (11.93%)  | 36 (33.03%) | 60 (55.05%) | 0        |
| matK_rbcLa         | Species     | Regional | 109       | 28 (25.69%)  | 53 (48.62%) | 28 (25.69%) | 0        |
| matK_rbcLa         | Species     | OLFS     | 109       | 63 (57.8%)   | 31 (28.44%) | 15 (13.76%) | 0        |
| matK_trnH          | Family      | GenBank  | 57        | 53 (92.98%)  | 0           | 4 (7.02%)   | 0        |
| matK_trnH          | Family      | Regional | 57        | 52 (91.23%)  | 0           | 5 (8.77%)   | 0        |
| matK_trnH          | Family      | OLFS     | 57        | 44 (77.19%)  | 0           | 13 (22.81%) | 0        |
| matK_trnH          | Genus       | GenBank  | 57        | 39 (68.42%)  | 0           | 18 (31.58%) | 0        |
| matK_trnH          | Genus       | Regional | 57        | 40 (70.18%)  | 1 (1.75%)   | 16 (28.07%) | 0        |
| matK_trnH          | Genus       | OLFS     | 57        | 28 (49.12%)  | 0           | 29 (50.88%) | 0        |
| matK_trnH          | Species     | GenBank  | 57        | 15 (26.32%)  | 4 (7.02%)   | 38 (66.67%) | 0        |
| matK_trnH          | Species     | Regional | 57        | 13 (22.81%)  | 2 (3.51%)   | 42 (73.68%) | 0        |
| matK_trnH          | Species     | OLFS     | 57        | 21 (36.84%)  | 0           | 36 (63.16%) | 0        |
| rbcLa_trnH         | Family      | GenBank  | 126       | 114 (90.48%) | 4 (3.17%)   | 8 (6.35%)   | 0        |
| rbcLa_trnH         | Family      | Regional | 126       | 121 (96.03%) | 0           | 5 (3.97%)   | 0        |
| rbcLa_trnH         | Family      | OLFS     | 126       | 119 (94.44%) | 0           | 7 (5.56%)   | 0        |
| rbcLa_trnH         | Genus       | GenBank  | 126       | 82 (65.08%)  | 27 (21.43%) | 17 (13.49%) | 0        |
| rbcLa_trnH         | Genus       | Regional | 126       | 91 (72.22%)  | 22 (17.46%) | 13 (10.32%) | 0        |
| rbcLa_trnH         | Genus       | OLFS     | 126       | 100 (79.37%) | 7 (5.56%)   | 19 (15.08%) | 0        |
| rbcLa_trnH         | Species     | GenBank  | 126       | 29 (23.02%)  | 40 (31.75%) | 57 (45.24%) | 0        |
| rbcLa_trnH         | Species     | Regional | 126       | 32 (25.4%)   | 47 (37.3%)  | 47 (37.3%)  | 0        |
| rbcLa_trnH         | Species     | OLFS     | 126       | 73 (57.94%)  | 28 (22.22%) | 25 (19.84%) | 0        |
| ITS_matK_rbcLa     | Family      | GenBank  | 73        | 65 (89.04%)  | 2 (2.74%)   | 6 (8.22%)   | 0        |
| ITS_matK_rbcLa     | Family      | Regional | 73        | 66 (90.41%)  | 0           | 7 (9.59%)   | 0        |
| ITS_matK_rbcLa     | Family      | OLFS     | 73        | 68 (93.15%)  | 0           | 5 (6.85%)   | 0        |
| ITS_matK_rbcLa     | Genus       | GenBank  | 73        | 44 (60.27%)  | 8 (10.96%)  | 21 (28.77%) | 0        |
| ITS_matK_rbcLa     | Genus       | Regional | 73        | 61 (83.56%)  | 3 (4.11%)   | 9 (12.33%)  | 0        |
| ITS_matK_rbcLa     | Genus       | OLFS     | 73        | 64 (87.67%)  | 1 (1.37%)   | 8 (10.96%)  | 0        |
| ITS_matK_rbcLa     | Species     | GenBank  | 73        | 13 (17.81%)  | 21 (28.77%) | 39 (53.42%) | 0        |
| ITS_matK_rbcLa     | Species     | Regional | 73        | 35 (47.95%)  | 19 (26.03%) | 19 (26.03%) | 0        |
| ITS_matK_rbcLa     | Species     | OLFS     | 73        | 56 (76.71%)  | 7 (9.59%)   | 10 (13.7%)  | 0        |
| ITS_matK_trnH      | Family      | GenBank  | 41        | 40 (97.56%)  | 0           | 1 (2.44%)   | 0        |
| ITS_matK_trnH      | Family      | Regional | 41        | 40 (97.56%)  | 0           | 1 (2.44%)   | 0        |
| ITS_matK_trnH      | Family      | OLFS     | 41        | 40 (97.56%)  | 0           | 1 (2.44%)   | 0        |
| ITS_matK_trnH      | Genus       | GenBank  | 41        | 31 (75.61%)  | 0           | 10 (24.39%) | 0        |
| ITS_matK_trnH      | Genus       | Regional | 41        | 37 (90.24%)  | 2 (4.88%)   | 2 (4.88%)   | 0        |
| ITS_matK_trnH      | Genus       | OLFS     | 41        | 30 (73.17%)  | 0           | 11 (26.83%) | 0        |
| ITS_matK_trnH      | Species     | GenBank  | 41        | 18 (43.9%)   | 4 (9.76%)   | 19 (46.34%) | 0        |
| ITS_matK_trnH      | Species     | Regional | 41        | 22 (53.66%)  | 7 (17.07%)  | 12 (29.27%) | 0        |
| ITS_matK_trnH      | Species     | OLFS     | 41        | 27 (65.85%)  | 0           | 14 (34.15%) | 0        |
| ITS_rbcLa_trnH     | Family      | GenBank  | 91        | 83 (91.21%)  | 6 (6.59%)   | 2 (2.2%)    | 0        |
| ITS_rbcLa_trnH     | Family      | Regional | 91        | 88 (96.7%)   | 1 (1.1%)    | 2 (2.2%)    | 0        |
| ITS_rbcLa_trnH     | Family      | OLFS     | 91        | 87 (95.6%)   | 0           | 4 (4.4%)    | 0        |
| ITS_rbcLa_trnH     | Genus       | GenBank  | 91        | 67 (73.63%)  | 13 (14.29%) | 11 (12.09%) | 0        |
| ITS_rbcLa_trnH     | Genus       | Regional | 91        | 73 (80.22%)  | 8 (8.79%)   | 10 (10.99%) | 0        |
| ITS_rbcLa_trnH     | Genus       | OLFS     | 91        | 76 (83.52%)  | 4 (4.4%)    | 11 (12.09%) | 0        |
| ITS_rbcLa_trnH     | Species     | GenBank  | 91        | 32 (35.16%)  | 23 (25.27%) | 36 (39.56%) | 0        |
| ITS_rbcLa_trnH     | Species     | Regional | 91        | 41 (45.05%)  | 18 (19.78%) | 32 (35.16%) | 0        |
| ITS_rbcLa_trnH     | Species     | OLFS     | 91        | 63 (69.23%)  | 9 (9.89%)   | 19 (20.88%) | 0        |
| matK_rbcLa_trnH    | Family      | GenBank  | 56        | 51 (91.07%)  | 1 (1.79%)   | 4 (7.14%)   | 0        |
| matK_rbcLa_trnH    | Family      | Regional | 56        | 54 (96.43%)  | 0           | 2 (3.57%)   | 0        |
| matK_rbcLa_trnH    | Family      | OLFS     | 56        | 55 (98.21%)  | 0           | 1 (1.79%)   | 0        |
| matK_rbcLa_trnH    | Genus       | GenBank  | 56        | 39 (69.64%)  | 2 (3.57%)   | 15 (26.79%) | 0        |
| matK_rbcLa_trnH    | Genus       | Regional | 56        | 45 (80.36%)  | 7 (12.5%)   | 4 (7.14%)   | 0        |
| matK_rbcLa_trnH    | Genus       | OLFS     | 56        | 48 (85.71%)  | 2 (3.57%)   | 6 (10.71%)  | 0        |

(Continues)

**APPENDIX 3.** Continued

| Locus <sup>a</sup>  | Taxon level | Database | Sequences | Confident   | Ambiguous   | Incorrect   | No match |
|---------------------|-------------|----------|-----------|-------------|-------------|-------------|----------|
| matK_rbcLa_trnH     | Species     | GenBank  | 56        | 16 (28.57%) | 8 (14.29%)  | 32 (57.14%) | 0        |
| matK_rbcLa_trnH     | Species     | Regional | 56        | 18 (32.14%) | 17 (30.36%) | 21 (37.5%)  | 0        |
| matK_rbcLa_trnH     | Species     | OLFS     | 56        | 34 (60.71%) | 14 (25%)    | 8 (14.29%)  | 0        |
| ITS_matK_rbcLa_trnH | Family      | GenBank  | 40        | 39 (97.5%)  | 0           | 1 (2.5%)    | 0        |
| ITS_matK_rbcLa_trnH | Family      | Regional | 40        | 39 (97.5%)  | 0           | 1 (2.5%)    | 0        |
| ITS_matK_rbcLa_trnH | Family      | OLFS     | 40        | 39 (97.5%)  | 0           | 1 (2.5%)    | 0        |
| ITS_matK_rbcLa_trnH | Genus       | GenBank  | 40        | 31 (77.5%)  | 0           | 9 (22.5%)   | 0        |
| ITS_matK_rbcLa_trnH | Genus       | Regional | 40        | 35 (87.5%)  | 3 (7.5%)    | 2 (5%)      | 0        |
| ITS_matK_rbcLa_trnH | Genus       | OLFS     | 40        | 36 (90%)    | 1 (2.5%)    | 3 (7.5%)    | 0        |
| ITS_matK_rbcLa_trnH | Species     | GenBank  | 40        | 16 (40%)    | 5 (12.5%)   | 19 (47.5%)  | 0        |
| ITS_matK_rbcLa_trnH | Species     | Regional | 40        | 20 (50%)    | 8 (20%)     | 12 (30%)    | 0        |
| ITS_matK_rbcLa_trnH | Species     | OLFS     | 40        | 32 (80%)    | 3 (7.5%)    | 5 (12.5%)   | 0        |

Note: OLFS = database of species sampled from Oak Lake Field Station.

<sup>a</sup>Multi-region barcodes are indicated by an underscore.**APPENDIX 4.** Identification success for tested barcodes (individual and concatenated regions) for tallgrass prairie plant species in Asteraceae, Cyperaceae, Fabaceae, and Poaceae, listing the number of retrieved sequences and the number of correctly identified sequences at the family, genus, and species levels for the three tested databases.

| Family     | Locus <sup>a</sup> | Taxon level | Database | Sequences | Confident   | Ambiguous   | Incorrect   | No match |
|------------|--------------------|-------------|----------|-----------|-------------|-------------|-------------|----------|
| Asteraceae | ITS                | Family      | GenBank  | 28        | 25 (89.29%) | 3 (10.71%)  | 0           | 0        |
| Asteraceae | ITS                | Family      | Regional | 28        | 28 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS                | Family      | OLFS     | 28        | 28 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS                | Genus       | GenBank  | 28        | 21 (75%)    | 6 (21.43%)  | 1 (3.57%)   | 0        |
| Asteraceae | ITS                | Genus       | Regional | 28        | 26 (92.86%) | 2 (7.14%)   | 0           | 0        |
| Asteraceae | ITS                | Genus       | OLFS     | 28        | 26 (92.86%) | 1 (3.57%)   | 1 (3.57%)   | 0        |
| Asteraceae | ITS                | Species     | GenBank  | 28        | 6 (21.43%)  | 17 (60.71%) | 5 (17.86%)  | 0        |
| Asteraceae | ITS                | Species     | Regional | 28        | 15 (53.57%) | 11 (39.29%) | 2 (7.14%)   | 0        |
| Asteraceae | ITS                | Species     | OLFS     | 28        | 23 (82.14%) | 3 (10.71%)  | 2 (7.14%)   | 0        |
| Asteraceae | trnH               | Family      | GenBank  | 32        | 31 (96.88%) | 0           | 1 (3.12%)   | 0        |
| Asteraceae | trnH               | Family      | Regional | 32        | 31 (96.88%) | 0           | 1 (3.12%)   | 0        |
| Asteraceae | trnH               | Family      | OLFS     | 32        | 31 (96.88%) | 0           | 1 (3.12%)   | 0        |
| Asteraceae | trnH               | Genus       | GenBank  | 32        | 24 (75%)    | 1 (3.12%)   | 7 (21.88%)  | 0        |
| Asteraceae | trnH               | Genus       | Regional | 32        | 20 (62.5%)  | 1 (3.12%)   | 11 (34.38%) | 0        |
| Asteraceae | trnH               | Genus       | OLFS     | 32        | 15 (46.88%) | 0           | 17 (53.12%) | 0        |
| Asteraceae | trnH               | Species     | GenBank  | 32        | 10 (31.25%) | 4 (12.5%)   | 18 (56.25%) | 0        |
| Asteraceae | trnH               | Species     | Regional | 32        | 8 (25%)     | 2 (6.25%)   | 22 (68.75%) | 0        |
| Asteraceae | trnH               | Species     | OLFS     | 32        | 11 (34.38%) | 0           | 21 (65.62%) | 0        |
| Asteraceae | matK               | Family      | GenBank  | 35        | 35 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK               | Family      | Regional | 35        | 35 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK               | Family      | OLFS     | 35        | 35 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK               | Genus       | GenBank  | 35        | 7 (20%)     | 6 (17.14%)  | 22 (62.86%) | 0        |
| Asteraceae | matK               | Genus       | Regional | 35        | 15 (42.86%) | 1 (2.86%)   | 19 (54.29%) | 0        |
| Asteraceae | matK               | Genus       | OLFS     | 35        | 7 (20%)     | 0           | 28 (80%)    | 0        |
| Asteraceae | matK               | Species     | GenBank  | 35        | 1 (2.86%)   | 3 (8.57%)   | 31 (88.57%) | 0        |
| Asteraceae | matK               | Species     | Regional | 35        | 3 (8.57%)   | 1 (2.86%)   | 31 (88.57%) | 0        |
| Asteraceae | matK               | Species     | OLFS     | 35        | 5 (14.29%)  | 0           | 30 (85.71%) | 0        |
| Asteraceae | rbcLa              | Family      | GenBank  | 43        | 36 (83.72%) | 7 (16.28%)  | 0           | 0        |
| Asteraceae | rbcLa              | Family      | Regional | 43        | 40 (93.02%) | 3 (6.98%)   | 0           | 0        |
| Asteraceae | rbcLa              | Family      | OLFS     | 43        | 43 (100%)   | 0           | 0           | 0        |
| Asteraceae | rbcLa              | Genus       | GenBank  | 43        | 12 (27.91%) | 30 (69.77%) | 1 (2.33%)   | 0        |
| Asteraceae | rbcLa              | Genus       | Regional | 43        | 21 (48.84%) | 20 (46.51%) | 2 (4.65%)   | 0        |
| Asteraceae | rbcLa              | Genus       | OLFS     | 43        | 35 (81.4%)  | 3 (6.98%)   | 5 (11.63%)  | 0        |
| Asteraceae | rbcLa              | Species     | GenBank  | 43        | 2 (4.65%)   | 36 (83.72%) | 5 (11.63%)  | 0        |
| Asteraceae | rbcLa              | Species     | Regional | 43        | 4 (9.3%)    | 26 (60.47%) | 13 (30.23%) | 0        |
| Asteraceae | rbcLa              | Species     | OLFS     | 43        | 15 (34.88%) | 20 (46.51%) | 8 (18.6%)   | 0        |
| Asteraceae | ITS_trnH           | Family      | GenBank  | 22        | 22 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_trnH           | Family      | Regional | 22        | 22 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_trnH           | Family      | OLFS     | 22        | 22 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_trnH           | Genus       | GenBank  | 22        | 16 (72.73%) | 3 (13.64%)  | 3 (13.64%)  | 0        |
| Asteraceae | ITS_trnH           | Genus       | Regional | 22        | 17 (77.27%) | 3 (13.64%)  | 2 (9.09%)   | 0        |
| Asteraceae | ITS_trnH           | Genus       | OLFS     | 22        | 14 (63.64%) | 1 (4.55%)   | 7 (31.82%)  | 0        |

(Continues)

**APPENDIX 4.** Continued

| Family     | Locus <sup>a</sup> | Taxon level | Database | Sequences | Confident   | Ambiguous   | Incorrect   | No match |
|------------|--------------------|-------------|----------|-----------|-------------|-------------|-------------|----------|
| Asteraceae | ITS_trnH           | Species     | GenBank  | 22        | 6 (27.27%)  | 4 (18.18%)  | 12 (54.55%) | 0        |
| Asteraceae | ITS_trnH           | Species     | Regional | 22        | 7 (31.82%)  | 7 (31.82%)  | 8 (36.36%)  | 0        |
| Asteraceae | ITS_trnH           | Species     | OLFS     | 22        | 11 (50%)    | 2 (9.09%)   | 9 (40.91%)  | 0        |
| Asteraceae | ITS_matK           | Family      | GenBank  | 20        | 19 (95%)    | 1 (5%)      | 0           | 0        |
| Asteraceae | ITS_matK           | Family      | Regional | 20        | 20 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK           | Family      | OLFS     | 20        | 20 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK           | Genus       | GenBank  | 20        | 8 (40%)     | 4 (20%)     | 8 (40%)     | 0        |
| Asteraceae | ITS_matK           | Genus       | Regional | 20        | 20 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK           | Genus       | OLFS     | 20        | 9 (45%)     | 0           | 11 (55%)    | 0        |
| Asteraceae | ITS_matK           | Species     | GenBank  | 20        | 2 (10%)     | 6 (30%)     | 12 (60%)    | 0        |
| Asteraceae | ITS_matK           | Species     | Regional | 20        | 11 (55%)    | 7 (35%)     | 2 (10%)     | 0        |
| Asteraceae | ITS_matK           | Species     | OLFS     | 20        | 8 (40%)     | 1 (5%)      | 11 (55%)    | 0        |
| Asteraceae | ITS_rbcLa          | Family      | GenBank  | 24        | 21 (87.5%)  | 3 (12.5%)   | 0           | 0        |
| Asteraceae | ITS_rbcLa          | Family      | Regional | 24        | 24 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_rbcLa          | Family      | OLFS     | 24        | 24 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_rbcLa          | Genus       | GenBank  | 24        | 19 (79.17%) | 4 (16.67%)  | 1 (4.17%)   | 0        |
| Asteraceae | ITS_rbcLa          | Genus       | Regional | 24        | 23 (95.83%) | 1 (4.17%)   | 0           | 0        |
| Asteraceae | ITS_rbcLa          | Genus       | OLFS     | 24        | 22 (91.67%) | 1 (4.17%)   | 1 (4.17%)   | 0        |
| Asteraceae | ITS_rbcLa          | Species     | GenBank  | 24        | 6 (25%)     | 13 (54.17%) | 5 (20.83%)  | 0        |
| Asteraceae | ITS_rbcLa          | Species     | Regional | 24        | 13 (54.17%) | 9 (37.5%)   | 2 (8.33%)   | 0        |
| Asteraceae | ITS_rbcLa          | Species     | OLFS     | 24        | 19 (79.17%) | 3 (12.5%)   | 2 (8.33%)   | 0        |
| Asteraceae | matK_rbcLa         | Family      | GenBank  | 35        | 35 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK_rbcLa         | Family      | Regional | 35        | 33 (94.29%) | 2 (5.71%)   | 0           | 0        |
| Asteraceae | matK_rbcLa         | Family      | OLFS     | 35        | 35 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK_rbcLa         | Genus       | GenBank  | 35        | 8 (22.86%)  | 8 (22.86%)  | 19 (54.29%) | 0        |
| Asteraceae | matK_rbcLa         | Genus       | Regional | 35        | 16 (45.71%) | 17 (48.57%) | 2 (5.71%)   | 0        |
| Asteraceae | matK_rbcLa         | Genus       | OLFS     | 35        | 27 (77.14%) | 3 (8.57%)   | 5 (14.29%)  | 0        |
| Asteraceae | matK_rbcLa         | Species     | GenBank  | 35        | 3 (8.57%)   | 4 (11.43%)  | 28 (80%)    | 0        |
| Asteraceae | matK_rbcLa         | Species     | Regional | 35        | 4 (11.43%)  | 19 (54.29%) | 12 (34.29%) | 0        |
| Asteraceae | matK_rbcLa         | Species     | OLFS     | 35        | 12 (34.29%) | 15 (42.86%) | 8 (22.86%)  | 0        |
| Asteraceae | matK_trnH          | Family      | GenBank  | 24        | 24 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK_trnH          | Family      | Regional | 24        | 24 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK_trnH          | Family      | OLFS     | 24        | 24 (100%)   | 0           | 0           | 0        |
| Asteraceae | matK_trnH          | Genus       | GenBank  | 24        | 14 (58.33%) | 0           | 10 (41.67%) | 0        |
| Asteraceae | matK_trnH          | Genus       | Regional | 24        | 16 (66.67%) | 1 (4.17%)   | 7 (29.17%)  | 0        |
| Asteraceae | matK_trnH          | Genus       | OLFS     | 24        | 10 (41.67%) | 0           | 14 (58.33%) | 0        |
| Asteraceae | matK_trnH          | Species     | GenBank  | 24        | 6 (25%)     | 1 (4.17%)   | 17 (70.83%) | 0        |
| Asteraceae | matK_trnH          | Species     | Regional | 24        | 6 (25%)     | 1 (4.17%)   | 17 (70.83%) | 0        |
| Asteraceae | matK_trnH          | Species     | OLFS     | 24        | 8 (33.33%)  | 0           | 16 (66.67%) | 0        |
| Asteraceae | rbcLa_trnH         | Family      | GenBank  | 29        | 29 (100%)   | 0           | 0           | 0        |
| Asteraceae | rbcLa_trnH         | Family      | Regional | 29        | 29 (100%)   | 0           | 0           | 0        |
| Asteraceae | rbcLa_trnH         | Family      | OLFS     | 29        | 29 (100%)   | 0           | 0           | 0        |
| Asteraceae | rbcLa_trnH         | Genus       | GenBank  | 29        | 22 (75.86%) | 4 (13.79%)  | 3 (10.34%)  | 0        |
| Asteraceae | rbcLa_trnH         | Genus       | Regional | 29        | 21 (72.41%) | 5 (17.24%)  | 3 (10.34%)  | 0        |
| Asteraceae | rbcLa_trnH         | Genus       | OLFS     | 29        | 20 (68.97%) | 1 (3.45%)   | 8 (27.59%)  | 0        |
| Asteraceae | rbcLa_trnH         | Species     | GenBank  | 29        | 8 (27.59%)  | 5 (17.24%)  | 16 (55.17%) | 0        |
| Asteraceae | rbcLa_trnH         | Species     | Regional | 29        | 8 (27.59%)  | 5 (17.24%)  | 16 (55.17%) | 0        |
| Asteraceae | rbcLa_trnH         | Species     | OLFS     | 29        | 13 (44.83%) | 7 (24.14%)  | 9 (31.03%)  | 0        |
| Asteraceae | ITS_matK_rbcLa     | Family      | GenBank  | 20        | 19 (95%)    | 1 (5%)      | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa     | Family      | Regional | 20        | 20 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa     | Family      | OLFS     | 20        | 20 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa     | Genus       | GenBank  | 20        | 9 (45%)     | 4 (20%)     | 7 (35%)     | 0        |
| Asteraceae | ITS_matK_rbcLa     | Genus       | Regional | 20        | 20 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa     | Genus       | OLFS     | 20        | 19 (95%)    | 0           | 1 (5%)      | 0        |
| Asteraceae | ITS_matK_rbcLa     | Species     | GenBank  | 20        | 2 (10%)     | 6 (30%)     | 12 (60%)    | 0        |
| Asteraceae | ITS_matK_rbcLa     | Species     | Regional | 20        | 11 (55%)    | 7 (35%)     | 2 (10%)     | 0        |
| Asteraceae | ITS_matK_rbcLa     | Species     | OLFS     | 20        | 17 (85%)    | 1 (5%)      | 2 (10%)     | 0        |
| Asteraceae | ITS_matK_trnH      | Family      | GenBank  | 17        | 17 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK_trnH      | Family      | Regional | 17        | 17 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK_trnH      | Family      | OLFS     | 17        | 17 (100%)   | 0           | 0           | 0        |
| Asteraceae | ITS_matK_trnH      | Genus       | GenBank  | 17        | 11 (64.71%) | 0           | 6 (35.29%)  | 0        |
| Asteraceae | ITS_matK_trnH      | Genus       | Regional | 17        | 15 (88.24%) | 1 (5.88%)   | 1 (5.88%)   | 0        |

(Continues)

## APPENDIX 4. Continued

| Family     | Locus <sup>a</sup>  | Taxon level | Database | Sequences | Confident   | Ambiguous  | Incorrect   | No match |
|------------|---------------------|-------------|----------|-----------|-------------|------------|-------------|----------|
| Asteraceae | ITS_matK_trnH       | Genus       | OLFS     | 17        | 7 (41.18%)  | 0          | 10 (58.82%) | 0        |
| Asteraceae | ITS_matK_trnH       | Species     | GenBank  | 17        | 4 (23.53%)  | 1 (5.88%)  | 12 (70.59%) | 0        |
| Asteraceae | ITS_matK_trnH       | Species     | Regional | 17        | 6 (35.29%)  | 4 (23.53%) | 7 (41.18%)  | 0        |
| Asteraceae | ITS_matK_trnH       | Species     | OLFS     | 17        | 6 (35.29%)  | 0          | 11 (64.71%) | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Family      | GenBank  | 20        | 20 (100%)   | 0          | 0           | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Family      | Regional | 20        | 20 (100%)   | 0          | 0           | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Family      | OLFS     | 20        | 20 (100%)   | 0          | 0           | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Genus       | GenBank  | 20        | 16 (80%)    | 1 (5%)     | 3 (15%)     | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Genus       | Regional | 20        | 17 (85%)    | 1 (5%)     | 2 (10%)     | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Genus       | OLFS     | 20        | 16 (80%)    | 1 (5%)     | 3 (15%)     | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Species     | GenBank  | 20        | 5 (25%)     | 2 (10%)    | 13 (65%)    | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Species     | Regional | 20        | 7 (35%)     | 4 (20%)    | 9 (45%)     | 0        |
| Asteraceae | ITS_rbcLa_trnH      | Species     | OLFS     | 20        | 13 (65%)    | 2 (10%)    | 5 (25%)     | 0        |
| Asteraceae | matK_rbcLa_trnH     | Family      | GenBank  | 24        | 24 (100%)   | 0          | 0           | 0        |
| Asteraceae | matK_rbcLa_trnH     | Family      | Regional | 24        | 24 (100%)   | 0          | 0           | 0        |
| Asteraceae | matK_rbcLa_trnH     | Family      | OLFS     | 24        | 24 (100%)   | 0          | 0           | 0        |
| Asteraceae | matK_rbcLa_trnH     | Genus       | GenBank  | 24        | 15 (62.5%)  | 1 (4.17%)  | 8 (33.33%)  | 0        |
| Asteraceae | matK_rbcLa_trnH     | Genus       | Regional | 24        | 17 (70.83%) | 5 (20.83%) | 2 (8.33%)   | 0        |
| Asteraceae | matK_rbcLa_trnH     | Genus       | OLFS     | 24        | 18 (75%)    | 1 (4.17%)  | 5 (20.83%)  | 0        |
| Asteraceae | matK_rbcLa_trnH     | Species     | GenBank  | 24        | 7 (29.17%)  | 2 (8.33%)  | 15 (62.5%)  | 0        |
| Asteraceae | matK_rbcLa_trnH     | Species     | Regional | 24        | 7 (29.17%)  | 4 (16.67%) | 13 (54.17%) | 0        |
| Asteraceae | matK_rbcLa_trnH     | Species     | OLFS     | 24        | 11 (45.83%) | 7 (29.17%) | 6 (25%)     | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Family      | GenBank  | 17        | 17 (100%)   | 0          | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Family      | Regional | 17        | 17 (100%)   | 0          | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Family      | OLFS     | 17        | 17 (100%)   | 0          | 0           | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Genus       | GenBank  | 17        | 13 (76.47%) | 0          | 4 (23.53%)  | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Genus       | Regional | 17        | 16 (94.12%) | 0          | 1 (5.88%)   | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Genus       | OLFS     | 17        | 15 (88.24%) | 0          | 2 (11.76%)  | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Species     | GenBank  | 17        | 5 (29.41%)  | 1 (5.88%)  | 11 (64.71%) | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Species     | Regional | 17        | 7 (41.18%)  | 3 (17.65%) | 7 (41.18%)  | 0        |
| Asteraceae | ITS_matK_rbcLa_trnH | Species     | OLFS     | 17        | 13 (76.47%) | 0          | 4 (23.53%)  | 0        |
| Cyperaceae | ITS                 | Family      | GenBank  | 7         | 6 (85.71%)  | 1 (14.29%) | 0           | 0        |
| Cyperaceae | ITS                 | Family      | Regional | 7         | 7 (100%)    | 0          | 0           | 0        |
| Cyperaceae | ITS                 | Family      | OLFS     | 7         | 7 (100%)    | 0          | 0           | 0        |
| Cyperaceae | ITS                 | Genus       | GenBank  | 7         | 6 (85.71%)  | 1 (14.29%) | 0           | 0        |
| Cyperaceae | ITS                 | Genus       | Regional | 7         | 7 (100%)    | 0          | 0           | 0        |
| Cyperaceae | ITS                 | Genus       | OLFS     | 7         | 7 (100%)    | 0          | 0           | 0        |
| Cyperaceae | ITS                 | Species     | GenBank  | 7         | 1 (14.29%)  | 2 (28.57%) | 4 (57.14%)  | 0        |
| Cyperaceae | ITS                 | Species     | Regional | 7         | 2 (28.57%)  | 2 (28.57%) | 3 (42.86%)  | 0        |
| Cyperaceae | ITS                 | Species     | OLFS     | 7         | 4 (57.14%)  | 1 (14.29%) | 2 (28.57%)  | 0        |
| Cyperaceae | trnH                | Family      | GenBank  | 5         | 2 (40%)     | 0          | 3 (60%)     | 0        |
| Cyperaceae | trnH                | Family      | Regional | 5         | 2 (40%)     | 0          | 3 (60%)     | 0        |
| Cyperaceae | trnH                | Family      | OLFS     | 5         | 0           | 0          | 4 (80%)     | 1 (20%)  |
| Cyperaceae | trnH                | Genus       | GenBank  | 5         | 2 (40%)     | 0          | 3 (60%)     | 0        |
| Cyperaceae | trnH                | Genus       | Regional | 5         | 0           | 0          | 4 (80%)     | 1 (20%)  |
| Cyperaceae | trnH                | Genus       | OLFS     | 5         | 0           | 0          | 5 (100%)    | 0        |
| Cyperaceae | trnH                | Species     | OLFS     | 5         | 0           | 0          | 4 (80%)     | 1 (20%)  |
| Cyperaceae | matK                | Family      | GenBank  | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Family      | Regional | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Family      | OLFS     | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Genus       | GenBank  | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Genus       | Regional | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Genus       | OLFS     | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Species     | GenBank  | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Species     | Regional | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | matK                | Species     | OLFS     | 1         | 0           | 0          | 1 (100%)    | 0        |
| Cyperaceae | rbcLa               | Family      | GenBank  | 24        | 23 (95.83%) | 0          | 1 (4.17%)   | 0        |
| Cyperaceae | rbcLa               | Family      | Regional | 24        | 23 (95.83%) | 0          | 1 (4.17%)   | 0        |
| Cyperaceae | rbcLa               | Family      | OLFS     | 24        | 23 (95.83%) | 0          | 1 (4.17%)   | 0        |
| Cyperaceae | rbcLa               | Genus       | GenBank  | 24        | 22 (91.67%) | 1 (4.17%)  | 1 (4.17%)   | 0        |

(Continues)

**APPENDIX 4.** Continued

| <b>Family</b> | <b>Locus<sup>a</sup></b> | <b>Taxon level</b> | <b>Database</b> | <b>Sequences</b> | <b>Confident</b> | <b>Ambiguous</b> | <b>Incorrect</b> | <b>No match</b> |
|---------------|--------------------------|--------------------|-----------------|------------------|------------------|------------------|------------------|-----------------|
| Cyperaceae    | rbcLa                    | Genus              | Regional        | 24               | 22 (91.67%)      | 1 (4.17%)        | 1 (4.17%)        | 0               |
| Cyperaceae    | rbcLa                    | Genus              | OLFS            | 24               | 23 (95.83%)      | 0                | 1 (4.17%)        | 0               |
| Cyperaceae    | rbcLa                    | Species            | GenBank         | 24               | 0                | 5 (20.83%)       | 19 (79.17%)      | 0               |
| Cyperaceae    | rbcLa                    | Species            | Regional        | 24               | 2 (8.33%)        | 6 (25%)          | 16 (66.67%)      | 0               |
| Cyperaceae    | rbcLa                    | Species            | OLFS            | 24               | 8 (33.33%)       | 3 (12.5%)        | 13 (54.17%)      | 0               |
| Cyperaceae    | ITS_trnH                 | Family             | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Family             | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Family             | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Genus              | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Genus              | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Genus              | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Species            | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Species            | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_trnH                 | Species            | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Family             | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Family             | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Family             | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Genus              | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Genus              | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Genus              | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Species            | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Species            | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK                 | Species            | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Family             | GenBank         | 7                | 6 (85.71%)       | 1 (14.29%)       | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Family             | Regional        | 7                | 7 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Family             | OLFS            | 7                | 7 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Genus              | GenBank         | 7                | 6 (85.71%)       | 1 (14.29%)       | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Genus              | Regional        | 7                | 7 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Genus              | OLFS            | 7                | 7 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_rbcLa                | Species            | GenBank         | 7                | 1 (14.29%)       | 2 (28.57%)       | 4 (57.14%)       | 0               |
| Cyperaceae    | ITS_rbcLa                | Species            | Regional        | 7                | 2 (28.57%)       | 2 (28.57%)       | 3 (42.86%)       | 0               |
| Cyperaceae    | ITS_rbcLa                | Species            | OLFS            | 7                | 5 (71.43%)       | 1 (14.29%)       | 1 (14.29%)       | 0               |
| Cyperaceae    | matK_rbcLa               | Family             | GenBank         | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_rbcLa               | Family             | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | matK_rbcLa               | Family             | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | matK_rbcLa               | Genus              | GenBank         | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_rbcLa               | Genus              | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | matK_rbcLa               | Genus              | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | matK_rbcLa               | Species            | GenBank         | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_rbcLa               | Species            | Regional        | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_rbcLa               | Species            | OLFS            | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Family             | GenBank         | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Family             | Regional        | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Family             | OLFS            | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Genus              | GenBank         | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Genus              | Regional        | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Genus              | OLFS            | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Species            | GenBank         | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Species            | Regional        | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | matK_trnH                | Species            | OLFS            | 1                | 0                | 0                | 1 (100%)         | 0               |
| Cyperaceae    | rbcLa_trnH               | Family             | GenBank         | 4                | 2 (50%)          | 0                | 2 (50%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Family             | Regional        | 4                | 3 (75%)          | 0                | 1 (25%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Family             | OLFS            | 4                | 3 (75%)          | 0                | 1 (25%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Genus              | GenBank         | 4                | 2 (50%)          | 0                | 2 (50%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Genus              | Regional        | 4                | 3 (75%)          | 0                | 1 (25%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Genus              | OLFS            | 4                | 3 (75%)          | 0                | 1 (25%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Species            | GenBank         | 4                | 0                | 1 (25%)          | 3 (75%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Species            | Regional        | 4                | 0                | 2 (50%)          | 2 (50%)          | 0               |
| Cyperaceae    | rbcLa_trnH               | Species            | OLFS            | 4                | 2 (50%)          | 0                | 2 (50%)          | 0               |
| Cyperaceae    | ITS_matK_rbcLa           | Family             | GenBank         | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK_rbcLa           | Family             | Regional        | 1                | 1 (100%)         | 0                | 0                | 0               |
| Cyperaceae    | ITS_matK_rbcLa           | Family             | OLFS            | 1                | 1 (100%)         | 0                | 0                | 0               |

(Continues)

**APPENDIX 4.** Continued

| Family     | Locus <sup>a</sup>  | Taxon level | Database | Sequences | Confident   | Ambiguous  | Incorrect  | No match  |
|------------|---------------------|-------------|----------|-----------|-------------|------------|------------|-----------|
| Cyperaceae | ITS_matK_rbcLa      | Genus       | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa      | Genus       | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa      | Genus       | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa      | Species     | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa      | Species     | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa      | Species     | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Family      | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Family      | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Family      | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Genus       | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Genus       | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Genus       | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Species     | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Species     | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_trnH       | Species     | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Family      | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Family      | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Family      | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Genus       | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Genus       | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Genus       | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Species     | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Species     | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_rbcLa_trnH      | Species     | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Family      | GenBank  | 1         | 0           | 0          | 1 (100%)   | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Family      | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Family      | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Genus       | GenBank  | 1         | 0           | 0          | 1 (100%)   | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Genus       | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Genus       | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Species     | GenBank  | 1         | 0           | 0          | 1 (100%)   | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Species     | Regional | 1         | 0           | 0          | 1 (100%)   | 0         |
| Cyperaceae | matK_rbcLa_trnH     | Species     | OLFS     | 1         | 0           | 0          | 1 (100%)   | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Family      | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Family      | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Family      | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Genus       | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Genus       | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Genus       | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Species     | GenBank  | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Species     | Regional | 1         | 1 (100%)    | 0          | 0          | 0         |
| Cyperaceae | ITS_matK_rbcLa_trnH | Species     | OLFS     | 1         | 1 (100%)    | 0          | 0          | 0         |
| Fabaceae   | ITS                 | Family      | GenBank  | 15        | 14 (93.33%) | 1 (6.67%)  | 0          | 0         |
| Fabaceae   | ITS                 | Family      | Regional | 15        | 14 (93.33%) | 0          | 1 (6.67%)  | 0         |
| Fabaceae   | ITS                 | Family      | OLFS     | 15        | 14 (93.33%) | 0          | 0          | 1 (6.67%) |
| Fabaceae   | ITS                 | Genus       | GenBank  | 15        | 11 (73.33%) | 1 (6.67%)  | 3 (20%)    | 0         |
| Fabaceae   | ITS                 | Genus       | Regional | 15        | 11 (73.33%) | 0          | 4 (26.67%) | 0         |
| Fabaceae   | ITS                 | Genus       | OLFS     | 15        | 11 (73.33%) | 0          | 3 (20%)    | 1 (6.67%) |
| Fabaceae   | ITS                 | Species     | GenBank  | 15        | 7 (46.67%)  | 4 (26.67%) | 4 (26.67%) | 0         |
| Fabaceae   | ITS                 | Species     | Regional | 15        | 9 (60%)     | 1 (6.67%)  | 5 (33.33%) | 0         |
| Fabaceae   | ITS                 | Species     | OLFS     | 15        | 9 (60%)     | 1 (6.67%)  | 4 (26.67%) | 1 (6.67%) |
| Fabaceae   | trnH                | Family      | GenBank  | 4         | 4 (100%)    | 0          | 0          | 0         |
| Fabaceae   | trnH                | Family      | Regional | 4         | 4 (100%)    | 0          | 0          | 0         |
| Fabaceae   | trnH                | Family      | OLFS     | 4         | 4 (100%)    | 0          | 0          | 0         |
| Fabaceae   | trnH                | Genus       | GenBank  | 4         | 4 (100%)    | 0          | 0          | 0         |
| Fabaceae   | trnH                | Genus       | Regional | 4         | 0           | 0          | 4 (100%)   | 0         |
| Fabaceae   | trnH                | Genus       | OLFS     | 4         | 0           | 0          | 4 (100%)   | 0         |
| Fabaceae   | trnH                | Species     | GenBank  | 4         | 0           | 0          | 4 (100%)   | 0         |
| Fabaceae   | trnH                | Species     | Regional | 4         | 0           | 0          | 4 (100%)   | 0         |
| Fabaceae   | trnH                | Species     | OLFS     | 4         | 0           | 0          | 4 (100%)   | 0         |
| Fabaceae   | matK                | Family      | GenBank  | 13        | 13 (100%)   | 0          | 0          | 0         |
| Fabaceae   | matK                | Family      | Regional | 13        | 13 (100%)   | 0          | 0          | 0         |

(Continues)

**APPENDIX 4.** Continued

| Family   | Locus <sup>a</sup> | Taxon level | Database | Sequences | Confident   | Ambiguous  | Incorrect   | No match |
|----------|--------------------|-------------|----------|-----------|-------------|------------|-------------|----------|
| Fabaceae | matK               | Family      | OLFS     | 13        | 13 (100%)   | 0          | 0           | 0        |
| Fabaceae | matK               | Genus       | GenBank  | 13        | 11 (84.62%) | 0          | 2 (15.38%)  | 0        |
| Fabaceae | matK               | Genus       | Regional | 13        | 8 (61.54%)  | 0          | 5 (38.46%)  | 0        |
| Fabaceae | matK               | Genus       | OLFS     | 13        | 5 (38.46%)  | 0          | 8 (61.54%)  | 0        |
| Fabaceae | matK               | Species     | GenBank  | 13        | 1 (7.69%)   | 3 (23.08%) | 9 (69.23%)  | 0        |
| Fabaceae | matK               | Species     | Regional | 13        | 2 (15.38%)  | 0          | 11 (84.62%) | 0        |
| Fabaceae | matK               | Species     | OLFS     | 13        | 3 (23.08%)  | 0          | 10 (76.92%) | 0        |
| Fabaceae | rbcLa              | Family      | GenBank  | 20        | 20 (100%)   | 0          | 0           | 0        |
| Fabaceae | rbcLa              | Family      | Regional | 20        | 19 (95%)    | 0          | 0           | 1 (5%)   |
| Fabaceae | rbcLa              | Family      | OLFS     | 20        | 19 (95%)    | 0          | 0           | 1 (5%)   |
| Fabaceae | rbcLa              | Genus       | GenBank  | 20        | 11 (55%)    | 6 (30%)    | 3 (15%)     | 0        |
| Fabaceae | rbcLa              | Genus       | Regional | 20        | 16 (80%)    | 1 (5%)     | 2 (10%)     | 1 (5%)   |
| Fabaceae | rbcLa              | Genus       | OLFS     | 20        | 16 (80%)    | 1 (5%)     | 2 (10%)     | 1 (5%)   |
| Fabaceae | rbcLa              | Species     | GenBank  | 20        | 2 (10%)     | 13 (65%)   | 5 (25%)     | 0        |
| Fabaceae | rbcLa              | Species     | Regional | 20        | 5 (25%)     | 10 (50%)   | 4 (20%)     | 1 (5%)   |
| Fabaceae | rbcLa              | Species     | OLFS     | 20        | 8 (40%)     | 8 (40%)    | 3 (15%)     | 1 (5%)   |
| Fabaceae | ITS_trnH           | Family      | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Family      | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Family      | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Genus       | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Genus       | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Genus       | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Species     | GenBank  | 3         | 2 (66.67%)  | 1 (33.33%) | 0           | 0        |
| Fabaceae | ITS_trnH           | Species     | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_trnH           | Species     | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK           | Family      | GenBank  | 8         | 8 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK           | Family      | Regional | 8         | 8 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK           | Family      | OLFS     | 8         | 8 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK           | Genus       | GenBank  | 8         | 6 (75%)     | 0          | 2 (25%)     | 0        |
| Fabaceae | ITS_matK           | Genus       | Regional | 8         | 6 (75%)     | 0          | 2 (25%)     | 0        |
| Fabaceae | ITS_matK           | Genus       | OLFS     | 8         | 6 (75%)     | 0          | 2 (25%)     | 0        |
| Fabaceae | ITS_matK           | Species     | GenBank  | 8         | 2 (25%)     | 3 (37.5%)  | 3 (37.5%)   | 0        |
| Fabaceae | ITS_matK           | Species     | Regional | 8         | 4 (50%)     | 1 (12.5%)  | 3 (37.5%)   | 0        |
| Fabaceae | ITS_matK           | Species     | OLFS     | 8         | 4 (50%)     | 1 (12.5%)  | 3 (37.5%)   | 0        |
| Fabaceae | ITS_rbcLa          | Family      | GenBank  | 15        | 14 (93.33%) | 1 (6.67%)  | 0           | 0        |
| Fabaceae | ITS_rbcLa          | Family      | Regional | 15        | 15 (100%)   | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa          | Family      | OLFS     | 15        | 15 (100%)   | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa          | Genus       | GenBank  | 15        | 11 (73.33%) | 1 (6.67%)  | 3 (20%)     | 0        |
| Fabaceae | ITS_rbcLa          | Genus       | Regional | 15        | 12 (80%)    | 0          | 3 (20%)     | 0        |
| Fabaceae | ITS_rbcLa          | Genus       | OLFS     | 15        | 12 (80%)    | 0          | 3 (20%)     | 0        |
| Fabaceae | ITS_rbcLa          | Species     | GenBank  | 15        | 6 (40%)     | 5 (33.33%) | 4 (26.67%)  | 0        |
| Fabaceae | ITS_rbcLa          | Species     | Regional | 15        | 10 (66.67%) | 1 (6.67%)  | 4 (26.67%)  | 0        |
| Fabaceae | ITS_rbcLa          | Species     | OLFS     | 15        | 10 (66.67%) | 1 (6.67%)  | 4 (26.67%)  | 0        |
| Fabaceae | matK_rbcLa         | Family      | GenBank  | 13        | 13 (100%)   | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa         | Family      | Regional | 13        | 13 (100%)   | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa         | Family      | OLFS     | 13        | 13 (100%)   | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa         | Genus       | GenBank  | 13        | 11 (84.62%) | 0          | 2 (15.38%)  | 0        |
| Fabaceae | matK_rbcLa         | Genus       | Regional | 13        | 11 (84.62%) | 0          | 2 (15.38%)  | 0        |
| Fabaceae | matK_rbcLa         | Genus       | OLFS     | 13        | 11 (84.62%) | 0          | 2 (15.38%)  | 0        |
| Fabaceae | matK_rbcLa         | Species     | GenBank  | 13        | 2 (15.38%)  | 5 (38.46%) | 6 (46.15%)  | 0        |
| Fabaceae | matK_rbcLa         | Species     | Regional | 13        | 3 (23.08%)  | 7 (53.85%) | 3 (23.08%)  | 0        |
| Fabaceae | matK_rbcLa         | Species     | OLFS     | 13        | 4 (30.77%)  | 7 (53.85%) | 2 (15.38%)  | 0        |
| Fabaceae | matK_trnH          | Family      | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_trnH          | Family      | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_trnH          | Family      | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_trnH          | Genus       | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_trnH          | Genus       | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_trnH          | Genus       | OLFS     | 3         | 1 (33.33%)  | 0          | 2 (66.67%)  | 0        |
| Fabaceae | matK_trnH          | Species     | GenBank  | 3         | 0           | 0          | 3 (100%)    | 0        |
| Fabaceae | matK_trnH          | Species     | Regional | 3         | 0           | 0          | 3 (100%)    | 0        |
| Fabaceae | matK_trnH          | Species     | OLFS     | 3         | 0           | 0          | 3 (100%)    | 0        |
| Fabaceae | rbcLa_trnH         | Family      | GenBank  | 4         | 4 (100%)    | 0          | 0           | 0        |

(Continues)

## APPENDIX 4. Continued

| Family   | Locus <sup>a</sup>  | Taxon level | Database | Sequences | Confident   | Ambiguous  | Incorrect   | No match |
|----------|---------------------|-------------|----------|-----------|-------------|------------|-------------|----------|
| Fabaceae | rbcLa_trnH          | Family      | Regional | 4         | 4 (100%)    | 0          | 0           | 0        |
| Fabaceae | rbcLa_trnH          | Family      | OLFS     | 4         | 4 (100%)    | 0          | 0           | 0        |
| Fabaceae | rbcLa_trnH          | Genus       | GenBank  | 4         | 4 (100%)    | 0          | 0           | 0        |
| Fabaceae | rbcLa_trnH          | Genus       | Regional | 4         | 4 (100%)    | 0          | 0           | 0        |
| Fabaceae | rbcLa_trnH          | Genus       | OLFS     | 4         | 4 (100%)    | 0          | 0           | 0        |
| Fabaceae | rbcLa_trnH          | Species     | GenBank  | 4         | 0           | 0          | 4 (100%)    | 0        |
| Fabaceae | rbcLa_trnH          | Species     | Regional | 4         | 2 (50%)     | 2 (50%)    | 0           | 0        |
| Fabaceae | rbcLa_trnH          | Species     | OLFS     | 4         | 2 (50%)     | 2 (50%)    | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa      | Family      | GenBank  | 8         | 8 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa      | Family      | Regional | 8         | 8 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa      | Family      | OLFS     | 8         | 8 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa      | Genus       | GenBank  | 8         | 6 (75%)     | 0          | 2 (25%)     | 0        |
| Fabaceae | ITS_matK_rbcLa      | Genus       | Regional | 8         | 6 (75%)     | 0          | 2 (25%)     | 0        |
| Fabaceae | ITS_matK_rbcLa      | Genus       | OLFS     | 8         | 6 (75%)     | 0          | 2 (25%)     | 0        |
| Fabaceae | ITS_matK_rbcLa      | Species     | GenBank  | 8         | 2 (25%)     | 3 (37.5%)  | 3 (37.5%)   | 0        |
| Fabaceae | ITS_matK_rbcLa      | Species     | Regional | 8         | 4 (50%)     | 1 (12.5%)  | 3 (37.5%)   | 0        |
| Fabaceae | ITS_matK_rbcLa      | Species     | OLFS     | 8         | 4 (50%)     | 1 (12.5%)  | 3 (37.5%)   | 0        |
| Fabaceae | ITS_matK_trnH       | Family      | GenBank  | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Family      | Regional | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Family      | OLFS     | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Genus       | GenBank  | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Genus       | Regional | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Genus       | OLFS     | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Species     | GenBank  | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Species     | Regional | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_trnH       | Species     | OLFS     | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Family      | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Family      | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Family      | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Genus       | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Genus       | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Genus       | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Species     | GenBank  | 3         | 2 (66.67%)  | 1 (33.33%) | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Species     | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_rbcLa_trnH      | Species     | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Family      | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Family      | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Family      | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Genus       | GenBank  | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Genus       | Regional | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Genus       | OLFS     | 3         | 3 (100%)    | 0          | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Species     | GenBank  | 3         | 0           | 0          | 3 (100%)    | 0        |
| Fabaceae | matK_rbcLa_trnH     | Species     | Regional | 3         | 1 (33.33%)  | 2 (66.67%) | 0           | 0        |
| Fabaceae | matK_rbcLa_trnH     | Species     | OLFS     | 3         | 1 (33.33%)  | 2 (66.67%) | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Family      | GenBank  | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Family      | Regional | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Family      | OLFS     | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Genus       | GenBank  | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Genus       | Regional | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Genus       | OLFS     | 2         | 2 (100%)    | 0          | 0           | 0        |
| Fabaceae | ITS_matK_rbcLa_trnH | Species     | OLFS     | 2         | 2 (100%)    | 0          | 0           | 0        |
| Poaceae  | ITS                 | Family      | GenBank  | 29        | 22 (75.86%) | 6 (20.69%) | 1 (3.45%)   | 0        |
| Poaceae  | ITS                 | Family      | Regional | 29        | 29 (100%)   | 0          | 0           | 0        |
| Poaceae  | ITS                 | Family      | OLFS     | 29        | 29 (100%)   | 0          | 0           | 0        |
| Poaceae  | ITS                 | Genus       | GenBank  | 29        | 15 (51.72%) | 7 (24.14%) | 7 (24.14%)  | 0        |
| Poaceae  | ITS                 | Genus       | Regional | 29        | 20 (68.97%) | 3 (10.34%) | 6 (20.69%)  | 0        |
| Poaceae  | ITS                 | Genus       | OLFS     | 29        | 21 (72.41%) | 2 (6.9%)   | 6 (20.69%)  | 0        |
| Poaceae  | ITS                 | Species     | GenBank  | 29        | 4 (13.79%)  | 8 (27.59%) | 17 (58.62%) | 0        |
| Poaceae  | ITS                 | Species     | Regional | 29        | 11 (37.93%) | 3 (10.34%) | 15 (51.72%) | 0        |
| Poaceae  | ITS                 | Species     | OLFS     | 29        | 15 (51.72%) | 5 (17.24%) | 9 (31.03%)  | 0        |

(Continues)

**APPENDIX 4.** Continued

| <b>Family</b> | <b>Locus<sup>a</sup></b> | <b>Taxon level</b> | <b>Database</b> | <b>Sequences</b> | <b>Confident</b> | <b>Ambiguous</b> | <b>Incorrect</b> | <b>No match</b> |
|---------------|--------------------------|--------------------|-----------------|------------------|------------------|------------------|------------------|-----------------|
| Poaceae       | trnH                     | Family             | GenBank         | 33               | 32 (96.97%)      | 0                | 1 (3.03%)        | 0               |
| Poaceae       | trnH                     | Family             | Regional        | 33               | 32 (96.97%)      | 0                | 1 (3.03%)        | 0               |
| Poaceae       | trnH                     | Family             | OLFS            | 33               | 32 (96.97%)      | 0                | 1 (3.03%)        | 0               |
| Poaceae       | trnH                     | Genus              | GenBank         | 33               | 17 (51.52%)      | 10 (30.3%)       | 6 (18.18%)       | 0               |
| Poaceae       | trnH                     | Genus              | Regional        | 33               | 14 (42.42%)      | 1 (3.03%)        | 18 (54.55%)      | 0               |
| Poaceae       | trnH                     | Genus              | OLFS            | 33               | 8 (24.24%)       | 0                | 25 (75.76%)      | 0               |
| Poaceae       | trnH                     | Species            | GenBank         | 33               | 3 (9.09%)        | 16 (48.48%)      | 14 (42.42%)      | 0               |
| Poaceae       | trnH                     | Species            | Regional        | 33               | 2 (6.06%)        | 3 (9.09%)        | 28 (84.85%)      | 0               |
| Poaceae       | trnH                     | Species            | OLFS            | 33               | 7 (21.21%)       | 0                | 26 (78.79%)      | 0               |
| Poaceae       | rbcLa                    | Family             | GenBank         | 34               | 34 (100%)        | 0                | 0                | 0               |
| Poaceae       | rbcLa                    | Family             | Regional        | 34               | 34 (100%)        | 0                | 0                | 0               |
| Poaceae       | rbcLa                    | Family             | OLFS            | 34               | 34 (100%)        | 0                | 0                | 0               |
| Poaceae       | rbcLa                    | Genus              | GenBank         | 34               | 9 (26.47%)       | 23 (67.65%)      | 2 (5.88%)        | 0               |
| Poaceae       | rbcLa                    | Genus              | Regional        | 34               | 14 (41.18%)      | 16 (47.06%)      | 4 (11.76%)       | 0               |
| Poaceae       | rbcLa                    | Genus              | OLFS            | 34               | 24 (70.59%)      | 6 (17.65%)       | 4 (11.76%)       | 0               |
| Poaceae       | rbcLa                    | Species            | GenBank         | 34               | 2 (5.88%)        | 22 (64.71%)      | 10 (29.41%)      | 0               |
| Poaceae       | rbcLa                    | Species            | Regional        | 34               | 4 (11.76%)       | 14 (41.18%)      | 16 (47.06%)      | 0               |
| Poaceae       | rbcLa                    | Species            | OLFS            | 34               | 14 (41.18%)      | 15 (44.12%)      | 5 (14.71%)       | 0               |
| Poaceae       | ITS_trnH                 | Family             | GenBank         | 27               | 21 (77.78%)      | 5 (18.52%)       | 1 (3.7%)         | 0               |
| Poaceae       | ITS_trnH                 | Family             | Regional        | 27               | 27 (100%)        | 0                | 0                | 0               |
| Poaceae       | ITS_trnH                 | Family             | OLFS            | 27               | 27 (100%)        | 0                | 0                | 0               |
| Poaceae       | ITS_trnH                 | Genus              | GenBank         | 27               | 13 (48.15%)      | 6 (22.22%)       | 8 (29.63%)       | 0               |
| Poaceae       | ITS_trnH                 | Genus              | Regional        | 27               | 18 (66.67%)      | 2 (7.41%)        | 7 (25.93%)       | 0               |
| Poaceae       | ITS_trnH                 | Genus              | OLFS            | 27               | 18 (66.67%)      | 1 (3.7%)         | 8 (29.63%)       | 0               |
| Poaceae       | ITS_trnH                 | Species            | GenBank         | 27               | 4 (14.81%)       | 7 (25.93%)       | 16 (59.26%)      | 0               |
| Poaceae       | ITS_trnH                 | Species            | Regional        | 27               | 12 (44.44%)      | 2 (7.41%)        | 13 (48.15%)      | 0               |
| Poaceae       | ITS_trnH                 | Species            | OLFS            | 27               | 13 (48.15%)      | 2 (7.41%)        | 12 (44.44%)      | 0               |
| Poaceae       | ITS_rbcLa                | Family             | GenBank         | 29               | 25 (86.21%)      | 4 (13.79%)       | 0                | 0               |
| Poaceae       | ITS_rbcLa                | Family             | Regional        | 29               | 29 (100%)        | 0                | 0                | 0               |
| Poaceae       | ITS_rbcLa                | Family             | OLFS            | 29               | 29 (100%)        | 0                | 0                | 0               |
| Poaceae       | ITS_rbcLa                | Genus              | GenBank         | 29               | 14 (48.28%)      | 12 (41.38%)      | 3 (10.34%)       | 0               |
| Poaceae       | ITS_rbcLa                | Genus              | Regional        | 29               | 19 (65.52%)      | 4 (13.79%)       | 6 (20.69%)       | 0               |
| Poaceae       | ITS_rbcLa                | Genus              | OLFS            | 29               | 23 (79.31%)      | 2 (6.9%)         | 4 (13.79%)       | 0               |
| Poaceae       | ITS_rbcLa                | Species            | GenBank         | 29               | 4 (13.79%)       | 12 (41.38%)      | 13 (44.83%)      | 0               |
| Poaceae       | ITS_rbcLa                | Species            | Regional        | 29               | 9 (31.03%)       | 4 (13.79%)       | 16 (55.17%)      | 0               |
| Poaceae       | ITS_rbcLa                | Species            | OLFS            | 29               | 16 (55.17%)      | 6 (20.69%)       | 7 (24.14%)       | 0               |
| Poaceae       | rbcLa_trnH               | Family             | GenBank         | 32               | 32 (100%)        | 0                | 0                | 0               |
| Poaceae       | rbcLa_trnH               | Family             | Regional        | 32               | 32 (100%)        | 0                | 0                | 0               |
| Poaceae       | rbcLa_trnH               | Family             | OLFS            | 32               | 32 (100%)        | 0                | 0                | 0               |
| Poaceae       | rbcLa_trnH               | Genus              | GenBank         | 32               | 13 (40.62%)      | 13 (40.62%)      | 6 (18.75%)       | 0               |
| Poaceae       | rbcLa_trnH               | Genus              | Regional        | 32               | 16 (50%)         | 12 (37.5%)       | 4 (12.5%)        | 0               |
| Poaceae       | rbcLa_trnH               | Genus              | OLFS            | 32               | 23 (71.88%)      | 5 (15.62%)       | 4 (12.5%)        | 0               |
| Poaceae       | rbcLa_trnH               | Species            | GenBank         | 32               | 4 (12.5%)        | 16 (50%)         | 12 (37.5%)       | 0               |
| Poaceae       | rbcLa_trnH               | Species            | Regional        | 32               | 5 (15.62%)       | 14 (43.75%)      | 13 (40.62%)      | 0               |
| Poaceae       | rbcLa_trnH               | Species            | OLFS            | 32               | 16 (50%)         | 10 (31.25%)      | 6 (18.75%)       | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Family             | GenBank         | 27               | 24 (88.89%)      | 3 (11.11%)       | 0                | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Family             | Regional        | 27               | 27 (100%)        | 0                | 0                | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Family             | OLFS            | 27               | 27 (100%)        | 0                | 0                | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Genus              | GenBank         | 27               | 12 (44.44%)      | 9 (33.33%)       | 6 (22.22%)       | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Genus              | Regional        | 27               | 18 (66.67%)      | 3 (11.11%)       | 6 (22.22%)       | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Genus              | OLFS            | 27               | 21 (77.78%)      | 2 (7.41%)        | 4 (14.81%)       | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Species            | GenBank         | 27               | 4 (14.81%)       | 11 (40.74%)      | 12 (44.44%)      | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Species            | Regional        | 27               | 10 (37.04%)      | 4 (14.81%)       | 13 (48.15%)      | 0               |
| Poaceae       | ITS_rbcLa_trnH           | Species            | OLFS            | 27               | 15 (55.56%)      | 4 (14.81%)       | 8 (29.63%)       | 0               |

Note: OLFS = database of species sampled from Oak Lake Field Station.

<sup>a</sup>Multi-region barcodes are indicated by underscore.