

Variables affecting DNA preservation in archival plant specimens¹

Kurt M. Neubig,^{2,3} W. Mark Whitten,² J. Richard Abbott,⁴ Savannah Elliott,² Douglas E. Soltis,^{2,5} and Pamela S. Soltis²

ABSTRACT

Although the use of plant DNA in scientific research is now decades old now, we still know very little about the best methods of DNA preservation. A few studies have addressed aspects of DNA quality and preservation, but a broad sense of the quality of DNA in herbarium specimens and tissues specifically preserved for DNA usage is still lacking. This article reviews the basic concepts of DNA preservation, factors affecting DNA in herbarium specimens, optimal methods for DNA extraction, and storage. To address lingering questions about the nature of DNA quality in archival plant specimens, we also evaluated ~2000 samples from herbarium specimens, as well as silica-dried and frozen tissues specifically preserved for DNA usage. For herbarium specimens, we used materials spanning much of angiosperm phylogenetic diversity to ascertain general trends of DNA degradation over time and to assess whether different clades had different rates of "expiration." Our results indicate different rates of DNA degradation, but for most samples, no matter the taxonomic group, we were unlikely to recover high-quality DNA after 30–40 years. Nonetheless, given rapid technological advances and changes in DNA sequencing methods, even herbarium specimens with highly degraded DNA may still be of use in molecular analyses. Additional herbarium specimens were sampled for two different collection sets with drastically different curation histories but of approximately the same age. This analysis showed a very marked difference in DNA quality between the two sets: the well-curated specimens had excellent-quality DNA while the poorly curated samples had low-quality DNA. For frozen tissues, virtually all samples had excellent quality, whether storage was at -20° or -80°C and whether tissue was pulverized or intact. For silica-dried tissues, the results were much more heterogeneous, depending on the storage vessel. Samples stored in vessels with poor seals yielded lower-quality DNA. To address the long-term viability of frozen DNA extracts, we compared a set of 7–12-year-old DNA extracts with new extracts of the same original silica-dried tissues and with fresh, greenhouse-collected tissues of the same original material; these comparisons indicated less degradation in the extracts than in the silica-preserved samples, although the differences were subtle. We also found that 15-year-old photo-bleached, silica-dried samples still had small amounts of high-molecular-weight DNA. The long-term preservation of DNA in archival samples remains problematic, but we can best preserve DNA by limiting the factors that degrade it. Although the botanical community has been preserving tissues and DNA extracts for a limited timespan, we can infer some basic conclusions from this and other studies. Recommended best practices are to freeze tissues and extracts at as low a temperature as possible (e.g., -80°C), but if that is not possible or practical, room temperature storage of tissues with silica in tightly sealed containers is a viable alternative, at least for the short term.

¹ We thank Wendy Applequist for the invitation to submit this article. Specimen curation was provided primarily by Kent Perkins at the PLAS herbarium in the Florida Museum of Natural History and by Jim Solomon at the Missouri Botanical Garden herbarium. We thank White Oak Plantation for their herbarium donation, which provided a valuable resource for curation standards in DNA quality. Lowell Urbatsch generously provided frozen tissue samples for this study, and Wendy Zomlefer provided silica-dried tissue samples. This project was supported in part by the Open Tree of Life (NSF Grant DEB-1301828) to DES and by iDigBio (NSF Cooperative Agreement EF-1115210) to PSS.

² Florida Museum of Natural History, University of Florida, P.O. Box 117800, Gainesville, FL 32611-7800, U.S.A.

³ author for correspondence: kneubig@flmnh.ufl.edu

⁴ Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri, 63166-0299, U.S.A.

⁵ Department of Biology, University of Florida, P.O. Box 118526, Gainesville, FL 32611-8526, U.S.A.

INTRODUCTION AND REVIEW

Advances in DNA sequencing technology have revolutionized the fields of plant evolutionary biology and systematics during the last 30 years, but methods for collecting and preserving plant herbarium specimens have changed little since the time of Linnaeus. Early molecular phylogenetic work in the 1980s usually relied upon extraction of DNA from fresh material from exemplar taxa, but as the difficulty and cost of DNA sequencing dropped, botanists sought to expand taxon sampling to many species that were not in cultivation. Attempts to extract DNA from herbarium specimens usually resulted in degraded DNA, which was still suitable in many cases for many types of analyses. Botanists experimented with various methods of preserving tissue in the field to supplement herbarium specimens. The simplest technique to preserve DNA was rapid, gentle drying (Doyle & Dickson, 1987), but Chase and Hills (1991) reported difficulties in extracting usable DNA from dried tissues of Malpighiaceae and Orchidaceae. They realized that rapid desiccation of tissues (<24 hr) was critical to DNA preservation and demonstrated that tissues dried in fine-grain silica gel yielded high-quality DNA across a wide range of taxa. Similar techniques (e.g., CTAB-NaCl gel; FTA paper) were also demonstrated to preserve DNA by rapidly removing water from tissue. These techniques are now routinely used to supplement standard herbarium collections, with minimal added cost and space requirements. With the emphasis on molecular data in plant systematics, these supplementary preserved tissue samples are important, just as their matching herbarium specimens are.

Although methods for archival storage of herbarium specimens are standardized, there is no commonly accepted standard method for storage of the matching tissue samples.

Cryogenic liquid nitrogen storage facilities are accepted as best practice for storage of DNA extracts and tissues, but many institutions do not currently have costly cryogenic facilities (although many larger institutions currently accept samples for cryogenic storage; see Endara et al., this volume). Nearly every plant systematics lab has hundreds to thousands of Ziploc® bags or vials containing silica-dried tissue, largely uncurated, linked to herbarium specimens. What should we do with the thousands of samples stored in this manner? How long can we store them, and what are the best conditions for storage for high yields of good-quality DNA over time? How quickly does DNA degrade in these dried samples, and what is the least expensive, most effective way to store them? Are there simple, relatively low-cost storage methods that might increase the lifespan of the DNA these dried samples contain?

This paper will focus on DNA preservation in plants and is divided into six parts: 1) a review of conditions affecting DNA preservation in herbarium specimens; 2) a review of methods for archiving tissues/DNA supplementary to herbarium specimens; 3) a review of DNA extraction methods for plant tissues; 4) a review of tissue/DNA storage; 5) a review of what is known of tissue/DNA storage at different temperatures; and 6) a new comparison of DNA extracted from sets of tissue sample stored for varying lengths of time and under different conditions, with the goal of revealing storage variables that affect DNA quality over time.

1. HERBARIUM SPECIMENS AS SOURCES OF DNA

DNA from herbarium specimens - The philosophical debate of using herbarium specimens as a DNA resource has been discussed (Metsger, 1999; Miller, 1999; Mueller, 199

Wood et al., 1999). Policies governing the use of herbarium specimens as a DNA resource have been contentious, but it is now a widely accepted practice, given some limitations. Predicting whether a given specimen will yield high-quality DNA is often subjective and often depends upon unrecorded details about how the specimen was pressed and stored. Factors that affect DNA quality in herbarium specimens are briefly outlined as follows.

Specimen Preparation – If properly dried plant tissues are ideal for DNA preservation, then well-prepared herbarium specimens alone might be an excellent source of archival DNA. Herbarium specimens have become an invaluable resource for systematists and phylogeneticists since dried herbarium specimens were first investigated for their utility as DNA sources (Liston et al., 1990; Savolainen et al., 1995; Bridson and Forman, 1998). Unfortunately, many factors during the preparation and storage of a herbarium specimen may result in highly degraded DNA. A plant specimen is collected and dried in a plant press, usually within a sheet of newspaper between sheets of blotter paper and cardboard. The drying conditions are critical to the quality of DNA that will remain in the pressed specimen. DNA degradation (due to decomposition of the plant material itself) can occur in specimens that dry too slowly; degradation can also occur if drying temperatures are too high. Field methods for plant drying using forced air electric heaters (Blanco et al., 2006), propane burners (Mori, 2011), or solar collectors (Sinnott, 1983) are methods for moving warm (60–100°C) air through the press and drying the tissues to crispness within 12–24 hours. Field work in remote, wet habitats may make field drying impractical, and botanists may resort to field pressing specimens and then soaking them in alcohol or formalin for transport prior to drying (Mori, 2011).

Such preservatives protect specimens against decomposition, but they destroy DNA present in tissues. Such field-pickled specimens almost never yield usable DNA after drying and mounting. As discussed elsewhere, tissues stored in aqueous ethanol have shown moderate to strong degradation of DNA quality (Adams and Baker, 2011), so it is not surprising that DNA degradation occurs in freshly collected specimens exposed to dilute alcohols for weeks.

Length of storage in acidic newspaper – Specimens are eventually mounted on archival paper, but in most (underfunded) herbaria, specimens often remain in yellowing newspaper for months or years before being mounted and accessioned. Newspaper is highly acidic and probably contributes to DNA degradation, especially in specimens stored for long periods. Best practice should include pressing specimens in archival, acid-free paper, but we are unaware of vendors that sell acid-free paper for plant pressing. Specimens should be removed from newspaper and mounted on archival paper as soon as possible after drying.

Specimen age – Early studies showed that age of herbarium specimens was a major limiting factor in obtaining high-quality DNA (Savolainen et al., 1995). Most workers assumed that greenness of tissue was associated with high-molecular-weight (HMW) DNA. However, studies reveal that greenness is only loosely correlated with DNA quality, and that age, in combination with the specimen curation history, is a more important factor in estimating quality of DNA (Erkens et al., 2008).

Using 454 sequences of amplicons from nuclear, plastid, and mitochondrial regions, Staats et al. (2011) measured the damage to DNA in herbarium specimens. They compared fresh, recently dried specimens and those up to 114 years old. They concluded that over

95% of DNA is irretrievably damaged during the plant drying process, with only about 1% of amplifiable copies remaining in old herbarium specimens. They observed increased levels of C to T /G to A transitions in plastid DNA of old herbarium specimens as compared to recently dried or fresh tissues. They concluded that ambient humidity in herbarium storage allows for rehydration of DNA that leads to hydrolytic DNA damage over time. While demonstrating that older herbarium specimens can provide sequenceable DNA in spite of damage, the authors advocated for supplemental collection of tissues in silica gel for modern collections.

Humidity – Ambient humidity during specimen storage is probably one of the most important conditions affecting specimens, although controlled studies are limited (Adams, 2011). Most herbaria attempt to keep humidity below 50% to help control pests, but smaller herbaria in tropical areas may not have air conditioning. Also, during transport, specimens will typically be exposed to temperatures and humidity well above standard curation norms for several days or even weeks.

Pest control chemicals and treatments – Herbaria occasionally use chemicals to control insect pests. Pyrethroid insecticides, paradichlorobenzene and naphthalene have no gross adverse effects on DNA quality (Neubig, pers. obs. at FLAS). Vikane (sulfuryl fluoride)/chloropicrin, used for building/collection fumigation) is not harmful to herbarium DNA (Whitten et al., 1999). Microwave ovens have been used for both initial specimen drying (Bacci et al., 1985) and for control of pest infestations (Hill, 1983; Philbrick, 1984), but because microwaves act by heating tissues, their use should probably be avoided. The deleterious effect of microwaves on DNA is apparently small, although noticeable (Lander et al., 2013).

Oxidation – While the oxidative effect of ambient air is well known (Daniel, 1995), storage of herbarium specimens in reduced-oxygen environments is impractical except for extremely rare historical objects. It has been suggested that DNA isolated from herbarium specimens degrades more rapidly than DNA isolated from fresh tissue (Jansen et al., 1999). In our experience, after many years of using herbarium-extracted DNA, we have no evidence to support this claim, especially if DNAs are stored in TE (Tris-EDTA; see below) buffer.

2. SUPPLEMENTARY COLLECTIONS SPECIFICALLY FOR DNA PRESERVATION

Staats et al. (2011) compared DNA damage in fresh, newly dried, and ca. 100-year-old herbarium specimens. They assessed strand breaks and other sorts of polymerase non-bypassable damage by quantitative real-time PCR and concluded that most of the DNA damage was caused by heating during the specimen drying process and not by aging during specimen storage. Given that herbarium drying techniques are unlikely to change in the near future, their results demonstrate the necessity to preserve supplementary samples specifically for DNA preservation. We concur with Gaudel and Rouhan (2013) that supplementary material for DNA preservation should be collected along with all new plant vouchers. Given the high cost of herbarium specimen collection and curation, specimen value can be greatly enhanced at minimal additional cost by supplementing the specimen with tissues preserved specifically for DNA extraction.

Storage of flash-frozen fresh tissues in cryogenic banks is ideal but is not feasible for most field projects. Various methods of tissue preservation have been proposed to minimize

DNA degradation over time (Nagy, 2010). The primary causes of DNA degradation are high humidity, light (especially sunlight or fluorescent lights that leak significant levels of ultraviolet wavelengths), oxidation, low pH, endogenous DNases, and exposure to chemical “preservatives” that can alter or destroy DNA. Most preservation methods remove water from tissues and/or buffer cell contents against DNA degradation.

Silica gel – Silica gel has been the most commonly used desiccant to preserve tissues for DNA usage (Chase and Hills, 1991). The most important variables affecting the rate of tissue desiccation are 1) particle size of silica gel; 2) the size/surface area of tissue pieces; and 3) the ratio of tissue to silica gel. Smaller grades of silica gel have greater surface area and therefore dehydrate tissues more rapidly; large beads of silica gel perform poorly compared to finer grades (i.e., 150-200 mesh). Tissues should be torn or cut into very small pieces before being placed into silica gel. Succulents or CAM plants can close their stomata when placed in silica gel; unless the tissues are cut into thin strips, they can take weeks to dehydrate. The weight of silica used should be at least 10 times the amount of tissue. Silica gel often includes colored indicator beads that change color when the silica is saturated with water. Cobalt chloride indicator changes from blue to pink; methyl violet changes from orange to green; iron (III) salts change from orange to clear. Silica gel that has become saturated with water can be regenerated by heating at 120°C for several hours in an oven or by microwaving. Reuse of silica gel carries the risk of cross-contamination of samples with small tissue fragments or pollen, and the use of silica gel is not without disadvantages. Silica gel is potentially hazardous, and inhaling the dust (especially from fine grades) carries risk of silicosis. The blue cobalt chloride indi-

cator is a carcinogen and is being replaced by iron (III) indicators (e.g., Sorbead Orange[®]) or methyl violet indicators. The cost of silica gel has increased dramatically in recent years, especially in grades sold by scientific vendors. Cheaper alternatives that work just as well are sold as desiccants for flower drying by florists (e.g., <http://www.deltaadsorbents.com/>). Silica gel works well with thin tissues, but tissues with thick cuticles or abundant mucilage or secondary compounds may dry slowly.

Many botanists use small Ziploc[™] polyethylene bags to store tissues in silica gel. Although convenient, such bags do not seal tightly, and the contents quickly become saturated with water, depending upon relative humidity. To slow the hydration of these bags, we place bundles of bags in larger bags used for food storage (see Appendix 1 for further detail); these food preservation bags can be heat-sealed after evacuation of air. Such vacuum-sealed bags can then be stored in a freezer or in a cool, dark space.

NaCl – Granular sodium chloride is an alternative to silica gel that is both cheaper and readily available around the world (Carrió and Rosselló, 2013). Using NaCl provides significant advantages in remote tropical regions. Silica gel desiccates tissues faster than NaCl (Carrió and Rosselló, 2013), but salt may be an acceptable alternative for most uses. The long-term stability of DNA in salt-dried tissues (relative to silica gel) is unknown.

NaCl-CTAB – Succulent or mucilaginous plants are slow to desiccate in silica gel. An alternative strategy is to use a saturated gel composed of NaCl and cetyltrimethylammonium bromide (CTAB) (Rogstad, 1992). The components for this preservative can be carried in the field as a dry powder and hydrated prior to use, in order to form the gel. This colloidal gel is extremely thick, and tissues should be

sliced into thin sections or (if possible) vacuum-infiltrated.

FTA paper – An alternative to drying tissues is the use of FTA (FTA is an acronym for fast technology for analysis of nucleic acids; Whatman, Inc., Clifton, NJ) paper (Lin et al., 2000; Smith and Burgoine, 2004; Mbogori et al., 2006; Rajendram et al., 2006). FTA paper is thick, adsorbent paper impregnated with buffers and chemical preservatives that retard DNA degradation. Tissues are squeezed or abraded, and the plant juice is adsorbed onto the paper, followed by air-drying. The paper can be stored at room temperature or in a freezer. Samples are cut from the paper for DNA extraction. This is a rapid and space-conservative method, but the quantities of DNA preserved may be relatively small (compared to tissue preservation) unless large pieces of paper are used. Commercially available FTA paper can be relatively expensive compared to silica gel. But a cheap and similar alternative can be made very easily (1 g SDS, 1 ml EDTA at 0.5M, 3 ml Tris at 1 M, add water to 50 ml and mix, soak solution onto filter paper, then dry) for a small fraction of the cost (Klemchin, 2008). These samples, like all others, should be kept in a cool, dry, dark, oxygen-poor location.

RNAlater – RNAlater is a commercial medium for preserving “all” nucleic acids within a tissue (Mutter et al., 2004), including DNA as well as RNA. Its high cost (ca. \$1/ml) makes it an expensive alternative to other methods unless RNA preservation is required. Long-term stability of DNA at room temperature is not well studied, and freezer storage is recommended.

Alcohols – Simple alcohols are frequently used as DNA preservatives for animal tissues, but they have not been effective in plants. Insects

in particular are frequently preserved in 100% ethanol, with several changes of alcohol needed to remove water and dehydrate tissues. Usable DNA may even be extracted from the alcohol preservative fluids of insect specimens (Shokrolla et al., 2010). Short-term storage (less than a year) of plant tissues in almost any alcohol can yield high-quality DNA (Flournoy et al., 1996). However, long-term storage of many years yields highly degraded DNA, at least in spinach (Adams and Baker, 2011). Although isopropanol is the best out of a suite of different alcohols examined, it is still mediocre at best (Adams and Baker, 2011). Plant tissues to be used in developmental or structural studies are frequently fixed (as spirit collections) in a formalin-glacial acetic acid-ethanol mixture (FAA) that fixes cellular contents (Simmons, 1995). This mixture is highly acidic and hence destructive to DNA (Doyle and Dickson, 1987; Pyle and Adams, 1989). However, extractions from formalin-fixed and paraffin-embedded human tissues have yielded high-quality DNA (Shi et al., 2004). Such success is probably attributable to the use of buffered formalin that is rapidly removed after fixation (rather than long-term storage as for plant tissues). There is some evidence that ethanol pretreatment might be useful in some applications. Soaking tissues in high ethanol concentrations (70% or higher) and then putting them in silica yields higher quantities of DNA, probably due to the rapid removal of water (Akinnagbe et al., 2011). Studies by Adams et al. (1999) showed that some plant (Brassicaceae, Poaceae) have potent DNases that are not inactivated by standard DNA extraction protocols, resulting in degraded DNA. Pretreatment of fresh tissue with ethanol prior to extraction increases DNA yields perhaps due to inactivation of DNases (Adams et al., 1999).

Acetone – Acetone is a superior preservativ-

to ethanol for insect tissues (Fukatsu, 1999). We have not found any literature on the preservation of plant tissues in acetone, but the flammability, volatility, and toxicity of acetone make it an unlikely candidate for wide use.

3. METHODS FOR DNA EXTRACTION FROM PLANTS

Tissue grinding – The first step in preparation for DNA extraction of fresh or dried tissue is the disruption of the cell wall. With dried tissue, this is most effectively done using beads (glass, zirconia, steel, etc.) in tubes (we use thick-walled plastic 2-ml tubes with gasketed screw caps) to create a fine powder from the tissue. There are many “bead beater” style machines available; they make extraction processes more streamlined and efficient and can be used for 96-well plate formats. However, such instruments may be relatively expensive (\$600-\$16,000; http://www.biospec.com/laboratory_cell_disrupters/), and so cheaper alternatives, such as reciprocating saws (Alexander et al., 2006) or electric drills (Zhang and Stewart, 2000), have been proposed.

The use of mortar and pestle to hand-grind tissue is effective for fresh or dried samples, but is very time consuming. Many older protocols call for the addition of liquid nitrogen at this stage of hand pulverization (Gawel and Jarret, 1991). Liquid nitrogen pulverization has multiple uses: one is to make fresh tissues very brittle and more easily ground into smaller pieces, and the second is to retard enzymatic or oxidative degradation until the addition of hot extraction buffer. For silica-dried tissues, grinding in liquid nitrogen serves little purpose, except perhaps to aid the physical grinding of tissues.

Extraction protocols – The most widely used plant DNA extraction is the “modified CTAB method” (Doyle and Doyle, 1987) that uses

CTAB (a detergent) combined with NaCl and buffers to disrupt organellar membranes and dissolve DNA. A chloroform liquid-liquid extraction is used to remove lipoidal compounds, and DNA is precipitated from the aqueous phase by addition of isopropanol or ethanol. A basic protocol is presented in Appendix 2. The basic CTAB extraction works well with a majority of plant taxa, but the great variation in plant secondary chemistry results in failures for various taxa. There are numerous published variant protocols to overcome these problems created by secondary chemicals. Examples include:

- In *Drosera*, there is a recipe that uses sorbitol, Tris-HCl, EDTA, and sodium bisulfite (Bekesiova et al., 1999).
- In *Saccharum*, there is a recipe more or less similar to a “basic CTAB” solution, but with polyethylene glycol instead of CTAB (Honeycutt et al., 1992).
- For Proteaceae, there are suggestions for modifying CTAB concentration, or the number of chloroform/isoamyl alcohol washes (Maguire et al., 1994).
- For Cactaceae, including additives like sorbitol in the CTAB buffer and additional chloroform washes remove inhibitory compounds (Tel-Zur et al., 1999).
- For algae, because of the complex mix of unusual polysaccharides, DNA extraction can be problematic and may require special pre-treatment of the tissue to remove secondary compounds. Polyvinylpyrrolidone (PVP) can be added to the CTAB buffer (Maeda et al., 2013)</style><style face="italic" font="default" size="100%">Saccharina</style></title><secondary-title>Journal of Applied Phycology</secondary-title></titles><periodical><-

full-title>Journal of Applied Phycology</full-title></periodical><dates><year>2012</year></dates><urls></urls><electronic-source-num>10.1007/s10811-012-9868-3</electronic-resource-num></record></Cite></EndNote>

- For *Ipomoea*, including multiple washes with chloroform removes inhibitory compounds (Kim and Hamada, 2005).
- For *Dalbergia*, adding PVP binds the phenolic compounds, a high molar concentration of NaCl inhibits co-precipitation of polysaccharides and DNA, and LiCl removes RNA by selective precipitation (Ribeiro and Lovato, 2007).
- A generalized extraction protocol for fungi and plants that avoids use of chloroform has also been proposed (Mahuku, 2004).
- For the diverse secondary compounds found in many rosids, a modified CTAB-based protocol was also developed (Soltis et al., 1991).

Various protocols have been developed to remove undesirable polysaccharides in DNA samples (Do and Adams, 1991; Jobes et al., 1995; Porebski et al., 1997). Essentially, these protocols suggest silica column purification (discussed below) or the use of PVP in the extraction buffer to bind to polysaccharides or polyphenolic compounds, thus removing them from the solution prior to DNA precipitation. For taxa with strong mucilaginous compounds, a 3X CTAB protocol is recommended with repeated chloroform extractions, followed by a second precipitation of DNA after treatment with RNase (Cota-Sánchez et al., 2006). For taxa with abundant latex, extraction of etiolated tissues may reduce the amount of secondary metabolites relative to

DNA (Michiels et al., 2003). Because of the degraded nature of ancient DNA, extractions are sensitive to loss and contamination (Rohland and Hofreiter, 2007; Särkinen et al., 2012).

Beta-mercaptoethanol is often added to CTAB extractions; it is a strong reducing agent that can remove tannins and other polyphenols often present in the crude plant extract. It is an irritant with a very strong odor, so its inclusion should be avoided unless it is demonstrated to be beneficial.

DNA extraction methods from plants, animals, and other eukaryotic organisms are reviewed by Knebelberger and Stoger (2012). Binding DNA to magnetic beads followed by washing is a technique amenable to automation and high-throughput sequencing utilized in DNA barcoding (Rudi et al., 1997; Levison et al., 1998; Oster et al., 2001; Palecek and Fojta, 2007). The resulting DNA is very pure, but yield is relatively low, and the technique is not oriented towards archival museum storage. Other high-throughput methods utilize silica-containing filters, hereafter referred to as silica columns (Whitlock et al., 2008).

The CTAB method involves one to several liquid phase extractions with chloroform/isoamyl alcohol (24:1) that are very effective in removing polar secondary compounds that interfere with DNA precipitation. Unfortunately, chloroform is carcinogenic and toxic and involves added expense for disposal. Various commercial plant DNA extraction kits (Qiagen DNeasy, Invitrogen ChargeSwitch, Nucleospin) utilize proprietary reagents combined with silica membrane columns, magnetic beads, or anion exchange resins to avoid the use and hazard of chloroform. Särkinen et al. (2012) compared the most commonly used commercial kits to several variants of CTAB extraction, measuring DNA yield, purity, and PCR amplification from the extracted DNA.

They noted that (especially for herbarium specimens) total quantity of DNA extracted is not as important as the purity of the resulting DNA and the ability of the DNA to yield amplicons. Their and other comparative studies showed that for optimal PCR amplification of various DNA loci, a CTAB extraction combined with silica column binding purification gave the best results (Csaiikl et al., 1998; Rohland and Hofreiter, 2007; Särkinen et al., 2012).

Telle and Thines (2008) exhaustively compared 19 different extraction methods or kits and 15 different polymerases, measuring their ability to amplify a ~620-bp oomycete (plant pathogen) mitochondrial fragment from herbarium specimens up to 129 years old. Their results were complex, but demonstrated that several of the commercial DNA extraction kits (Geneclean Kit for Ancient DNA, Bio 101; Genelute Plant Genomic DNA kit, Sigma; innuPREP Plant DNA Kit, Analytikjena; DNeasy Plant Mini Kit, Qiagen; Invisorb Spin Plant Mini Kit, Invitek; E.N.Z.A. Forensic DNA Isolation Kit, VWR) outperformed basic CTAB + silica column extractions as measured by the amplification of 300-600-bp amplicons. They also demonstrated that choice of polymerase was crucial to successful amplification. They did not sequence the amplicons, so DNA damage could not be compared.

Activation of DNases during extraction – Proteinase K is routinely used in extraction of animal tissues; the enzyme not only degrades structural proteins but also inactivates proteinases that digest DNA (Knebelsberger and Stoger, 2012). Proteinase K is not inactivated by chelating reagents such as EDTA or detergents such as SDS and is active over a wide range of pH (4-12.5). Adams et al. (1999) showed that some taxa (Poaceae, Brassicaceae) exhibit rapid degradation of DNA during ex-

traction in normal protocols from both fresh or silica-dried tissues. They hypothesized the degradation was caused by exceptionally stable DNases that could tolerate desiccation and that consumed DNA during the extraction process. They showed that addition of proteinase K during the initial incubation stage of DNA extraction likewise destroys any endonucleases, resulting in high yields of HMW DNA. Other workers have noted the beneficial effects of proteinase K in extraction buffers (Crowe et al., 1991; Zhang and Stewart, 2000).

4. ARCHIVAL STORAGE OF PLANT TISSUES OR EXTRACTS TO MAXIMIZE QUALITY

Should we store tissue samples or just DNA extracts? Given that we cannot anticipate all future uses of archival specimens, it makes sense to preserve portions in their most natural form (leaves, flowers, seeds), because these contain not only DNA but also all the other biomolecules that survive drying. Anchordquy (2007) notes the increasing importance of epigenetics and suggests that archival storage methods should include preservation of other biomolecules in addition to DNA. However, if DNA degrades inside intact tissues during archival storage, then DNA should be extracted for archival storage. If space and curation facilities allow, storage of both tissues and extracted DNAs seems optimal.

5. STORAGE TEMPERATURE AND ROOM-TEMPERATURE ALTERNATIVES

Storage of tissues or DNA at liquid nitrogen temperatures (-196°C) is optimal because degradation is minimized relative to all other storage techniques (Lee et al., 2010). The high quality of preservation comes at a relatively high cost due to initial cost of the Dewar tanks and the specialized cryogenic vials and

labels that must be used, combined with the perpetual costs of liquid nitrogen. Mechanical freezers (-60° to -80°C) are an alternative but are relatively expensive, and mechanical failure must be anticipated for archival storage. Storage in -20°C freezers is much more common; some large herbaria (e.g., Missouri Botanical Garden) have walk-in freezers for storage of voucherized tissue samples. Commercial food freezers are suitable for specimen storage as long as they are not frost-free; the periodic thawing in frost-free freezers is harmful to extracted DNA and presumably to tissues and specimens as well (Surzycki, 2000).

Methods for storing DNA or tissue extracts at room temperature include FTA paper (mentioned above) and vitrification of DNA in trehalose [e.g., Qiasafe tubes (Qiagen, Inc.) or Gentegra (IntegenX, Inc.); Trehalose works by binding to DNA and stabilizing its structure (Zhu et al., 2007)]. Ivanova and Kuzmina (2013) compared Biomatrica DNAsable plates, homemade trehalose plates, and polyvinyl alcohol (PVA) plates for room-temperature and freezer preservation of insect DNA. They measured successful amplification and sequencing following various storage conditions up to 4 years old. Overall, the dry storage media (trehalose, Biomatrica, or PVA) provided sufficient protection for short-term storage of DNA at room temperature, thus enabling shipment and exchange of DNA extracts and PCR products between DNA barcoding facilities (Knebelsberger and Stoger, 2012). Although trehalose might be sufficient for short-term storage, they recommend Biomatrica and Tris-buffered PVA for long-term storage and for sample exchange with tropical countries. Desiccants should be used to minimize exposure to humidity. Temperature is a crucial factor for DNA quality, which has to be considered especially for long-term storage. Some have found that short-term DNA storage is best at 4°C or long-term at -70°C or

less. Others have concluded that DNA is best stored in trehalose, either at room temperature or at -80°C (Smith and Morin, 2005). However, Smith and Morin (2005) focused on extracted DNA and not storage of tissues, which behave differently because of the matrix of cellular contents. It is premature to advocate transition to DNA storage at room temperature, and that these storage methods should be viewed as adjuncts to archival freezer storage of tissues or DNAs.

Frippiat (2011) compared stability of human DNA stored at room temperature in Gentegra matrix vs. Qiasafe, using DNA extracted by phenol/chloroform vs. a magnetic bead protocol. Reduction in amplification was shown in the samples extracted with magnetic beads, suggesting that specific DNA isolation protocols are important in these anhydrous preservation methods.

Although many researchers resuspend DNA extracts in water, many references emphasize the beneficial effects of TE buffer (1 mM Tris-HCl, 1 mM EDTA, pH 8.0) for long-term storage of DNA. DNA is more stable under high pH and in the absence of metal ions that catalyze degradation. TE buffer provides a slightly alkaline environment, as EDTA is a chelating agent that binds metal ions (Surzycki, 2000; Anchordoquy and Milina, 2007).

6. WHAT QUESTIONS REMAIN ABOUT PLANT DNA RESOURCES, CURATION, AND LONG-TERM STORAGE?

Given the above review, some aspects of DNA curation, and storage are clear, but many questions remain.

- A. How is DNA quality related to methods of herbarium specimen curation (do higher temperature and humidity negatively affect DNA quality in herbarium specimens)? In a broad sense,

- of taxa, are there any plant groups that have consistently good or bad DNA quality in herbarium specimens (i.e., might certain families be particularly prone to DNA degradation)?
- B. What are the age limits of herbarium specimens that give "high-quality" DNA? Does this vary among different clades (families/genera)? Does tissue succulence play a role in DNA degradation in herbarium specimens (i.e., does the added water of thick tissues make DNA preservation poorer than in thin-leaved samples)?
 - C. How is storage of tissues in silica affected by type of storage vessel? How is DNA quality affected in tissues stored in silica vs. freezers?
 - D. How does DNA quality compare in DNA extracts stored at -20°C vs. silica-dried tissues stored at room temperature?
 - E. What are the effects of light on DNA quality of silica-dried tissues (i.e., effects of photobleaching)?

Here we present results of experiments designed to address these questions.

MATERIALS AND METHODS

In discussions of DNA and herbarium specimens, we realized that collectively we have sets of hundreds of herbarium specimens and/or specimens dried in silica gel collected up to 30 years ago that might facilitate a serendipitous experiment on the effects of specimen preparation and curation on DNA preservation. These specimens were collected for a variety of phylogenetic or floristic projects, but their curation history is known in sufficient detail for us to make correlations

between DNA quality, age, and specimen curation (Appendix 4). To examine the relative importance of such effects, we extracted DNA from these sets of specimens and used gel electrophoresis as a rough estimate of DNA quality among samples. We did not test amplification or sequencing success from these DNAs.

A. Effects of herbarium specimen curation on DNA quality: To address the impact of different herbarium curation variables (archival mounting paper, ambient temperature and humidity), we used two similar sets of taxa collected for Florida floristic studies from about the same time period (7-21 years old) but stored under different conditions. The first data set was collected to document the flora of the White Oak Plantation, Nassau Co., Florida. These specimens ($n = 461$, mean age of 13.6 years) were pressed carefully over gentle forced air, immediately mounted on archival herbarium paper, and stored in pest-free cabinets in an air-conditioned lab, under low humidity (ca. 45% RH) at cool temperatures (<20°C) for the lifetime of the collection.

The second set of specimens, hereafter referred to as the "Abbott collection" ($n = 192$, mean age 17.6 years), was collected by Richard Abbott and has been stored unmounted in the original newspapers and has never been mounted on archival paper. The collection was moved cross-country several times (Florida, California, Illinois, Missouri, Florida). Until recently, the specimens were not consistently in air-conditioned environments, thus were periodically subject to ambient humidity and temperature.

B. Effects of herbarium specimen age and leaf succulence on DNA quality: To determine if DNA degradation rates in herbarium specimens might vary among families or genera, we examined specimens of various ages of phylogenetically disparate families from specimens

with the same curation history: *Asimina* ($n = 128$, Annonaceae), *Mikania* ($n = 334$, Asteraceae), Polygalaceae ($n = 355$), Hibisceae ($n = 330$, Malvaceae), and *Portulaca* ($n = 40$, Portulacaceae).

C. Effect of silica drying and freezing on DNA preservation: We examined DNA quality in various tissues that had been stored with the specific intent of preserving DNA. Specimen sets included tissues dried in silica gel and then stored at room temperature (usually in the dark) for up to 20 years in falcon tubes (transparent 50-ml polypropylene centrifuge tubes with opaque blue caps) ($n = 63$), glass specimen jars ($n = 20$), and Ziploc® bags ($n = 91$). Unfortunately, no tissues older than this could be located, because this method of tissue storage only came into use in the early 1990s (Chase and Hills, 1991). We also analyzed various tissues stored for up to 24 years in -20°C ($n = 20$) or -80°C ($n = 38$) freezers to assess the effects of low temperatures on DNA quality.

D. Comparison of DNA preservation of orchid leaf samples in room temperature silica-dried tissues vs. DNA extracts frozen at -20°C for 7-12 years: Silica-dried samples ($n = 11$) were stored at room temperature for 7-12 years with a replicate of DNA extracts from the matching samples. Fresh tissues of the same silica-dried accessions were recollected from greenhouse-grown material at the present day (i.e., 2013) for comparison.

E. Effect of photobleaching: Orchid leaf tissues ($n = 8$) were stored in falcon tubes and exposed to ambient light (both indirect sunlight and fluorescent light) on a laboratory shelf for 15 years. These tubes seal tightly with little air infiltration. These samples are not directly compared to other DNA resources; they simply represent the demonstrable effect of light on tissues.

Extraction protocol – All samples were ground in a bead beater mill in 2-ml screwcap vials using glass beads. Extractions utilized a modified CTAB protocol (Doyle and Doyle, 1987); details are presented in Appendix 2. Because of the large number of samples, the DNAs were simply precipitated with isopropanol followed by two washes of the pellet with 70% ethanol. Neither RNase treatment nor silica column cleaning was performed prior to electrophoresis.

Electrophoresis – Because of cost and time considerations, we did perform exhaustive spectrophotometric analyses (e.g., Nanodrop, Qu-bit) or more sophisticated methods of nucleic acid analysis (e.g., Bioanalyzer). All total genomic DNAs were electrophoresed on 1.5% agarose gels using ethidium bromide and two size standards (a 100-bp hyperladder IV and lambda DNA digested with *Eco*RI and *Hind*III) run at 150 volts for ~30 minutes or 2 cm migration of loading dye (whichever was achieved first); details are in Appendix 3. Gels were photographed using a Canon A650IS camera. Gel images were analyzed using Photoshop software for basic image cropping and rotation. GelAnalyzer2010 was then used to produce a two-dimensional graph of absorbance vs. fragment length (<http://www.gelanalyzer.com/>).

Analysis – We regard the pattern of DNA fragment length revealed through gel electrophoresis as roughly indicative of overall DNA quality and extent of degradation. High-quality total DNA extracts have a strong peak of very HMW (>23 kbp), with relatively few low-molecular-weight fragments. Highly degraded DNA has no HMW fragments, with smears of fragments below 1000 bp. Based on the relative intensity of absorbance at high-, medium-, and low-molecular-weight regions of each trace (Fig. 1), we constructed 24 cat-

categories of DNA trace quality, ranging from highest to lowest quality (Fig. 2). We subjectively scored each sample's electrophoretic

trace and placed it into one of these quality categories. These quality scores were used to compare samples in the data analyses.

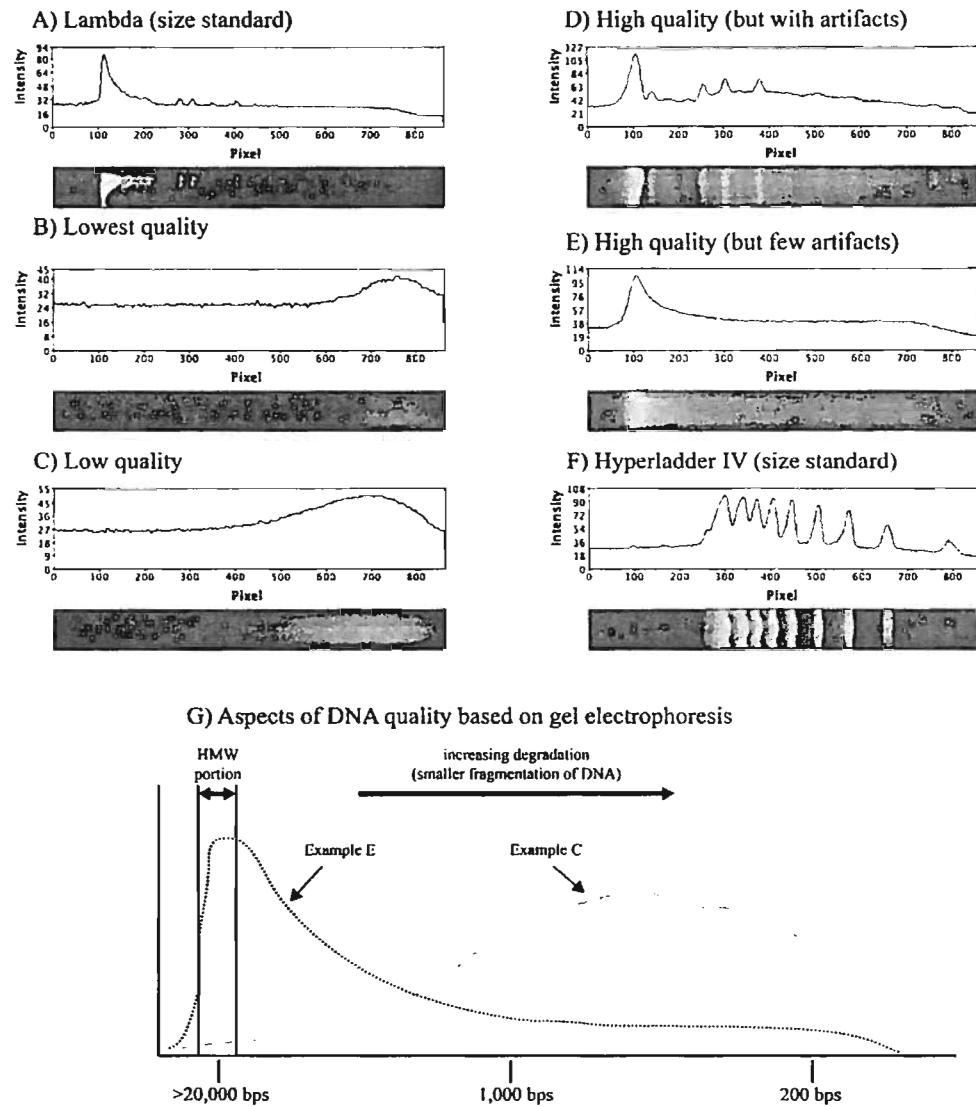


Figure 1. Electrophoretic profiles of exemplar samples demonstrating: A) Upper size limit. B) Lowest quality sample (except for a sample with no discernible DNA) with a low peak in the low-molecular-weight region indicating intense degradation. C) Another low quality sample, but with a broader smear with slightly higher molecular weight. D) High quality DNA with a HMW band, but peak artifacts at lower molecular weight (presumably ribosomal RNA). E) High-quality sample with a HMW peak and very little low-molecular-weight DNA, indicating little degradation. F) Size standard hyperladder showing peaks at 100-bp intervals from 1000 bp at left to 200 bp at right. Peak height is correlated with overall fluorescence and is not an absolute measurement of DNA.

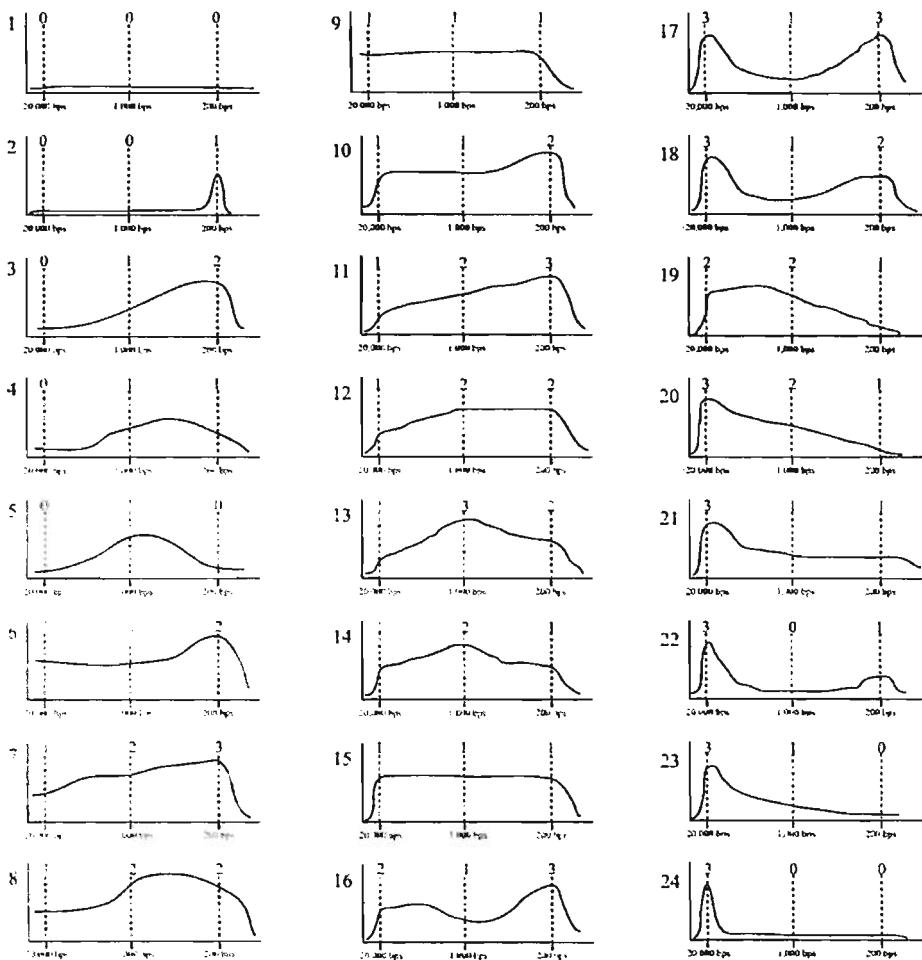


Figure 2. Character states of DNA quality as delimited by four aspects in gel electrophoresis imaging. The first aspect is the presence of a peak within the HMW region as indicated by Figure 1. The second, third, and fourth aspects are the relative fluorescence at the HMW, 1 kb, and 200 bp portions, respectively, of the total DNA extracts. The lowest-quality sample is exemplified by image 1; the highest-quality is exemplified by image 24.

RESULTS

For this study, we define “high-quality” DNA as a sample containing a relatively large amount of HMW fragments. Likewise, “degraded” DNA implies that there is relatively little HMW DNA with a majority of low-molecular-weight DNA. Our results are contingent upon the premise of DNA degradation shifting HMW DNA to low molecular weight (Fig. 1G) and are consistent with the

examples of degradation presented by Adan and Sharma (2010).

A) Comparison of the extracts from the two differentially curated herbaria (White Oak Plantation vs. Abbott) (Fig. 3) demonstrate that method of curation has a large effect on DNA quality. The White Oak Plantation samples had 89% of the samples with HMW bands, whereas the Abbott samples had only 16% of samples with HMW bands.

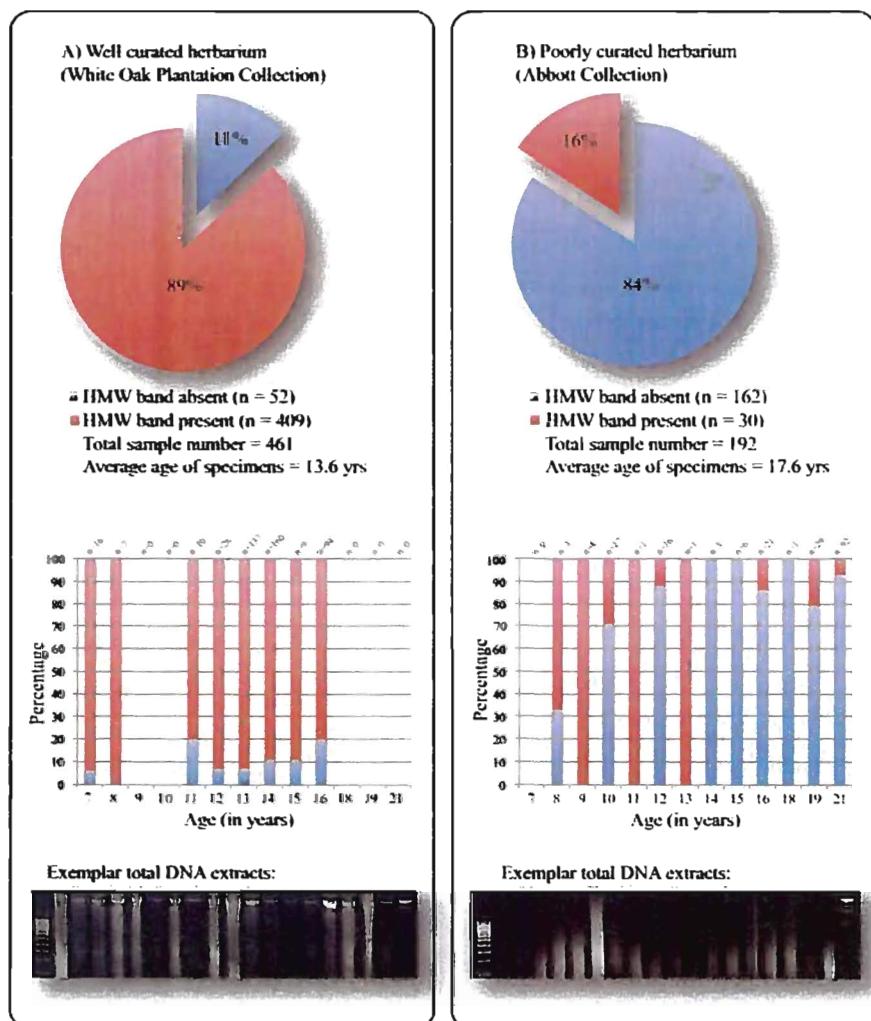
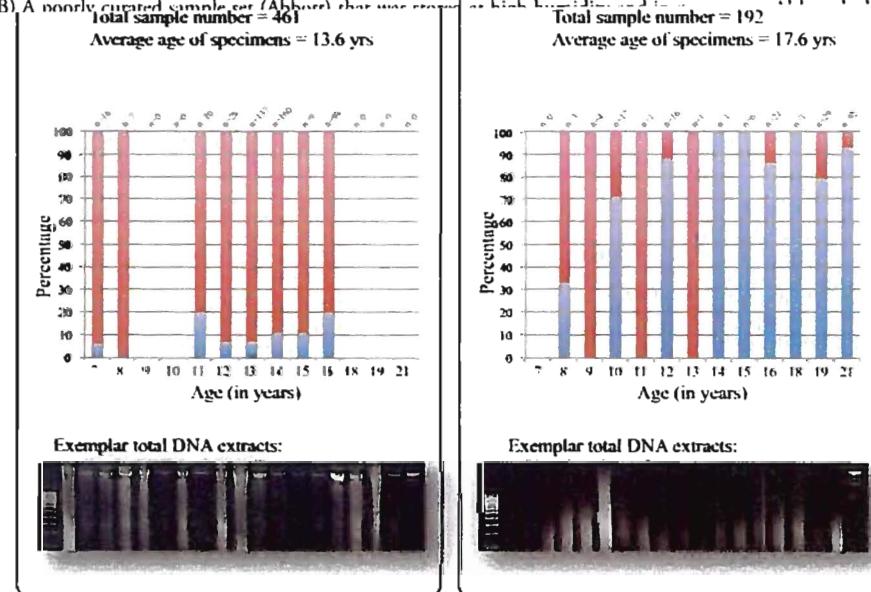
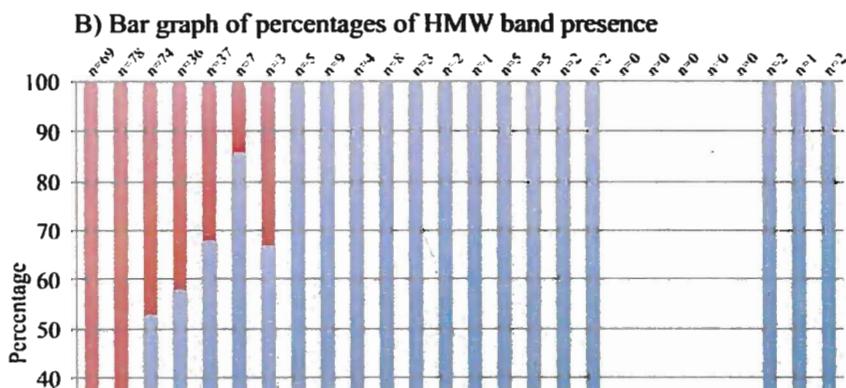
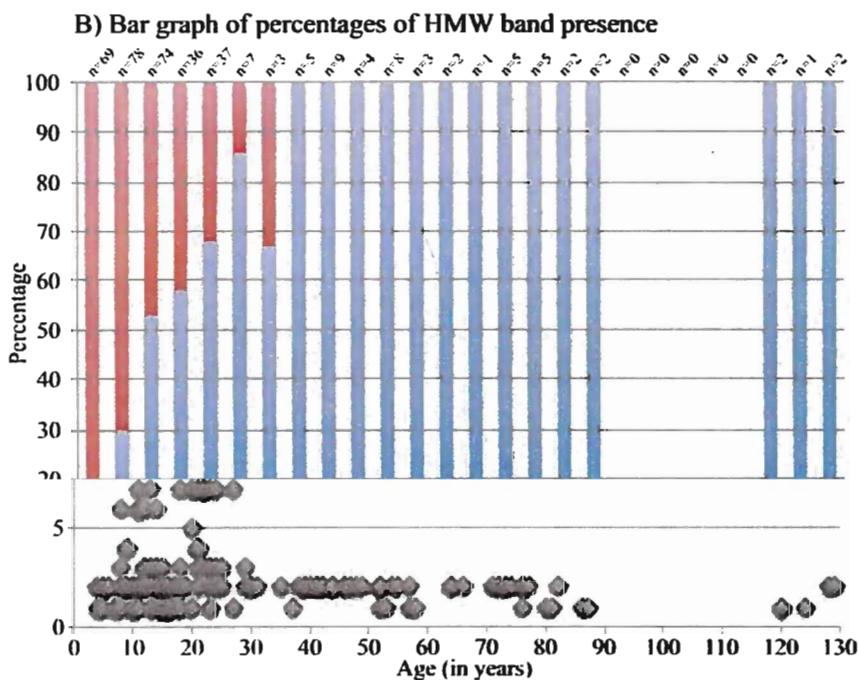
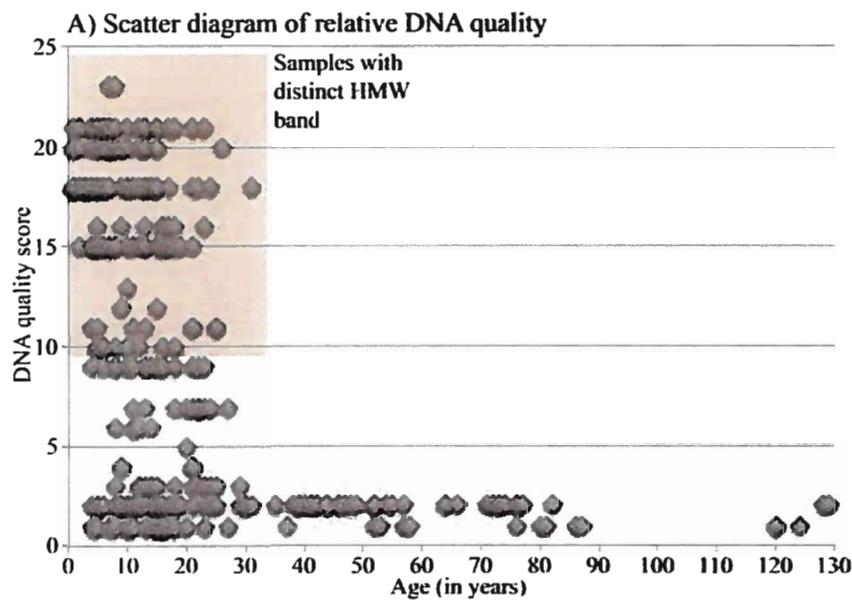
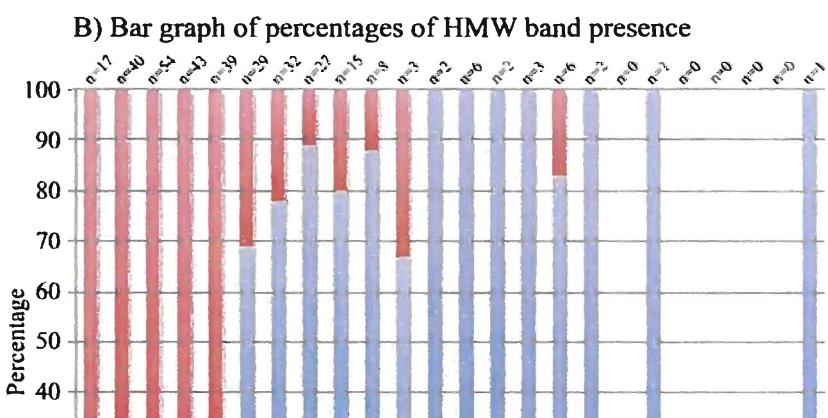
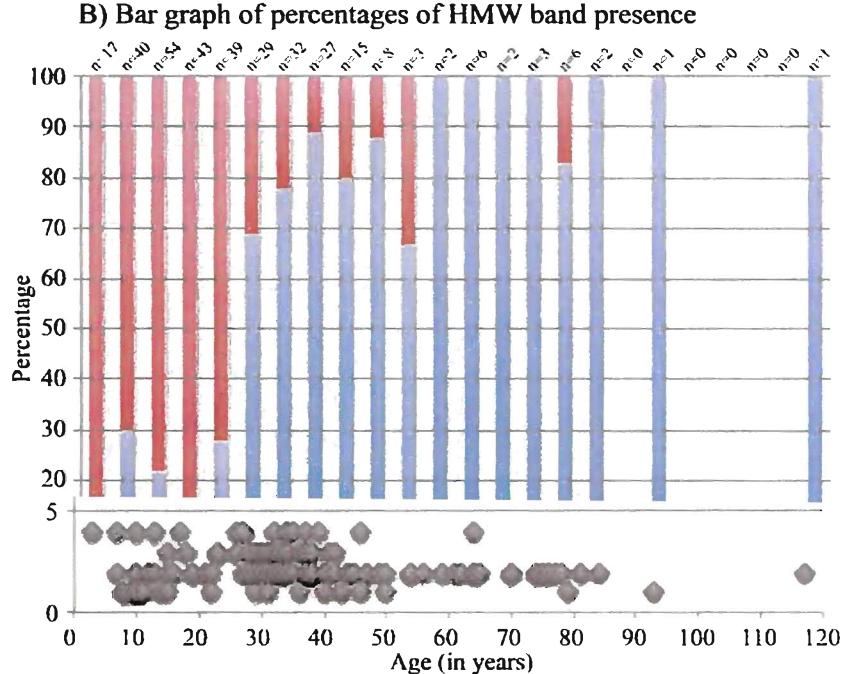
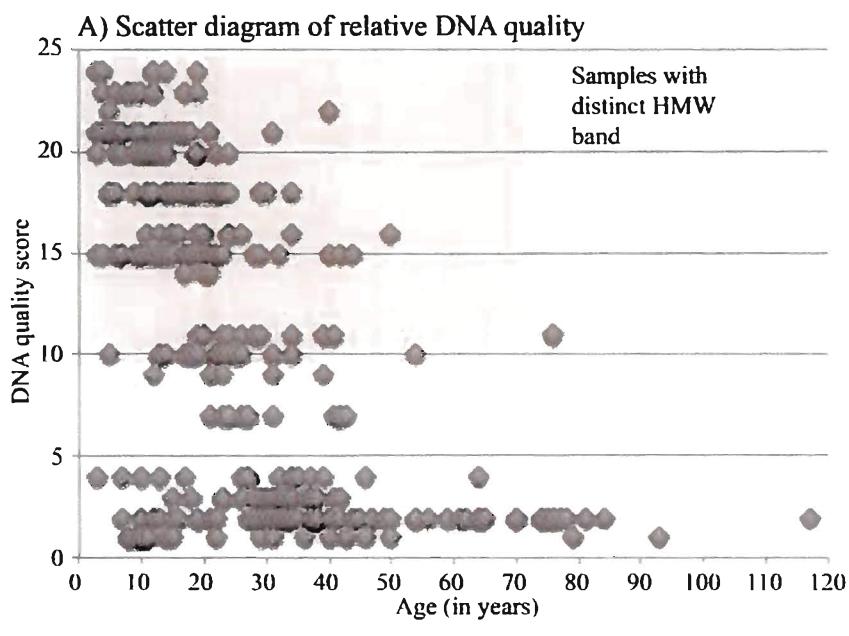
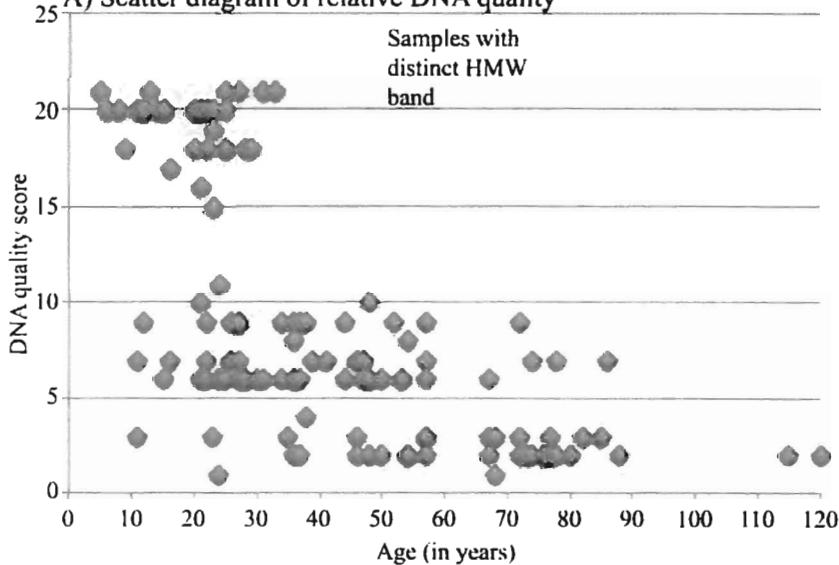
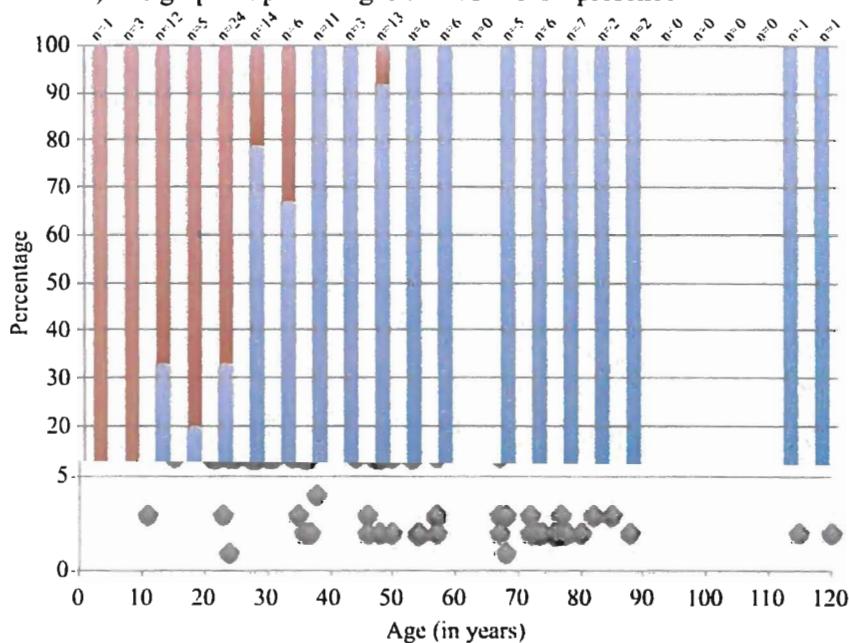
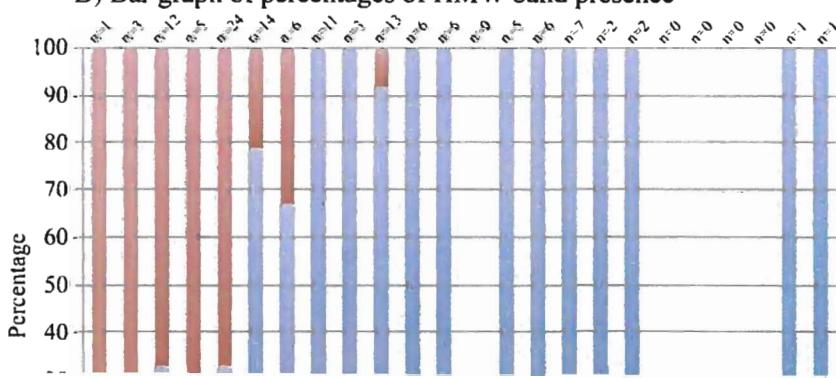


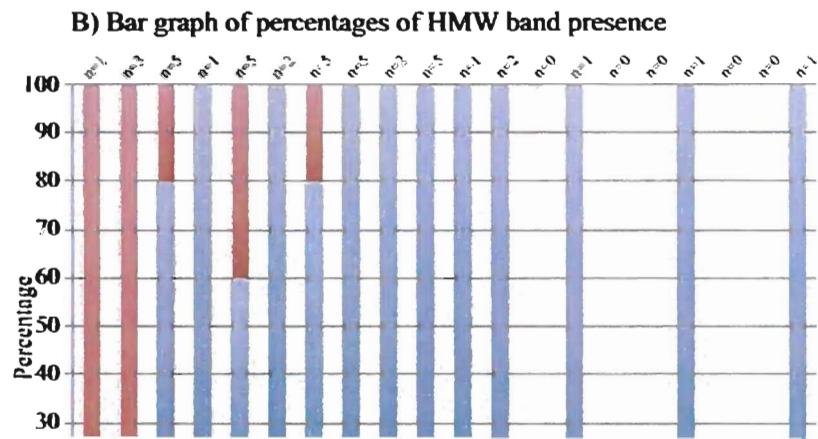
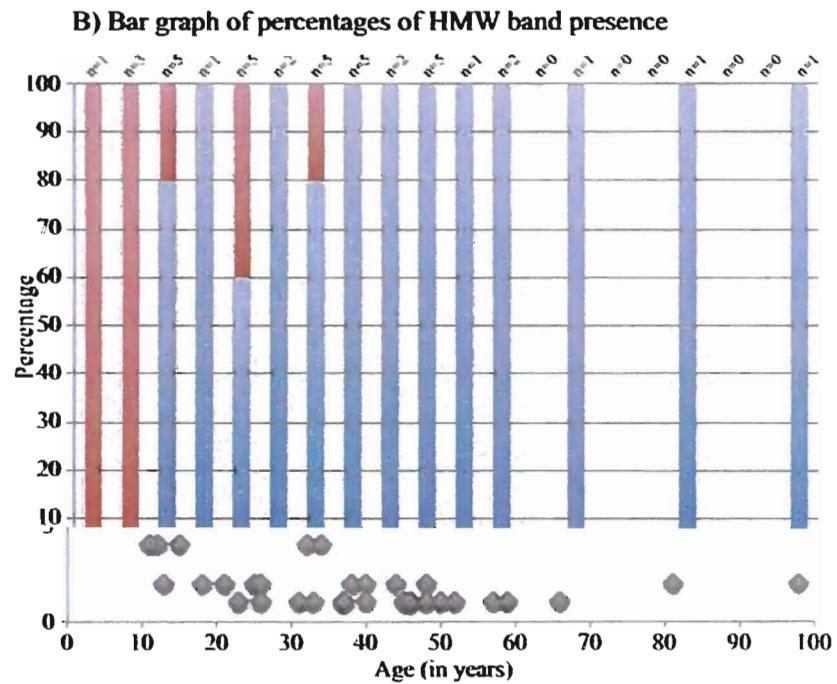
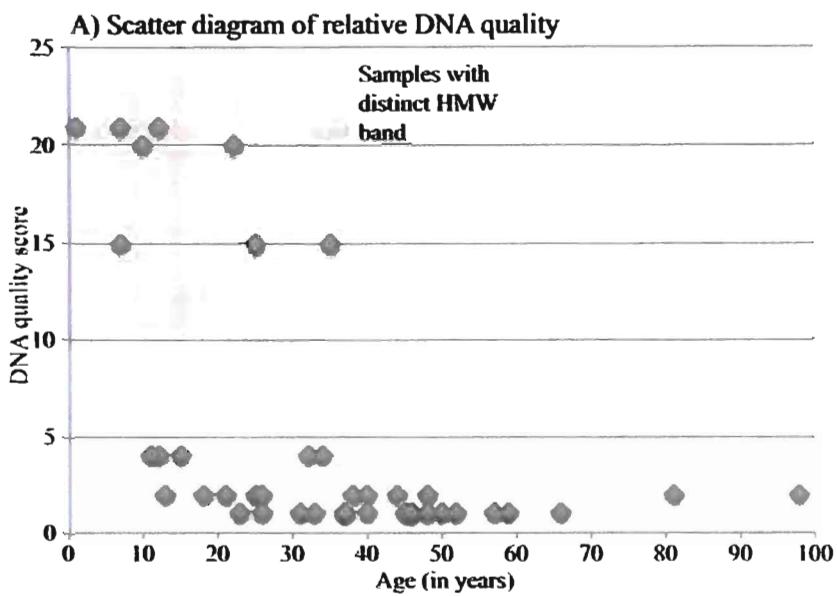
Figure 3. A comparison of the presence of HMW DNA in various herbarium collections to ascertain the influence of herbarium sample curation on DNA quality. A) A well-curated sample set (White Oak Plantation) that was stored at low humidity. B) A poorly curated sample set (Abbott) that was stored at high humidity.

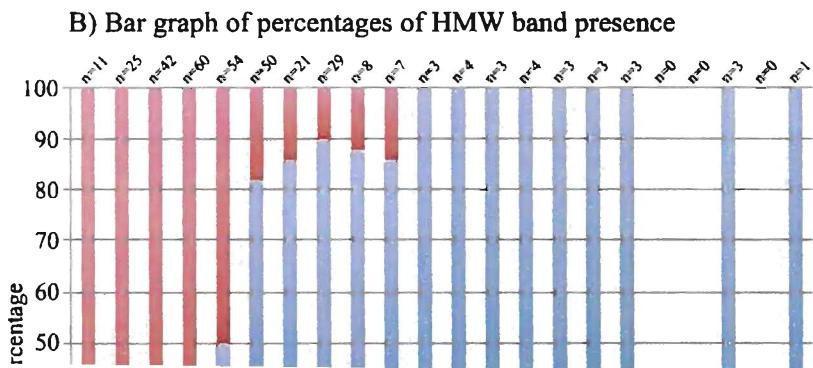
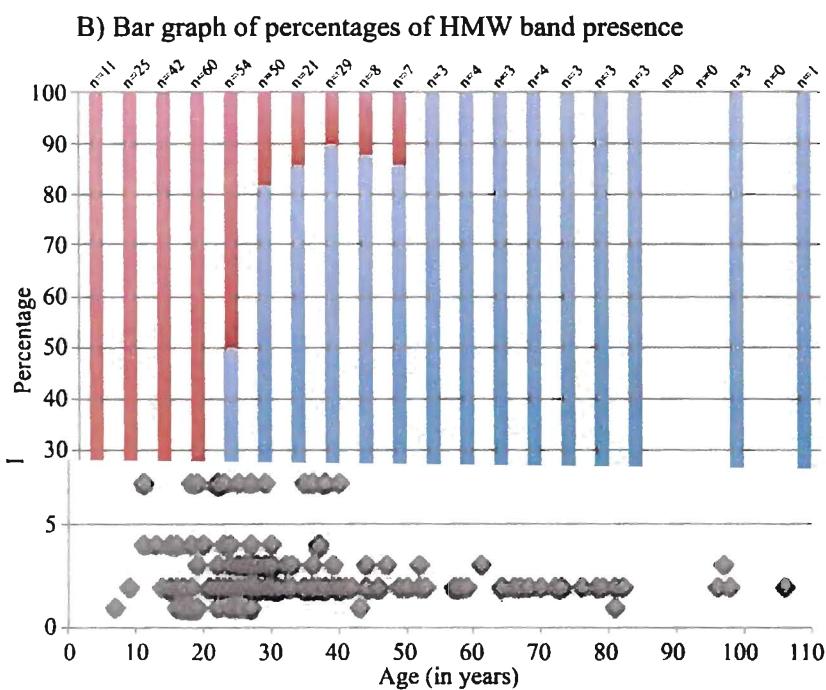
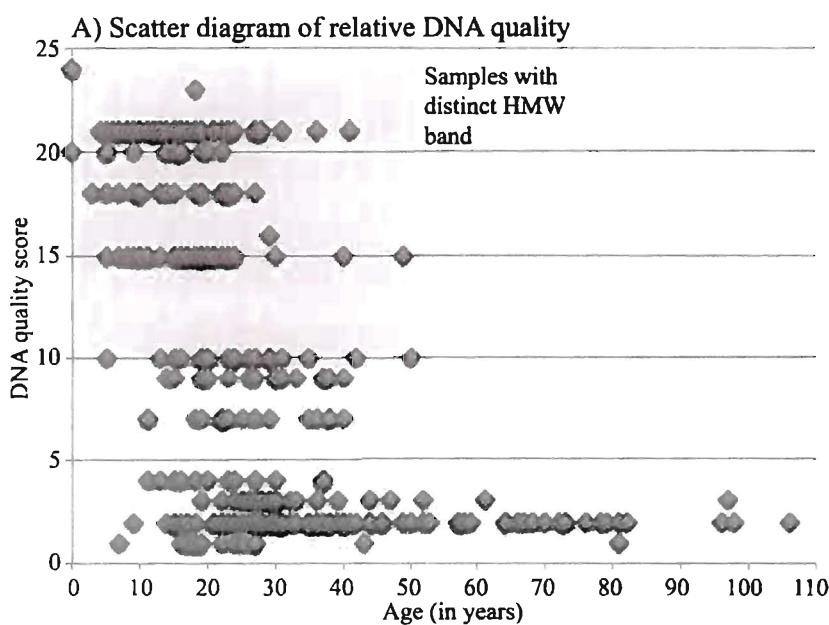




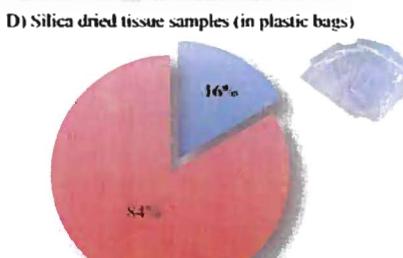
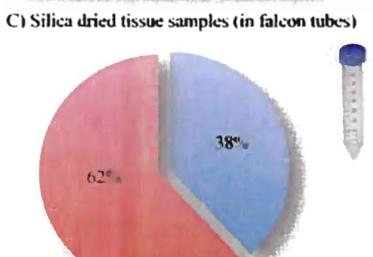
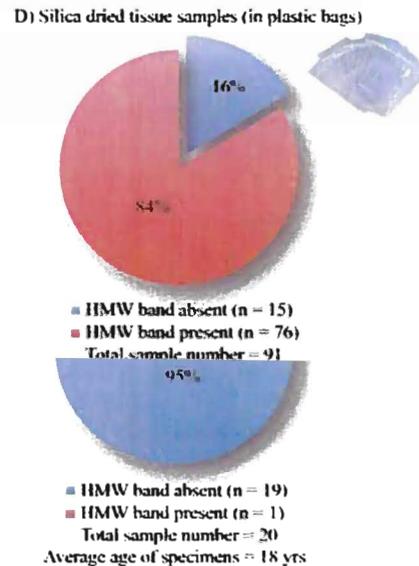
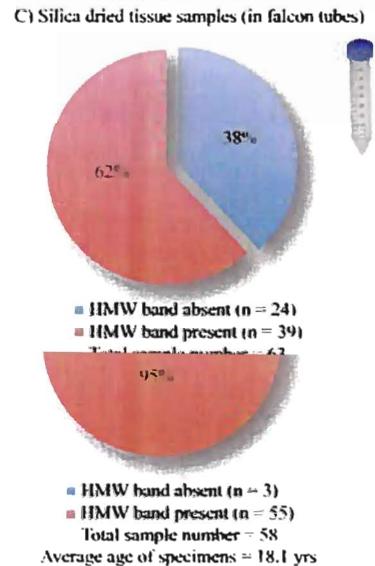
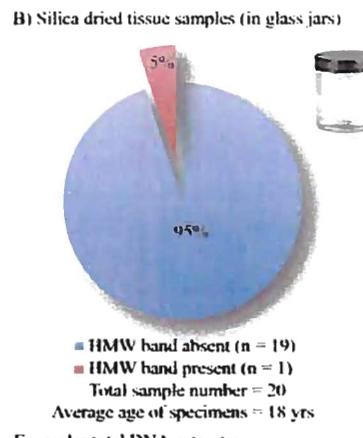
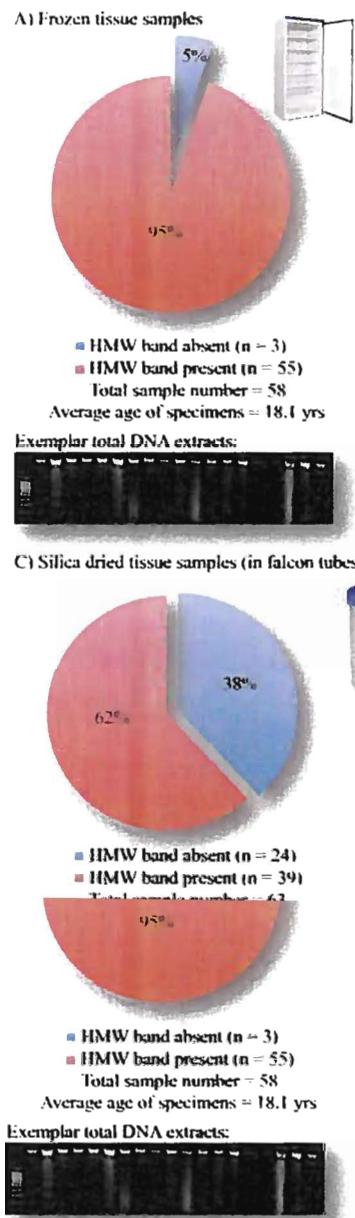


A) Scatter diagram of relative DNA quality**B) Bar graph of percentages of HMW band presence****B) Bar graph of percentages of HMW band presence**





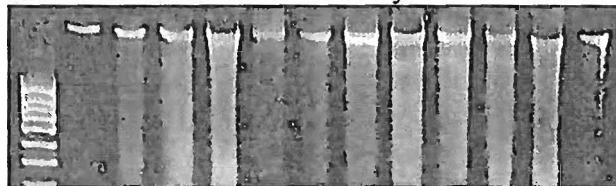
C) Frozen tissues gave consistently high-quality DNA (Fig. 9A), regardless of age. However, silica-dried tissues yielded extracts of widely varying DNA quality (Fig. 9B-D), suggesting that storage conditions of the silica-dried tissues might greatly affect long-term DNA quality.



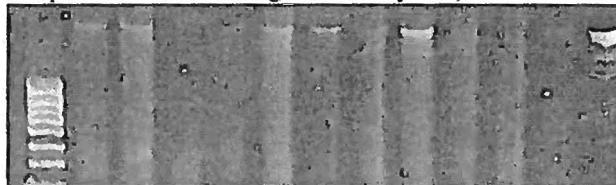
D) Figure 10 shows DNA extracts for a set of 11 orchid samples. The bottom row shows extracts from fresh tissue, the top row shows DNA extracts made from freshly silica-dried tissues 7-12 years ago (the total DNAs have been stored at -20°C since extraction), and the

middle row shows recent re-extractions of the same 7-12-year-old silica-dried tissues, illustrating a comparison of the first and second rows showing degradation of these samples over time.

A) Original DNA extracts (from silica-dried tissues) that were stored in -20°C for 7-12 years.



B) Secondary DNA extracts from the same silica-dried tissues as in part A (those tissues were stored at room temperature in silica bags for 7-12 years).



C) DNA extracts from fresh tissues of same material grown in greenhouse (collected present day).

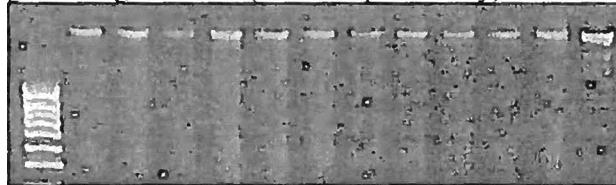


Figure 10. Comparison of A) original extracted DNA from silica-dried samples stored at -20°C, B) secondary extractions from the same silica-dried samples in present day, and C) freshly collected samples from greenhouse-grown living plant collection in present day. Size standards are in lanes 1 and 13 (hyperladder IV and lambda digest, respectively), identical sample replicates are in lanes 2-12.

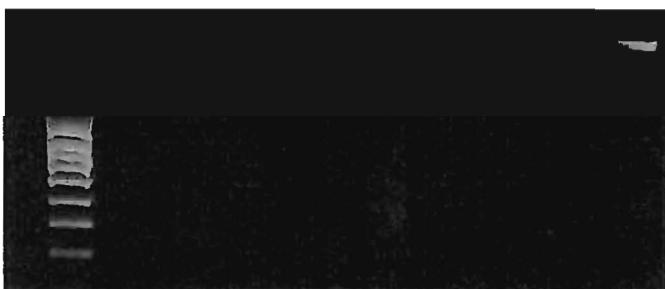
E) In Figure 11, the effects of photobleaching in silica-dried samples are depicted. Although very little DNA was recovered from these

samples, some of them still had some HMW DNA.

A) Exemplar photobleached sample



B) Electrophoretic gel image of photobleached samples



B) Electrophoretic gel image of photobleached samples



DISCUSSION

DNA measurements can be made by various means. Spectrophotometric analyses of DNA solutions measure the absorbance ratio at 260 vs. 280 nm; the ratio is indicative of the purity of the DNA (i.e., 1.8 for pure DNA) (Manchester, 1995). DNA-specific spectrophotometers include NanoVue UV-spectrophotometer (GE Healthcare), Nanodrop, and Qubit. These technologies assess DNA quantity, but not quality. So, low-quality samples can have high masses of DNA, and vice versa. Assays to measure damage to DNA strands generally involve sequencing of the DNA, usually through next-generation methods (Godden et al., 2012; Bi et al., 2013)K.</author><author>Linderroth, T.</author><author>Vanderpool, D.</author><author>Good, J. M.</author><author>Nielsen, R.</author><author>Moritz, C.</author></authors></contributors><titles><title>Unlocking the vault: next-generation museum population genomics</title><secondary-title>Molecular Ecology</secondary-title></titles><periodical><full-title>Molecular Ecology</full-title></periodical><pages>6018-6032</pages><volume>22</volume><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>.

Many studies have used PCR as a measure of DNA quantity and quality. DNA quality is estimated by simple success/failure ratios and the length of the target amplicon, with the assumption that higher-quality DNA is capable of yielding higher-molecular-weight amplicons (Erkens et al., 2008; Adams and Sharma, 2010; Särkinen et al., 2012; Ivanova and Kuzmina, 2013). Real-time PCR has also been applied as a means of more precisely quantifying DNA quality (Cankar et al., 2006). However, there are at least several potential problems with using PCR as a gauge of template quality. The first potential problem is

primer mismatch; if a primer does not match the template appropriately, a product will not be generated, thus giving a false negative for DNA quality. The second potential problem is inhibition; because PCR is a relatively sensitive enzymatic reaction, secondary compounds, when present within a total DNA extract, commonly inhibit PCR, also giving a false negative for DNA quality.

Highly accurate analyses of fragment length are provided by instruments such as the Bioanalyzer (Agilent Technologies, Inc.). However, the Bioanalyzer only analyzes fragments up to 17 kb, whereas HMW DNA tends to be >20 kb (Panaro et al., 2000), so it is severely limited for assessing high-quality DNA. A quicker and somewhat less precise method of assessing DNA quality through a proportionate measure of fragment length is by gel electrophoresis; the gel image provides a semi-quantitative assessment of DNA fragment size and abundance. We used gel electrophoresis to estimate DNA quality because it is relatively cheap and rapid.

Pertinent variables affecting DNA quality that were addressed in our study include herbarium specimen curation conditions, taxonomic variation versus time, leaf succulence and DNA preservation, silica storage, freezer storage, DNA extract storage, and photo-bleaching, as follows.

A) Effects of herbarium specimen curation on DNA quality

The comparison of the White Oak Plantation and Abbott collections showed different patterns in DNA quality. With these two sets of data, we attempted to eliminate as many variables as possible, such as inconsistency in collection process, by having a limited number of collectors for each set. Taxonomic biases were minimized because both sets represent the same north Florida flora, although sample

size and taxon sampling are not identical. The main variables between the two data sets were the environmental conditions during storage. Whereas the White Oak collection was mounted immediately after pressing and drying and then stored under very tightly controlled temperature and humidity, the Abbott collection has been stored in boxes and the original newspapers. Interestingly, this collection also sat in hot moving trucks (often exceeding 60°C) moving across the country from Florida to California, back to Florida, then to Illinois, then to Missouri, then ultimately back to Florida (all in this 21-year period with an average specimen age of 17.6 years). This collection was not stored under ideal conditions of low humidity. In the course of this collection's history, the specimens were subject to multiple pests. Most notably, while in California, one bundle of specimens became home to a small family of field mice, who chewed through the newspapers holding the specimens.

Not surprisingly, the two sets are strikingly different in DNA quality. Whereas the White Oak Plantation specimens included 89% of specimens with HMW DNA, only 16% of the Abbott collection had HMW DNA. Further, although these two data sets do differ slightly in age [the White Oak Plantation is an average of 13.6 years (7-16 years), while the Abbott collection is an average of 17.6 years (8-21 years)], all of the specimen ages are less than that of the typical thresholds of samples that give HMW DNA (see Figs. 4-8 and following section), if collection methods are standardized. These data demonstrate that the quality of collection and storage of herbarium specimens strongly affects DNA quality, at least for specimens 15-21 years old.

B) Effects of herbarium specimen age and leaf succulence on DNA quality

DNA quality in herbarium specimens is loose-

ly correlated with specimen age. Although high-quality samples are exclusively found in younger specimens, low-quality samples can be found throughout the age distribution, even in recently collected specimens.

Effect of succulence on DNA preservation
We selected subsets of herbarium specimens with different tissue thicknesses and rates of drying. Samples of *Asimina*, *Hibisceae*, and *Mikania* are typically thin-leaved, whereas those of *Portulaca* are succulent. *Polygalaceae* are usually thin-leaved, but more variable. Results showed that thin-leaved taxa generally have higher quality DNA than do thick-leaved or succulent specimens. Although a 35-year-old sample of *Portulaca* showed relatively high-quality DNA, most *Portulaca* samples of high quality were less than 15 years old. This is relatively young compared to the other taxonomic groups examined. This is consistent with Chase and Hills (1991), who showed low DNA yields in succulent herbarium specimens of orchids. Our own work on herbarium specimens of orchid taxa shows similar results in DNA quality of succulent versus thin-leaved orchid tissues (Neubig and Whitten, pers. obs.).

Taxon-specific variation in DNA preservation – Does DNA survive better in some families or genera than others? Aside from the effect of succulence causing slow drying, our uncontrolled data are not sufficient to demonstrate any clear correlations between taxonomic group and DNA preservation. Adams et al. (1999) reported rapid DNA degradation in some Poaceae and Brassicaceae, and they hypothesized that robust DNases degraded DNA rapidly in fresh and herbarium tissues. These effects may be restricted to relatively few taxa. We have sampled many taxa from Poaceae both in this study and others, and the vast majority of samples yield good-quality DNA (within a reasonable age limitation of 30 years or less). In contrast, Melastomataceae

exhibit very poor DNA preservation in herbarium specimens, even in recent collections. Our work on Melastomataceae (especially in tribe Miconieae) consistently shows low success rates of extractions from herbarium specimens, despite being relatively thin-leaved (Neubig, pers. obs.; Bécquer-Granados et al., 2003). The cause of this degradation in Melastomataceae is unclear. High success rates of extraction and amplification from old herbarium specimens of xeric Melanthiaceae, such as *Schoenocaulon*, led Zomlefer (2006) to speculate that physiological adaptations to xeric habitats might result in better DNA preservation than in mesic plants, but we are unaware of controlled studies.

C) Effect of silica drying and freezing on DNA preservation

We analyzed 58 samples of tissues that had been frozen (-20° or -80°C) for at least 10 years (Fig. 9). The samples are phylogenetically diverse and variable in preparation (pulverized or intact tissue). Almost all yielded extremely good-quality DNA. This is somewhat at odds with Smith and Morin (2005), who found significant degradation of samples stored at -20°C versus colder temperatures.

We presume that nearly all the silica-dried tissues initially contained high-quality DNA, because most of the samples used had been originally preserved for molecular laboratory use (i.e., plastid restriction site analysis, or PCR-based procedures). The great range of quality present in the ca. 20-year-old samples must be due to storage variables. The most obvious is the seal of the container. Specimens stored in tightly sealed containers (i.e., falcon tubes), often with an excess of silica gel, appeared to have higher-quality DNA. Loosely sealed specimen jars (such as jars used for spirit collections in herbaria) are very poorly sealed against air infiltration, and thus humid-

ity. Small Ziploc® bags are not airtight, and the silica will eventually equilibrate with atmospheric humidity. But, most of our sample from Ziploc® bags were triple sealed in airtight polypropylene boxes with silicone gaskets. So not surprisingly, the Ziploc® bags performed the best (Fig. 9D) because of the extra measures of protection taken, rather than the innate nature of Ziploc® bags.

D) Comparison of DNA preservation of orchid leaf samples stored in room temperature as silica-dried tissues vs. DNA extracts frozen at -20°C for 7-12 years

Comparison of DNA quality in Figure 10 shows that storage of DNA extracts (stored in TE at -20°C for 7-12 years) is superior to new extracts of silica-dried leaves stored at room temperature, sealed in containers in the dark, but without additional silica to absorb ambient moisture. Nevertheless, most of the silica-dried tissues retained some HMW DNA. The freshly collected tissues all exhibit relatively uniform and strong HMW DNA as expected. Therefore, we can conclude that ambient moisture, and potentially temperature, are the dominant contributing factors to DNA degradation in the silica-dried tissue samples.

E. Effect of photobleaching

Silica-dried tissues that have been stored in transparent containers (plastic bags or falcon tubes) are subject to the deleterious effects of light over long periods (Sinha and Häder, 2002). Such tissues exposed to light usually lose their green pigmentation. However, this bleaching does not necessarily affect HMW DNA. This reiterates the findings of Erkens et al. (2008), who correlated DNA quality with herbarium specimen color. They found a weak correlation with specimen "greenness" when

compared to DNA quality. Nevertheless, we recommend storage of tissues in the dark.

CONCLUSIONS

The wide variety of morphology and life history of organisms makes DNA and tissue preservation a complex topic (Seutin et al., 1990; Kilpatrick, 2002; Mitchell and Takacs-Vesbach, 2008; Zimmermann et al., 2008). Although very limited, these data indicate that best practice is to freeze tissues and DNA samples for maximum quality preservation at the lowest temperatures possible. Although cryogenic tissue banks that curate samples for archival storage are becoming more common (Corthals and Desalle, 2005; Hodkinson et al., 2007; Gemeinholzer et al., 2011; Endara et al., this volume; Godden and Soltis, this volume), they are still expensive for small institutions and are impractical for short term lab work where samples need to be accessed frequently.

Although the ability of next-generation sequencing technologies to produce useful data from fragmented DNA will undoubtedly increase, we should not use this technology as an excuse to abandon collection/curation methods that maximize DNA quality. Highly intact and undamaged DNA will always be preferred over degraded DNA of uncertain quality. We recommend that all herbarium collections should be supplemented with tissues dried in silica gel (or FTA paper, or both). If institutional resources permit, an aliquot of these tissues should be stored at the lowest temperature possible. If liquid nitrogen storage is not available, we recommend vacuum-packing of dried tissues as a low-cost alternative to minimize degradation over time. This procedure has been suggested before (Nagy, 2010), but we have tested this method over the past 10 years (see Appendix 1) and find it to be an effective way to preserve DNA quality in tissues,

yet still have them readily available for active use in the laboratory.

The emergence of room temperature storage methods such as trehalose vitrification or FTA paper is encouraging, but the available data do not justify using these methods to replace cryogenic banks and silica-dried tissues at present. They are useful supplements, but cannot be considered archival.

Although this and other studies have shown that DNA degrades over time in herbarium specimens (Erkens et al., 2008; Adams and Sharma, 2010; Adams, 2011), it is clear that we can retard degradation by optimizing the environmental conditions in which the specimens are stored. From the herbarium curator's perspective, we strongly recommend minimizing the length of time specimens are stored in acidic newspaper. Although specimen mounting backlogs are usually the result of budget constraints, herbarium curators should realize that leaving specimens in acidic newspapers is one of the greatest threats to specimen quality, analogous to dermestid beetles, on a molecular level.

Archival storage is expensive in terms of money, space, and time. Institutions need to set priorities to decide what to preserve and at what quality. Is it really necessary to store all DNA for eternity? If we store a sample on FTA paper or vitrified in trehalose that will be good for 200 years, is that good enough, or do we need to store everything in liquid nitrogen? How do we prioritize sample storage vs. cost? What are the effects of various storage techniques on DNA damage (i.e., "nicking", etc.)? Although liquid nitrogen storage is superior, relatively inexpensive methods can be used to improve the storage conditions of specimens and tissues, drastically slowing the rate of DNA degradation. With global extinction rates rising rapidly, our herbaria will soon contain the only remains of many extinct species or extirpated populations. The DNA in

these specimens contains the majority of the information on the species that we can leave to future generations. Anything we can do to minimize the loss of this information should be encouraged.

LITERATURE CITED

- Adams, R. P. 2011. DNA from herbarium specimens: II. Correlation of DNA degradation with humidity. *Phytologia* 93: 351–359.
- Adams, R. P. & L. N. Sharma. 2010. DNA from herbarium specimens: I. Correlation of DNA size with specimen age. *Phytologia* 92: 346–353.
- Adams, R. P. & L. Baker. 2011. Seventeen years storage of juniper and spinach leaves in alcohols: effects on DNA. *Phytologia* 93: 283–292.
- Adams, R. P., M. Zhong & Y. Fei. 1999. Preservation of DNA in plant specimens: inactivation and re-activation of DNases in field specimens. *Mol. Ecol.* 8: 681–684.
- Akinnagbe, A., X. B. Ji, M. Yang & D. Ewald. 2011. Ethanol pretreatment increases DNA yields from dried tree foliage. *Conserv. Genet. Resour.* 3: 409–411.
- Alexander, P. J., G. Rajanikanth, C. Bacon & C. D. Bailey. 2006. Recovery of plant DNA using a reciprocating saw and silica-based columns. *Mol. Ecol. Notes* 7: 5–9.
- Anchordoquy, T. J. & M. C. Molina. 2007. Frontiers in clinical research: preservation of DNA. *Cell Preserv. Technol.* 5: 180–188.
- Bacci, M., A. Checcucci, G. Checcucci & M. R. Palandri. 1985. Microwave drying of herbarium specimens. *Taxon* 34: 649–653.
- Bécquer-Granados, E., K. M. Neubig, W. S. Judd, F. A. Michelangeli, J. R. Abbott & D. S. Penneys. 2003. Preliminary molecular phylogenetic studies in *Pachyanthus* (Miconiacae, Melastomataceae). *Bot. Rev.* 74: 37–52.
- Bekesiova, I., J.-P. Nap & L. Milynarova. 1999. Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. *Plant Mol. Biol. Rep.* 17: 269–277.
- Bi, K., T. Linderroth, D. Vanderpool, J. M. Good, R. Nielsen & C. Moritz. 2013. Unlocking the vault: next-generation museum population genomics. *Mol. Ecol.* 22: 6018–6032.
- Blanco, M. A., W. M. Whitten, D. S. Penneys, N. H. Williams, K. M. Neubig & L. Endara. 2006. A simple and safe method for rapid drying of plant specimens using forced-air space heaters. *Selbyana* 27: 83–87.
- Bridson, D. & L. Forman. 1998. The Herbarium Handbook. Royal Botanical Gardens, Kew.
- Cankar, K., D. Štebih, T. Drej, J. Žel & K. Gruden. 2006. Critical points of DNA quantification by real-time PCR – effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. *BMC Biotechnol.* 6: 37.
- Carrió, E. & J. A. Rosselló. 2013. Salt drying: a low-cost, simple and efficient method for storing plants in the field and preserving biological repositories for DNA diversity research. *Mol. Ecol. Resour.* 14: 344–351.
- Chase, M. W. & H. H. Hills. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.
- Corthals, A. & R. Desalle. 2005. An application of tissue and DNA banking for genomics and conservation: The Ambrose Monell Cryo-Collection (AMCC). *Syst. Biol.* 54: 819–823.
- Cota-Sánchez, J. H., K. Remarchuk & K. Ubayesen. 2006. Ready-to-use DNA extracted with a CTAB method adapted for herbarium specimens and mucilaginous plant tissues. *Plant Mol. Biol. Rep.* 24: 161–167.
- Crowe, J. S., H. J. Cooper, M. A. Smith, M. J. Sims, D. Parker & D. Gewert. 1991. Improved cloning efficiency of polymerase chain reaction (PCR) products after proteinase K digestion. *Nucleic Acids Res.* 19: 184.
- Csaikl, U. M., H. Bastian, R. Brettschneider, S. Gauch, A. Meir, M. Schaurte, F. Scholz, C. Sperisen, B. Vornam & B. Ziegenhagen. 1998. Comparative analysis of different DNA extraction protocols: A fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Mol. Biol. Rep.* 16: 69–86.

- Daniel, V. 1995. Storage in low-oxygen environments. Pp. 147–155 in C. L. Rose, C. A. Hawks & H. H. Genoways (editors), *Storage of Natural History Collections: A Preventive Conservation Approach*. Society for the Preservation of Natural History Collections, York, PA.
- Do, N. & R. P. Adams. 1991. A simple technique for removing plant polysaccharide contaminants from DNA. *BioTechniques* 10: 162–166.
- Doyle, J. J. & E. E. Dickson. 1987. Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722.
- Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.
- Erkens, R. H. J., H. Cross, J. W. Maas, K. Hoenselaar & L. W. Chatrou. 2008. Assessment of age and greenness of herbarium specimens as predictors for successful extraction and amplification of DNA. *Blumea* 53: 407–428.
- Flournoy, I. E., R. P. Adams & R. N. Pandya. 1996. Interim and archival preservation of plant specimens in alcohols for DNA studies. *BioTechniques* 20: 657–660.
- Frippiat, C., S. Zorbo, D. Leonard, A. Marcotte, M. Chaput, C. Aelbrecht & F. Noel. 2011. Evaluation of novel forensic DNA storage methodologies. *Forensic Sci. Int. Genet.* 5: 386–392.
- Fukatsu, T. 1999. Acetone preservation: a practical technique for molecular analysis. *Mol. Ecol.* 8: 1935–1945.
- Gawel, N. J. & R. L. Jarret. 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Plant Mol. Biol. Rep.* 9: 262–266.
- Gemeinholzer, B., G. Droege, H. Zetsche, G. Haszprunar, H.-P. Klenk, A. Guntsch, W. G. Berendsohn & J.-W. Wagele. 2011. The DNA bank network: the start from a German initiative. *Bio-preserv. Biobank.* 9: 51–55.
- Godden, G. T., I. E. Jorden-Thaden, S. Chamala, A. A. Crowl, N. Garcia, C. C. Germain-Aubrey, J. M. Heaney, M. Latvis, X. Qi & M. A. Gitzendanner. 2012. Making next-generation sequencing work for you: approaches and practical considerations for marker development and phylogenetics. *Plant Ecol. Divers.* 5: 427–450.
- Hill, S. R. 1983. Microwave and the herbarium specimen: potential dangers. *Taxon* 32: 614–615.
- Hodkinson, T. R., S. Waldren, J. A. N. Parnell, C. T. Kelleher, K. Salamin & N. Salamin. 2007. DNA banking for plant breeding, biotechnology and biodiversity evaluation. *J. Plant Res.* 120: 17–29.
- Honeycutt, R. J., B. W. S. Sobral, P. Keim & J. E. Irvine. 1992. A rapid DNA extraction method for sugarcane and its relatives. *Plant Mol. Biol. Rep.* 10: 66–72.
- Ivanova, N. V. & M. L. Kuzmina. 2013. Protocols for dry DNA storage and shipment at room temperature. *Mol. Ecol. Resourc.* 13: 890–898.
- Jansen, R. K., D. J. Loockerman & H.-G. Kim. 1999. DNA sampling from herbarium material: A current perspective. Pp. 277–286 in D. A. Metsger & S. C. Byers (editors), *Managing the Modern Herbarium, an Interdisciplinary Approach*. Elton-Wolf Publishing, Vancouver.
- Jobes, D. V., D. L. Hurley & L. B. Thien. 1995. Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon* 44: 379–386.
- Kilpatrick, C. W. 2002. Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. *Biochem. Genet.* 40: 53–62.
- Kim, S.-H. & T. Hamada. 2005. Rapid and reliable method of extracting DNA and RNA from sweet-potato, *Ipomoea batatas* (L.). *Lam. Biotechnol. Lett.* 27: 1841–1845.
- Klemchin, D. 2008. Whatman's FTA paper and DNA archiving kits. <http://methodsandreagents.pbworks.com/w/page/20806952/Whatman%27s%20FTA%20paper%20and%20DNA%20archiving%20kits>. Accessed 6 Feb. 2014.
- Knebelberger, T. & I. Stoger. 2012. DNA extraction, preservation, and amplification. Pp. 311–338 in W. J. Kress & D. L. Erickson (editors), *DNA Barcodes: Methods and Protocols (Methods in Molecular Biology)*. Humana Press, New York.
- Lander, T. A., B. Dadonaite & A. K. Monroe. 2013. Microwave drying of plant material for herbarium specimens and genetic analysis. *Taxon* 62: 790–797.
- Lee, S. B., C. A. Crouse & M. C. Kline. 2010. Optimiz-

- ing storage and handing DNA extracts. *Forensic Sci. Rev.* 22: 131–144.
- Levison, P. R., S. E. Badger, J. Dennis, P. Hathi, M. J. Davies, I. J. Bruce & D. Schimkat. 1998. Recent developments of magnetic beads for use in nucleic acid purification. *J. Chromatogr. A* 816: 107–111.
- Lin, J.-J., R. Fleming, J. Kuo, B. F. Matthews & J. A. Saunders. 2000. Detection of plant genes using a rapid, nonorganic DNA purification method. *Bio-Techiques* 28: 346–350.
- Liston, A., L. H. Rieseberg, R. P. Adams, N. Do & G.-L. Zhu. 1990. A method for collecting dried plant specimens for DNA and isozyme analyses, and the results of a field test in Xinjiang, China. *Ann. Missouri Bot. Gard.* 77: 859–863.
- Maeda, T., T. Kawai, M. Nakaoka & N. Yotsukura. 2013. Effective DNA extraction method for fragment analysis using capillary sequencer of the kelp, *Saccharina*. *J. Appl. Phycol.* 25: 337–347.
- Maguire, T. L., G. G. Collins & M. Sedgley. 1994. A modified CTAB DNA extraction procedure for plants belonging to family Proteaceae. *Plant Mol. Biol. Rep.* 12: 106–109.
- Mahuku, G. S. 2004. A simple extraction method for PCR-based analysis of plant, fungal, and bacterial DNA. *Plant Mol. Biol. Rep.* 22: 71–81.
- Manchester, K. L. 1995. Value of A260/A180 ratios for measurement of purity of nucleic acids. *BioTechniques* 19: 208–210.
- Mbogori, M. N., M. Kimani, A. Kuria, M. Lagat & J. W. Danson. 2006. Optimization of I^{TA} technology for large scale plant DNA isolation for use in marker assisted selection. *Afr. J. Biotechnol.* 5: 693–696.
- Metsger, D. A. 1999. Recommendations on the use of herbarium and other museum materials for molecular research: A position paper. Pp. 345–350 in D. A. Metsger & S. C. Byers (editors), *Managing the Modern Herbarium, an Interdisciplinary Approach*. Elton-Wolf Publishing, Vancouver.
- Michiels, A., W. Van Den Ende, M. Tucker, L. Van Riet & A. Van Laere. 2003. Extraction of high-quality genomic DNA from latex-containing plants. *Anal. Biochem.* 315: 85–89.
- Miller, J. S. 1999. Banking desiccated leaf material as a resource for molecular phylogenetics. Pp. 331–344 in D. A. Metsger & S. C. Byers (editors), *Managing the Modern Herbarium, an Interdisciplinary Approach*. Elton-Wolf Publishing, Vancouver.
- Mitchell, K. R. & C. D. Takacs-Vesbach. 2008. A comparison of methods for total community DNA preservation and extraction from various thermal environments. *J. Ind. Microbiol. Biotechnol.* 35: 1139–1147.
- Mori, S. A. 2011. From the field. Pp. 131–190 in S. A. Mori, A. Berkov, C. A. Gracie & E. I. Hecklau (editors), *Tropical Plant Collecting: From the Field to the Internet*. TI:CC Editora, Florianopolis, Brazil.
- Mueller, G. M. 1999. A new challenge for mycological herbaria: Destructive sampling of specimens for molecular data. Pp. 287–300 in D. A. Metsger & S. C. Byers (editors), *Managing the Modern Herbarium, an Interdisciplinary Approach*. Elton-Wolf Publishing, Vancouver.
- Mutter, G. L., D. Zahrich, C. Liu, D. Neuberg, D. Finkelstein, H. E. Baker & J. A. Warrington. 2004. Comparison of frozen and RNA later solid tissue storage methods for use in RNA expression microarrays. *BMC Genomics* 5: 88.
- Nagy, Z. T. 2010. A hands-on overview of tissue preservation methods for molecular genetic analyses. *Org. Divers. Evol.* 10: 91–105.
- Oster, J., J. Parker & L. A. Brassard. 2001. Polyvinyl-alcohol-based magnetic beads for rapid and efficient separation of specific or unspecific nucleic acid sequences. *J. Magn. Magn. Mater.* 225: 145–150.
- Palcek, E., & M. Fojta. 2007. Magnetic beads as versatile tools for electrochemical DNA and protein biosensing. *Talanta* 74: 276–290.
- Panaro, N. J., P. K. Yuen, T. Sakazume, P. Fortina, L. J. Kricka & P. Wilding. 2000. Evaluation of DNA fragment sizing and quantification by the Agilent 2100 Bioanalyzer. *Clin. Chem.* 46: 1851–1853.
- Philbrick, C. T. 1984. Comments on the use of microwave as a method of herbarium insect control: Possible drawbacks. *Taxon* 33: 73–74.
- Porebski, S., L. G. Bailey & B. R. Baum. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.* 15: 8–15.

- Pyle, M. M. & R. P. Adams. 1989. In situ preservation of DNA in plant specimens. *Taxon* 38: 576–581.
- Rajendram, D., R. Ayenza, F. M. Holder, B. Moran, T. Long & H. N. Shah. 2006. Long-term storage and safe retrieval of DNA from microorganisms for molecular analysis using FTA matrix cards. *J. Microbiol. Meth.* 67: 582–592.
- Ribeiro, R. A. & M. B. Lovato. 2007. Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia*. *Genet. Mol. Res.* 6: 173–187.
- Rogstad, S. H. 1992. Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analysis. *Taxon* 41: 701–708.
- Rohland, N. & M. Hofreiter. 2007. Comparison and optimization of ancient DNA extraction. *BioTechniques* 42: 343–352.
- Rudi, K., M. Kroken, O. J. Dahlberg, A. Deggerdal, K. S. Jakobsen & F. Larsen. 1997. Rapid, universal method to isolate PCR-ready DNA using magnetic beads. *BioTechniques* 22: 506–511.
- Särkinen, T., M. Staats, J. E. Richardson, R. S. Cowan & F. T. Bakker. 2012. How to open the treasure chest? Optimising DNA extraction from herbarium specimens. *PLOS ONE* 7: e43808.
- Savolainen, V., P. Cuénoud, R. Spichiger, M. D. P. Martinez, M. Crèvecoeur & J.-F. Manen. 1995. The use of herbarium specimens in DNA phylogenetics: evaluation and improvement. *Plant Syst. Evol.* 197: 87–98.
- Scutin, G., B. N. White & P. T. Boag. 1990. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* 69: 82–90.
- Shi, S.-R., R. Datar, C. Liu, L. Wu, Z. Zhang, R. J. Cote & C. R. Taylor. 2004. DNA extraction from archival formalin-fixed, paraffin-embedded tissues: heat-induced retrieval in alkaline solution. *Histochem. Cell Biol.* 122: 211–218.
- Shokrolla, S., G. A. Singer & M. Hajibabaci. 2010. Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol. *BioTechniques* 48: 233–234.
- Siddappa, N. B., A. Avinash, M. Venkatramanan & U. Ranga. 2007. Regeneration of commercial nucleic acid extraction columns without the risk of carry-over contamination. *BioTechniques* 42: 186–192.
- Simmons, J. E. 1995. Storage in fluid preservatives. Pp. 161–186 in C. L. Rose, C. A. Hawks & H. H. Genoways (editors), *Storage of Natural History Collections: A Preventive Conservation Approach*. Society for the Preservation of Natural History Collections, York, PA.
- Sinha, R. P. & D.-P. Häder. 2002. UV-induced DNA damage and repair: a review. *Photochem. Photobiol. Sci.* 1: 225–236.
- Sinnott, Q. P. 1983. Solar thermoconvective plant dryer. *Taxon* 32: 611–613.
- Smith, L. M. & L. A. Burgoynie. 2004. Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecol.* 4: 4.
- Smith, S. & P. A. Morin. 2005. Optimal storage conditions for highly dilute DNA samples: a role for trehalose as a preserving agent. *J. Forensic Sci.* 50: 1–8.
- Soltis, D. E., P. S. Soltis, T. G. Collier & M. L. Edger-ton. 1991. Chloroplast DNA variation within and among genera of the *Heuchera* group (Saxifragaceae): Evidence for chloroplast transfer and paraphyly. *Am. J. Bot.* 78: 1091–1112.
- Surzycki, S. 2000. *Basic Techniques in Molecular Biology*. Springer, New York.
- Tel-Zur, N., S. Abbo, D. Myslabodski & Y. Mizrahi. 1999. Modified CTAB Procedure for DNA Isolation from Epiphytic Cacti of the Genera *Hylocereus* and *Selenicerus* (Cactaceae). *Plant Mol. Biol. Rep.* 17: 249–254.
- Telle, S. & M. Thines. 2008. Amplification of cox2 (~620 bp) from 2 mg of up to 129 years old herbarium specimens, comparing 19 extraction methods and 15 polymerases. *PLOS ONE* 3: e3584.
- Whitlock, R., H. Hipperson, M. Mannarelli & T. Burke. 2008. A high-throughput protocol for extracting high-purity genomic DNA from plants and animals. *Mol. Ecol. Res.* 8: 736–741.
- Whitten, W. M., N. H. Williams & K. V. Glover. 1999. Sulphuryl fluoride fumigation: Effect on DNA extraction and amplification from herbarium specimens. *Taxon* 48: 507–510.

- Wood, E. W., T. Eriksson & M. J. Donoghue. 1999. Guidelines for the use of herbarium materials in molecular research. Pp. 265–276 in D. A. Metsger & S. C. Byers (editors), *Managing the Modern Herbarium, an Interdisciplinary Approach*. Elton-Wolf Publishing, Vancouver.
- Zhang, J. & J. M. Stewart. 2000. Economical and rapid method for extracting cotton genomic DNA. *J. Cotton Sci.* 4: 193–201.
- Zhu, B., T. Furuki, T. Okuda & M. Sakurai. 2007. Natural DNA mixed with trehalose persists in B-form double-stranding even in the dry state. *J. Phys. Chem. B* 111: 5542–5544.
- Zimmermann, J., M. Hajibabaci, D. C. Blackburn, J. Hanken, E. Cantin, J. Posfai & T. C. Evans, Jr. 2008. DNA damage in preserved specimens and tissue samples: a molecular assessment. *Front. Zool.* 5: 18.
- Zomlefer, W. B., W. M. Whitten, N. H. Williams & W. S. Judd. 2006. Infrageneric phylogeny of *Schoenocaulon* (Liliales: Melanthiaceae) with clarification of cryptic species based on ITS sequence data and geographical distribution. *Am. J. Bot.* 93: 1178–1192.

Appendix 1. Vacuum bag storage for plant tissue preservation

In our combined efforts of storing plant tissues for many years, we have been concerned with the storage of plant tissues to maximally preserve DNA quality.

Over the course of the past 10 years we have used vacuum bags from the commercial food preservation industry with good success. Silica-dried tissues stored in these bags have routinely yielded high quality DNA (sample size of >5,000 ranging in age from 1-10 years old). As indicated in this paper, even silica-dried tissues that are exposed to ambient light, atmosphere and moisture quickly degrade in DNA quality. Storage in vacuum-sealed bags minimizes moisture and oxygen uptake in stored specimens.

A. Sample preparation

Ensure that each sample is labeled for future reference (ideally with a miniaturized copy of the label of the corresponding herbarium voucher). Although we routinely prefer to keep tissues in their original polyethylene collection bags with silica in those individual collection bags, paper envelopes can be used if they are acid-free and archival quality. Bundle the samples into groups that will conveniently fit inside the storage bags.

If storing at room temperature, we recommend including a small mesh bag of indicating silica gel in each vacuum bag. The indicator allows visual inspection of each vacuum bag for moisture infiltration over time; if the indicator shows the presence of moisture, the bag can be opened to replace the silica gel and resealed. We prefer a mesh bag for indicator because it allows maximal exchange with any tissues in the vacuum bag.

B. Vacuum and sealing

Although many commercial vacuum food preservation kits are available, we have used FoodSaver® brand with satisfactory results. This apparatus pumps air out of the vacuum bag and uses heat to melt and seal the ends. It is preferable to choose a vacuum bag product with the thickest available plastic to minimize air infiltration and durability.

Because the effects of hydration of silica at very low temperatures are not well known, it is not preferable to use silica in vacuum bags that are to be stored in freezers at low temperatures. This has the added benefit of reducing bulk in bags and taking less room in the freezer.

C. Storage

Freezers provide optimal storage conditions. If storing at room temperature, it is preferable to keep the bags in a cool dark place because sunlight and fluorescent lights are damaging to DNA. Opaque plastic storage bins or even cardboard boxes should be used to minimize exposure to light.

Appendix 2. DNA extraction protocol for plant tissue adapted from Doyle, J.J. and J.L. Doyle (1987).

Reagents and equipment: CTAB (cetyl trimethylammonium bromide) buffer (1 M Tris, pH 8.0, 20 ml; 0.25 M EDTA, pH 8.0, 20 ml; NaCl, 16.4 g; CTAB, 4.0 g; water, to 200 ml); proteinase K, 1g/ml stock; chloroform/isoamyl alcohol 24:1; 3 M sodium acetate, pH 4.8; 100% isopropanol; 70% ethanol; Tris-EDTA buffer (TE), pH 8.5 (10 mM Tris, 1 mM EDTA); microcentrifuge; 2.0 ml tubes with gasket screwcap; disposable pipettes; mortar and pestle, or beater tube and glass beads; heating block, prewarmed to 50-55 C; nitrile gloves; fume hood (for chloroform steps).

A. Tissue pulverization and extraction

Using Bead Beater:

1. Place 5-6 glass beads into beater tube; tough tissues may require use of steel or zirconia beads. Add 50-100 mg dried tissue. Grind in beater for 1-2 minutes or until tissue is reduced to a fine powder.
2. Add 2-5 µl of Proteinase K and 1.2 ml CTAB buffer. Vortex.

Or, using Pestle and Mortar:

1. Place 50-100 mg fresh tissue in mortar (about 1.0 cm square of petal or leaf tissue, or equivalent amount of dried tissue; too much tissue can reduce yield). Add 1.2 ml CTAB buffer and 2-5 µl of Proteinase K.

2. Grind tissue with mortar and pestle until tissue is completely homogenized (ignore tough fibers). Transfer 1.2 ml to a labeled 2.0 ml tube; vortex.
3. Incubate tube at 50°C in the heating block for at least 2 hours (heating too long may result in degraded DNA). Shake occasionally during incubation.
4. In fume hood, add 500 µl of chloroform/isoamyl alcohol, and vortex lightly until a milky suspension is obtained.
5. Centrifuge at 10,000 rpm for 4 minutes to separate phases. The chloroform layer is on the bottom; the aqueous layer (containing DNA) is on top.
6. Using 1000 µl pipet, carefully remove 750 µl of the aqueous (top) layer and transfer to a new labeled tube. Avoid sucking up any of the chloroform layer or debris. Discard the chloroform and homogenate into the waste bottle. (The chloroform extraction can be repeated if necessary.)

B. DNA precipitation

1. Add 3 M sodium acetate to the aqueous phase according to the following formula: volume aqueous phase in µl X 0.04 (e.g., $750 \times 0.04 = 30 \text{ }\mu\text{l}$). Add 100% isopropanol according to the formula: volume aqueous phase in µl X 0.65 (e.g., $780 \times 0.65 = \text{ca. } 510 \text{ }\mu\text{l}$). Rock tube gently to mix; continue until layers are completely mixed. Occasionally, you will see DNA strands precipitate at this point (especially with floral tissue). Many protocols recommend leaving tube in -20°C freezer overnight; this is generally not necessary, although it might help precipitate very dilute small fragments.
2. Centrifuge at maximum speed (13,000 g) for 20 minutes. After centrifuging, a pellet of DNA should be visible in the bottom of the tube.
3. Carefully pour off the alcohol without disturbing pellet. Wash pellet with 1 ml 70% ethanol; close cap and invert tube to wash the internal surface of tube. Carefully pour off ethanol (don't lose pellet; centrifuge again if pellet is loose). Repeat ethanol wash (if pellet

is large and mucilaginous, allow to stand in 70% EtOH for 30 minutes with occasional agitation). Drain tube on clean Kimwipe, and use the 100 µl pipet to remove any excess ethanol. We have found that drying the pellet under heat is unnecessary; often, the dried pellet is more difficult to resuspend. The residual amounts of ethanol do not seem to interfere with PCR, and the wet pellets resuspend much more rapidly.

4. Resuspend DNA pellet in 200 µl of 1X Tris-EDTA buffer (TE). Incubate at 65°C for 15 minutes to assure resuspension of DNA; finger-flick until pellet is dissolved. Store in fridge at 4°C, or in freezer for long-term storage. Resuspending DNA in water is not recommended, because DNA is most stable under basic conditions.

C. Silica column cleaning of total DNA

The total DNA extract from the above procedure is often clean enough for routine PCR, but often still contains many impurities that can interfere with downstream applications. There are various methods for cleaning DNA, but one of the most commonly used involves binding the DNA to a silica matrix in a special tube with chaotropic salts, washing the impurities away with buffered isopropanol, and then eluting the cleaned DNA with basic TE buffer. DNA purification columns and reagents are sold by various vendors including Qiagen, Promega, and Axygen. The columns (or their 96 well format equivalent) are relatively expensive (ca. \$1 each), and are the most expensive component in DNA extraction. To save cost, Siddappa et al. (2007) demonstrated that the columns can be cleaned and regenerated by washing and storage in dilute HCl; the acid destroys residual DNA on the columns. An alternative method of column cleaning bypasses the DNA precipitation step; this method is especially useful for weak or degraded samples where risk of losing the pellet is high. This alternative method begins after chloroform extraction (step A above):

B. Direct column cleaning without DNA precipitation

1. After centrifugation, remove 750 µl of aqueous

- layer and place in separate labeled tube.
2. Add 750 µl of binding buffer (Qiagen buffer PB or equivalent); vortex to mix.
 3. Add 2 µl of phenol red indicator. [This pH indicator is yellow below pH 6.8, and pink above pH 8.2; in order for DNA to bind to the silica column, the pH of the mixture must be slightly acidic (yellow)]. The initial color of the aqueous extract should be pink.
 4. Add 10 µl of 3M sodium acetate; mix and note change of color. Continue to add 10 µl aliquots of sodium acetate until the color of the tube changes from pink to yellow. When it is yellow, then proceed to the next step.
 5. Transfer 750 µl of mixture to clean silica spin column; spin for 30 seconds at high speed to push solution through the column. Discard flowthrough; DNA is bound to the silica matrix in the column.
 6. Add remaining mixture to spin column and spin through column. Discard flowthrough.
 7. Add 750 µl of wash buffer (Qiagen buffer PE or equivalent). Spin for 30 seconds to push through column; discard flowthrough. Repeat this wash if desired.
 8. Spin empty column for 2 minutes at high speed to remove all traces of wash buffer.
 9. Transfer spin column to a clean 1.5 ml Eppendorf tube. Add 100-200 µl of 1X TE buffer, pH 8.0 (The high pH of the buffer allows the cleaned DNA to be released from the silica matrix). Optional: warm in heating block for several minutes.
 10. Centrifuge column at 6000 rpm (high speed may cause hinge to break on Eppendorf). The clean DNA is in the bottom of the collecting tube. Step 9 can be repeated to remove residual DNA, and the aliquots pooled.

Appendix 3. Agarose gel electrophoresis protocol.
N.B.: Etidium bromide (EtBr) is hazardous and potentially mutagenic. Always wear nitrile gloves when handling EtBr solutions, gels, buffers, and anything that has contacted these. Use proper disposal techniques. Visualizing EtBr requires intense UV light; wear appropriate eye protection when visualizing DNA samples.

Reagents and equipment: 1X Sodium Boric Acid buffer (Brody et al., 2004; sodium hydroxide pellets, boric acid (powder), and water); 250 ml flask; agarose, molecular biology grade; gel rig; 1-10 µl pipeter and tips; dams and combs (for casting gel); ethidium bromide solution, 1%; strip of parafilm; loading dye; pipeter and tips; DNA samples.

A. Prepare sodium boric acid buffer (SBA) 20X stock.

1. Make a 0.2 M sodium hydroxide (NaOH) solution by adding 4 g of NaOH to 500 ml water; stir to dissolve.
2. Make a 1.0 M boric acid solution by adding 30 g boric acid to 500 ml water.
3. Adjust pH of NaOH solution to 8.0 by adding boric acid solution to NaOH solution approximately 1:1.
4. Use 50ml of the 20X stock solution and bring to 1000 ml with water to make a 1 X working solution for use in making gels and electrophoresis.

B. Prepare agarose gel.

1. Place gel rig in manufacturer's mold and insert combs as needed.
2. To create a 1.5% gel, measure 1.5 x the volume of buffer in grams of agarose (e.g., pour 75 ml of buffer into flask and weigh 1.13 g of agarose on balance). Pour agarose and buffer into flask and swirl to suspend agarose.
3. Heat flask in microwave just until it begins to boil (ca. 1.5 min); watch carefully to avoid explosive boiling.
4. When dissolved, add 2.0 µl of EtBr solution to flask. Swirl carefully to mix. Cool flask under running tap water until warm to the touch, but still liquid (hot agarose will cause the gel rig to warp and leak).
5. Pour agar solution into gel tray. Allow gel to cool undisturbed until solid (ca. 15 min.)

C. Loading the DNA samples in the agarose gel.

1. Place tray with gel into electrophoresis rig. Add 1X SBA buffer as needed to barely submerge gel. Carefully remove the combs.
2. Cut a strip of parafilm and press corners down onto tray. Pipet 5 µl droplets of loading dye

- onto the parafilm (one drop per sample) and 5 µl of DNA solution to drop of loading dye. Change tip after each sample to avoid contamination.
3. Set pipeter to 10 µl. Draw up sample into pipeter; load carefully into well (avoid puncturing bottom of well). Repeat for all samples. Place lid on gel rig and turn on power supply; power should read 150 volts. Allow to run for 10-30 minutes, noting migration of bromophenol blue dye front. Turn off power source and remove lid.
 5. Carefully remove gel tray, draining off excess buffer without letting gel slide off the tray. Place tray on UV transilluminator. Turn on UV viewer and examine; photograph as needed.

Appendix 4. Vouchers and assessed DNA quality. Each specimen includes the following information in this order: taxon, voucher information (herbarium), year of collection, age of specimen at time of extraction, HMW presence, peak at >20,000 bp region, peak at 1000 bp region, and peak at 200 bp region.

- A) White Oak Plantation Collection data set from herbarium specimens: *Elytraria caroliniensis* (J.F. Gmel.) Pers. (Acanthaceae), *Rider 258* (FLAS), 2000, 13, 1, 3, 1, 2; *Ruellia caroliniensis* (J.F. Gmel.) Steud. (Acanthaceae), *Rider 95* (FLAS), 1999, 14, 1, 3, 1, 2; *Sambucus canadensis* L. (Adoxaceae), *Mercurio & Wagamon 85* (FLAS), 1997, 16, 1, 3, 2, 1; *Viburnum* L. (Adoxaceae), *Rider 87* (FLAS), 1999, 14, 1, 3, 1, 1; *Viburnum obovatum* Walter (Adoxaceae), *Mercurio & Wagamon 138* (FLAS), 1997, 16, 1, 3, 2, 1; *Viburnum obovatum* Walter (Adoxaceae), *Rider 37* (FLAS), 1999, 14, 1, 3, 1, 1; *Sagittaria graminea* Michx. (Alismataceae), *Rider 3* (FLAS), 1998, 15, 1, 3, 1, 1; *Sagittaria lancifolia* L. (Alismataceae), *Rider 84* (FLAS), 1999, 14, 1, 3, 1, 1; *Liquidambar styraciflua* L. (Altingiaceae), *Rider 32* (FLAS), 1999, 14, 1, 3, 2, 1; *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), *Rider 243* (FLAS), 2000, 13, 1, 3, 1, 2; *Amaranthus australis* (A. Gray) J.D. Sauer (Amaranthaceae), *Rider 267* (FLAS), 2000, 13, 1, 3, 1, 2; *Hymenocallis crassifolia* Herb. (Amaryllidaceae), *Rider 225* (FLAS), 2000, 13, 1, 3, 1, 2; *Zephyranthes atamasco* (L.) Herb. (Amaryllidaceae), *Rider 50* (FLAS), 1999, 14, 1, 3, 1, 1; *Rhus copallina* L. (Anacardiaceae), *Rider 281* (FLAS), 2000, 13, 0, 0, 0, 1; *Rhus copallina* L. (Anacardiaceae), *Rider 139* (FLAS), 1999, 14, 1, 1, 1, 2; *Toxicodendron radicans* (L.) Kuntze (Anacardiaceae), *Rider 192* (FLAS), 2000, 13, 1, 1, 1, 2; *Toxicodendron pubescens* Mill. (Anacardiaceae), *Rider 193* (FLAS), 2000, 13, 1, 1, 1, 2; *Asimina incana* (W. Bartram) Exell (Annonaceae), *Rider 60* (FLAS), 1999, 14, 1, 3, 1, 2; *Cicuta mexicana* J.M. Coulter & Rose (Apiaceae), *Rider 137* (FLAS), 1999, 14, 1, 3, 1, 2; *Cicuta mexicana* J.M. Coulter & Rose (Apiaceae), *Rider 132* (FLAS), 1999, 14, 1, 3, 1, 2; *Eryngium baldwinii* Spreng. (Apiaceae), *Mercurio & Wagamon 10* (FLAS), 1997, 16, 1, 3, 1, 1; *Eryngium baldwinii* Spreng. (Apiaceae), *Proenza 429* (FLAS), 2006, 7, 1, 3, 1, 2; *Eryngium baldwinii* Spreng. (Apiaceae), *Rider 254* (FLAS), 2000, 13, 1, 3, 1, 2; *Eryngium yuccifolium* Michx. (Apiaceae), *Rider 127* (FLAS), 1999, 14, 1, 2, 1, 3; *Oxypolis filiformis* (Walter) Britton (Apiaceae), *Rider 161* (FLAS), 1999, 14, 1, 3, 1, 2; *Ptilimnium capillaceum* (Michx.) Raf. (Apiaceae), *Rider 115* (FLAS), 1999, 14, 1, 3, 1, 2; *Asclepias connivens* Baldwin ex Elliott (Apocynaceae), *Rider 151* (FLAS), 1999, 14, 1, 3, 1, 1; *Asclepias lanceolata* Walter (Apocynaceae), *Rider 108* (FLAS), 1999, 14, 1, 3, 1, 1; *Asclepias michauxii* Decne. (Apocynaceae), *Rider 229* (FLAS), 2000, 13, 1, 3, 1, 1; *Asclepias perennis* Walter (Apocynaceae), *Rider 105* (FLAS), 1999, 14, 1, 3, 1, 1; *Ilex cassine* L. (Araliaceae), *Rider 351* (FLAS), 2001, 12, 1, 3, 1, 2; *Ilex glabra* (L.) A. Gray (Araliaceae), *Mercurio & Wagamon 104* (FLAS), 1997, 16, 1, 3, 1, 2; *Ilex glabra* (L.) A. Gray (Araliaceae), *Mercurio & Wagamon 2* (FLAS), 1997, 16, 1, 3, 1, 2; *Ilex myrtifolia* Walter (Araliaceae), *Rider 5* (FLAS), 1998, 15, 1, 3, 1, 2; *Ilex opaca* Aiton (Araliaceae), *Rider 182* (FLAS), 2000, 13, 1, 3, 1, 2; *Ilex vomitoria* Aiton (Araliaceae), *Rider 188* (FLAS), 2000, 13, 1, 3, 1, 2; *Colocasia esculenta* (L.) Schott (Araceae), *Rider 388* (FLAS), 2002, 11, 1, 3, 1, 1; *Peltandra virginica* (L.) Schott (Araceae), *Rider 259* (FLAS), 2000, 13, 1, 3, 2, 1; *Aralia spinosa* L. (Araliaceae), *Rider 221* (FLAS), 2000, 13, 1, 3, 1, 2; *Hydrocotyle umbellata* L. (Araliaceae), *Mercurio & Wagamon 73* (FLAS), 1997, 16, 1, 3, 1, 2; *Sabal minor* (Jacq.) Pers. (Arecaceae), *Rider 270* (FLAS), 2000, 13, 1, 3, 2, 1; *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult. f. (Arecaceae), *Rider 269* (FLAS), 2000, 13, 1, 3, 2, 1; *Serenoa repens* (W. Bartram) Small (Arecaceae), *Rider 274* (FLAS), 2000, 13, 1, 3, 2, 1; *Washingtonia robusta* H. Wendl. (Arecaceae), *Rider 273* (FLAS), 2000, 13, 1, 3, 1, 2; *Yucca aloifolia* L. (Asparagaceae), *Rider 372* (FLAS), 2001, 12, 1, 3, 1, 2; *Yucca gloriosa* L. (Asparagaceae), *Rider 328* (FLAS), 2000, 13, 1, 3, 1, 2; *Arnoglossum ovatum* (Walter) H. Rob. (Asteraceae), *Rider 330* (FLAS), 2000, 13, 1, 3, 1, 2; *Baccharis glomeruliflora* Pers. (Asteraceae), *Rider 180* (FLAS), 1999, 14, 1, 3, 1, 2; *Baccharis halimifolia* L. (Asteraceae), *Rider 04* (FLAS), 1998, 15, 1, 3, 1, 2; *Baldwinia uniflora* Nutt. (Asteraceae), *Rider 157* (FLAS), 1999, 14, 1, 3, 1, 2; *Bidens alba* (L.) DC. (Asteraceae), *Rider 160* (FLAS), 1999, 14, 1, 3, 1, 1; *Bidens mitis* (Michx.) Sherff (Asteraceae), *Mercurio & Wagamon 162* (FLAS), 1997, 16, 1, 3, 1, 1; *Bigelowia nudata* (Michx.) DC. (Asteraceae), *Rider 167* (FLAS), 1999, 14, 0, 0, 0, 0; *Carphephorus corymbosus* (Nutt.) Torr. & A. Gray (Asteraceae), *Rider 323* (FLAS), 2000, 13, 1, 3, 1, 2; *Carphephorus odoratissimus* (J.F. Gmel.) H.J.-C. Hebert (Asteraceae), *Rider 295* (FLAS), 2000, 13, 1, 3, 1, 2; *Chaptalia tomentosa* Vent. (Asteraceae), *Rider 16* (FLAS), 1999, 14, 1, 3, 1, 0; *Cirsium horridulum* Michx. (Asteraceae), *Rider 66* (FLAS), 1999, 14, 1, 3, 1, 2; *Cirsium nuttallii* DC. (Asteraceae), *Rider 217* (FLAS), 2000, 13, 1, 3, 1, 1; *Conoclinium coelestinum* (L.) DC. (Asteraceae), *Rider 173* (FLAS), 1999, 14, 1, 3, 1, 2; *Coreopsis gladiata* Walter (Asteraceae), *Rider 170* (FLAS), 1999, 14, 1, 3, 1, 2; *Coreopsis gladiata* Walter (Asteraceae), *Rider 376* (FLAS), 2001, 12, 1, 3, 1, 2; *Coreopsis gladiata* Walter (Asteraceae), *Rider 381* (FLAS), 2001, 12, 1, 3, 1, 2; *Coreopsis gladiata* Walter (Asteraceae), *Rider 379* (FLAS), 2001, 12, 1, 3, 1, 2; *Coreopsis nudata*

- Nutt. (Asteraceae), *Rider 104* (FLAS), 1999, 14, 1, 3, 2, 1; *Elephantopus mollis* Kunth (Asteraceae), *Rider 333* (FLAS), 2000, 13, 1, 3, 1, 3; *Elephantopus elatus* Bertol. (Asteraceae), *Rider 310* (FLAS), 2000, 13, 1, 3, 1, 2; *Elephantopus nudatus* A. Gray (Asteraceae), *Rider 321* (FLAS), 2000, 13, 1, 3, 1, 1; *Erigeron vernus* (L.) Torr. & A. Gray (Asteraceae), *Rider 55* (FLAS), 1999, 14, 1, 3, 1, 1; *Eupatorium capillifolium* (Lam.) Small ex Porter & Britton (Asteraceae), *Mercurio & Wagamon 80* (FLAS), 1997, 16, 1, 2, 1, 3; *Eupatorium leucolepis* (DC.) Torr. & A. Gray (Asteraceae), *Rider 296* (FLAS), 2000, 13, 1, 2, 1, 3; *Eupatorium rotundifolium* L. (Asteraceae), *Rider 268* (FLAS), 2000, 13, 1, 3, 1, 2; *Eupatorium rotundifolium* L. (Asteraceae), *Mercurio & Wagamon 97* (FLAS), 1997, 16, 1, 2, 1, 3; *Eupatorium rotundifolium* L. (Asteraceae), *Mercurio & Wagamon 79* (FLAS), 1997, 16, 1, 2, 1, 3; *Euthamia caroliniana* (L.) Greene ex Porter & Britton (Asteraceae), *Rider 378* (FLAS), 2001, 12, 1, 3, 1, 1; *Gamochaeta pensylvanica* (Willd.) Cabrera (Asteraceae), *Rider 233* (FLAS), 2000, 13, 1, 3, 1, 2; *Helenium autumnale* L. (Asteraceae), *Mercurio & Wagamon 33* (FLAS), 1997, 16, 1, 3, 2, 1; *Helenium pinnatifidum* (Schwein. ex Nutt.) Rydb. (Asteraceae), *Rider 53* (FLAS), 1999, 14, 1, 3, 2, 1; *Krigia virginica* (L.) Willd. (Asteraceae), *Rider 204* (FLAS), 2000, 13, 1, 3, 1, 3; *Liatris chapmanii* Torr. & A. Gray (Asteraceae), *Rider 344* (FLAS), 2000, 13, 1, 3, 1, 1; *Liatris gracilis* Pursh (Asteraceae), *Rider 343* (FLAS), 2000, 13, 1, 3, 1, 2; *Liatris pauciflora* Pursh (Asteraceae), *Rider 348* (FLAS), 2000, 13, 1, 3, 1, 2; *Liatris spicata* (L.) Willd. (Asteraceae), *Rider 338* (FLAS), 2000, 13, 1, 3, 2, 1; *Liatris spicata* (L.) Willd. (Asteraceae), *Rider 168* (FLAS), 1999, 14, 1, 3, 1, 1; *Marshallia tenuifolia* Raf. (Asteraceae), *Rider 154* (FLAS), 1999, 14, 1, 3, 1, 1; *Marshallia tenuifolia* Raf. (Asteraceae), *Mercurio & Wagamon 98* (FLAS), 1997, 16, 1, 3, 2, 1; *Mikania scandens* (L.) Willd. (Asteraceae), *Rider 172* (FLAS), 1999, 14, 1, 3, 1, 1; *Mikania scandens* (L.) Willd. (Asteraceae), *Mercurio & Wagamon 66* (FLAS), 1997, 16, 1, 2, 1, 3; *Oclemena reticulata* (Pursh) G.L. Nesom (Asteraceae), *Rider 81* (FLAS), 1999, 14, 1, 3, 1, 2; *Pityopsis graminifolia* (Michx.) Nutt. (Asteraceae), *Rider 174* (FLAS), 1999, 14, 1, 3, 2, 1; *Pluchea foetida* (L.) DC. (Asteraceae), *Rider 282* (FLAS), 2000, 13, 1, 3, 2, 1; *Pluchea odorata* (L.) Cass. (Asteraceae), *Rider 327* (FLAS), 2000, 13, 1, 3, 1, 2; *Pluchea baccharis* (Mill.) Pruski (Asteraceae), *Rider 253* (FLAS), 2000, 13, 0, 1, 2, 1; *Pluchea baccharis* (Mill.) Pruski (Asteraceae), *Mercurio & Wagamon 3* (FLAS), 1997, 16, 1, 2, 1, 3; *Pteroaulon pycnostachyum* (Michx.) Elliott (Asteraceae), *Rider 93* (FLAS), 1999, 14, 1, 3, 2, 1; *Pteroaulon virgatum* (L.) DC. (Asteraceae), *Mercurio & Wagamon 81* (FLAS), 1997, 16, 1, 3, 2, 1; *Pyrrhopappus carolinianus* (Walter) DC. (Asteraceae), *Rider 143* (FLAS), 1999, 14, 1, 3, 2, 1; *Rudbeckia fulgida* Aiton (Asteraceae), *Rider 124* (FLAS), 1999, 14, 1, 3, 2, 1; *Solidago canadensis* L. (Asteraceae), *Rider 345* (FLAS), 2000, 13, 1, 3, 1, 1; *Solidago fistulosa* Mill. (Asteraceae), *Rider 324* (FLAS), 2000, 13, 1, 3, 2, 1; *Solidago odora* subsp. *chapmanii* (A. Gray) Semple (Asteraceae), *Rider 304* (FLAS), 2000, 13, 1, 3, 1, 1; *Solidago* L. (Asteraceae), *Mercurio & Wagamon 56* (FLAS), 1997, 16, 1, 2, 1, 3; *Solidago stricta* Aiton (Asteraceae), *Rider 377* (FLAS), 2001, 12, 1, 3, 1, 1; *Sonchus asper* (L.) Hill (Asteraceae), *Proenza 427* (FLAS), 2006, 7, 1, 3, 1, 1; *Sonchus asper* (L.) Hill (Asteraceae), *Rider 213* (FLAS), 2000, 13, 1, 3, 1, 1; *Sonchus oleraceus* L. (Asteraceae), *Rider 232* (FLAS), 2000, 13, 1, 3, 1, 1; *Sympyotrichum adnatum* (Nutt.) G.L. Nesom (Asteraceae), *Rider 09* (FLAS), 1998, 15, 1, 3, 1, 2; *Ampelaster carolinianus* (Walter) G.L. Nesom (Asteraceae), *Mercurio & Wagamon 43* (FLAS), 1997, 16, 1, 2, 1, 3; *Sympyotrichum dumosum* (L.) G.L. Nesom (Asteraceae), *Rider 382* (FLAS), 2001, 12, 1, 3, 1, 2; *Taraxacum officinale* F.H. Wigg. (Asteraceae), *Rider 18* (FLAS), 1999, 14, 1, 3, 1, 1; *Vernonia angustifolia* Michx. (Asteraceae), *Rider 147* (FLAS), 1999, 14, 1, 3, 1, 1; *Vernonia noveboracensis* (L.) Michx. (Asteraceae), *Rider 314* (FLAS), 2000, 13, 1, 3, 1, 1; *Youngia japonica* (L.) DC. (Asteraceae), *Rider 20* (FLAS), 1999, 14, 1, 3, 1, 1; *Azolla caroliniana* Willd. (Azollaceae), *Proenza 415* (FLAS), 2005, 8, 1, 3, 1, 1; *Carpinus caroliniana* Walter (Betulaceae), *Rider 31'* (FLAS), 1999, 14, 1, 3, 1, 1; *Bignonia capreolata* L. (Bignoniaceae), *Rider 57* (FLAS), 1999, 14, 1, 3, 1, 1; *Campsip radicans* (L.) Bureau (Bignoniaceae), *Rider 250* (FLAS), 2000, 13, 1, 3, 1, 1; *Campsip radicans* (L.) Bureau (Bignoniaceae), *Rider 249* (FLAS), 2000, 13, 1, 3, 1, 1; *Catalpa bignonioides* Walter (Bignoniaceae), *Rider 364* (FLAS), 2001, 12, 1, 3, 1, 1; *Lepidium campestre* (L.) W.T. Aiton (Brassicaceae), *Rider 231* (FLAS), 2000, 13, 1, 3, 1, 1; *Tillandsia bartramii* Elliott (Bromeliaceae), *Rider 15* (FLAS), 1999, 14, 1, 3, 1, 1; *Tillandsia usneoides* (L.) L. (Bromeliaceae), *Rider 238* (FLAS), 2000, 13, 0, 1, 1, 2; *Lobelia floridana* Chapm. (Campanulaceae), *Rider 88* (FLAS), 1999, 14, 1, 3, 1, 1; *Lobelia glandulosa* Walter (Campanulaceae), *Rider 179* (FLAS), 1999, 14, 1, 3, 1, 1; *Lobelia* L. (Campanulaceae), *Rider 2* (FLAS), 1998, 15, 1, 3, 1, 1; *Lobelia* L. (Campanulaceae), *Mercurio & Wagamon 30* (FLAS), 1997, 16, 1, 3, 1, 1; *Wahlenbergia marginata* (Thunb.) A. DC. (Campanulaceae), *Rider 239* (FLAS), 2000, 13, 1, 3, 1, 1; *Wahlenbergia marginata* (Thunb.) A. DC. (Campanulaceae), *Mercurio & Wagamon 72* (FLAS), 1997, 16, 1, 3, 1, 1; *Celtis laevigata* Willd. (Cannabaceae), *Rider 129* (FLAS), 1999, 14, 1, 3, 2, 1; *Canna flaccida* Salisb. (Cannaceae), *Rider 109* (FLAS), 1999, 14, 0, 1, 1, 2; *Lonicera japonica* Thunb. (Caprifoliaceae), *Rider 371* (FLAS), 2001, 12, 1, 3, 1, 2; *Lonicera sempervirens* L. (Caprifoliaceae), *Rider 195* (FLAS), 2000, 13, 1, 3, 1, 1; *Helianthemum nashii* Britton (Cistaceae), *Rider 209* (FLAS), 2000, 13, 1, 3, 1, 1; *Clethra alnifolia* L. (Clethraceae), *Mercurio & Wagamon 54* (FLAS), 1997, 16, 1, 3, 1, 1; *Commelinella diffusa* Burm. f. (Commelinaceae), *Rider 134* (FLAS), 1999, 14, 1, 3, 2, 1; *Commelinella diffusa* Burm. f. (Commelinaceae), *Mercurio & Wagamon 123* (FLAS), 1997, 16, 0, 1, 1, 2; *Calystegia sepium* (L.) R. Br. (Convolvulaceae), *Mercurio & Wagamon 21* (FLAS), 1997, 16, 1, 3, 1, 1; *Calystegia sepium* (L.) R. Br. (Convolvulaceae), *Rider 82* (FLAS), 1999, 14, 1, 3, 1, 1; *Cuscuta compacta* Juss. ex Choisy (Convolvulaceae), *Rider 260* (FLAS), 2000, 13, 1, 3, 1, 1; *Ipomoea cordatotriloba* Dennst. (Convolvulaceae), *Rider 334* (FLAS), 2000, 13, 1, 3, 1, 1; *Cornus florida* L. (Cornaceae), *Rider 38* (FLAS), 1999, 14, 1, 2, 3, 1; *Cornus foemina* Mill. (Cornaceae), *Mercurio & Wagamon 125* (FLAS), 1997, 16, 1, 3, 1, 1; *Juniperus virginiana* L. (Cupressaceae), *Rider 236* (FLAS), 2000, 13, 1, 3, 1, 1; *Taxodium distichum* var. *imbricatum* (Nutt.) Croon (Cupressaceae), *Rider 216* (FLAS), 2000, 13, 1, 3, 1, 2; *Taxodium distichum* (L.) Rich. (Cupressaceae), *Mercurio & Wagamon 22* (FLAS), 1997, 16, 0, 1, 1, 2; *Carex complanata* Torr. & Hook. (Cyperaceae), *Proenza 432* (FLAS), 2006, 7, 1, 3, 2, 1; *Carex glaucescens* Elliott (Cyperaceae), *Mercurio & Wagamon 76* (FLAS), 1997, 16, 1, 3, 1, 2; *Carex glaucescens* Elliott (Cyperaceae), *Rider 308* (FLAS), 2000, 13, 1, 3, 1, 2; *Carex louisianica* L.H. Bailey (Cyperaceae), *Proenza 440* (FLAS), 2006, 7, 1, 3,

- 2, 1; *Carex lupulina* Muhl. ex Willd. (Cyperaceae), *Rider* 241 (FLAS), 2000, 13, 1, 3, 2, 1; *Carex* L. (Cyperaceae), *Proenza* 441 (FLAS), 2006, 7, 1, 3, 1, 1; *Carex stipata* Muhl. ex Willd. (Cyperaceae), *Rider* 210 (FLAS), 2000, 13, 1, 3, 1, 2; *Cladium jamaicense* Crantz (Cyperaceae), *Rider* 153 (FLAS), 1999, 14, 1, 3, 1, 1; *Cyperus alternifolius* L. (Cyperaceae), *Mercurio & Wagamon* 70 (FLAS), 1997, 16, 1, 1, 1, 2; *Cyperus croceus* Vahl (Cyperaceae), *Rider* 275 (FLAS), 2000, 13, 1, 3, 1, 2; *Cyperus involucratus* Rottb. (Cyperaceae), *Rider* 276 (FLAS), 2000, 13, 1, 3, 1, 2; *Cyperus odoratus* L. (Cyperaceae), *Rider* 305 (FLAS), 2000, 13, 1, 3, 1, 2; *Eleocharis vivipara* Link (Cyperaceae), *Proenza* 418 (FLAS), 2005, 8, 1, 3, 1, 2; *Fuirena pumila* (Torr.) Spreng. (Cyperaceae), *Rider* 322 (FLAS), 2000, 13, 1, 3, 1, 2; *Rhynchospora careyana* Fernald (Cyperaceae), *Mercurio & Wagamon* 119 (FLAS), 1997, 16, 1, 3, 1, 2; *Rhynchospora cephalantha* A. Gray (Cyperaceae), *Mercurio & Wagamon* 113 (FLAS), 1997, 16, 0, 1, 1, 2; *Rhynchospora colorata* (L.) H. Pfeiff. (Cyperaceae), *Rider* 76 (FLAS), 1999, 14, 1, 3, 1, 2; *Rhynchospora inundata* (Oakes) Fernald (Cyperaceae), *Rider* 309 (FLAS), 2000, 13, 1, 3, 1, 2; *Rhynchospora latifolia* (Baldwin) W.W. Thomas (Cyperaceae), *Rider* 99 (FLAS), 1999, 14, 1, 3, 1, 2; *Rhynchospora Vahl* (Cyperaceae), *Proenza* 433 (FLAS), 2006, 7, 1, 3, 1, 2; *Rhynchospora tracyi* Britton (Cyperaceae), *Rider* 318 (FLAS), 2000, 13, 1, 3, 1, 2; *Scirpus cyperinus* (L.) Kunth (Cyperaceae), *Rider* 292 (FLAS), 2000, 13, 1, 3, 1, 2; *Schoenoplectus pungens* var. *pungens* (Cyperaceae), *Rider* 315 (FLAS), 2000, 13, 1, 3, 1, 2; *Cyrilla racemiflora* L. (Cyrillaceae), *Rider* 116 (FLAS), 1999, 14, 0, 1, 1, 1; *Drosera brevifolia* Pursh (Droseraceae), *Rider* 63 (FLAS), 1999, 14, 0, 0, 0; *Drosera capillaris* Poir. (Droseraceae), *Rider* 103 (FLAS), 1999, 14, 0, 1, 1, 1; *Diospyros virginiana* L. (Ebenaceae), *Rider* 89 (FLAS), 1999, 14, 1, 1, 1, 1; *Diospyros virginiana* L. (Ebenaceae), *Rider* 90 (FLAS), 1999, 14, 1, 3, 2, 1; *Bejaria racemosa* Vent. (Ericaceae), *Mercurio & Wagamon* 19 (FLAS), 1997, 16, 0, 0, 1, 1; *Kalmia hirsuta* Walter (Ericaceae), *Mercurio & Wagamon* 90 (FLAS), 1997, 16, 0, 1, 1, 1; *Lyonia lucida* (Lam.) K. Koch (Ericaceae), *Mercurio & Wagamon* 16 (FLAS), 1997, 16, 1, 1, 1, 1; *Lyonia lucida* (Lam.) K. Koch (Ericaceae), *Rider* 35 (FLAS), 1999, 14, 1, 3, 1, 1; *Rhododendron canescens* (Michx.) Sweet (Ericaceae), *Rider* 44 (FLAS), 1999, 14, 1, 3, 1, 1; *Vaccinium corymbosum* L. (Ericaceae), *Rider* 26 (FLAS), 1999, 14, 1, 3, 1, 1; *Vaccinium corymbosum* L. (Ericaceae), *Mercurio & Wagamon* 96 (FLAS), 1997, 16, 1, 2, 1, 3; *Vaccinium corymbosum* L. (Ericaceae), *Mercurio & Wagamon* 141 (FLAS), 1997, 16, 1, 2, 1, 3; *Eriocaulon decangulare* L. (Eriocaulaceae), *Rider* 264 (FLAS), 2000, 13, 1, 3, 1, 2; *Lachnocalon anceps* (Walter) Morong (Eriocaulaceae), *Rider* 235 (FLAS), 2000, 13, 1, 3, 1, 2; *Lachnocalon* Kunth (Eriocaulaceae), *Mercurio & Wagamon* 14 (FLAS), 1997, 16, 1, 3, 1, 1; *Cnidoscolus urens* (L.) Arthur (Euphorbiaceae), *Mercurio & Wagamon* 71 (FLAS), 1997, 16, 1, 3, 2, 1; *Sapium sebiferum* (L.) Roxb. (Euphorbiaceae), *Mercurio & Wagamon* 39 (FLAS), 1997, 16, 0, 1, 1, 1; *Sapium sebiferum* (L.) Roxb. (Euphorbiaceae), *Rider* 8 (FLAS), 1998, 15, 1, 1, 1, 1; *Stillingia sylvatica* Garden ex L. (Euphorbiaceae), *Rider* 117 (FLAS), 1999, 14, 0, 1, 2, 3; *Amorpha fruticosa* L. (Fabaceae), *Mercurio & Wagamon* 25 (FLAS), 1997, 16, 1, 1, 1, 2; *Amorpha fruticosa* L. (Fabaceae), *Rider* 74 (FLAS), 1999, 14, 1, 3, 1, 2; *Baptisia* Vent. (Fabaceae), *Rider* 54 (FLAS), 1999, 14, 1, 3, 1, 1; *Centrosema virginianum* (L.) Benth. (Fabaceae), *Rider* 126 (FLAS), 1999, 14, 1, 3, 1, 1; *Cercis canadensis* L. (Fabaceae), *Rider* 42 (FLAS), 1999, 14, 1, 3, 1, 1; *Cercis canadensis* L. (Fabaceae), *Mercurio & Wagamon* 147 (FLAS), 1997, 16, 1, 1, 1, 1; *Crotalaria rotundifolia* J.F. Gmel. (Fabaceae), *Rider* 61 (FLAS), 1999, 14, 1, 3, 1, 1; *Crotalaria spectabilis* Roth (Fabaceae), *Rider* 1 (FLAS), 1998, 15, 1, 1, 1; *Dalea carnea* (Michx.) Poir. (Fabaceae), *Rider* 150 (FLAS), 1999, 14, 1, 3, 1, 1; *Erythrina herbacea* L. (Fabaceae), *Rider* 247 (FLAS), 2000, 13, 1, 3, 1, 1; *Galactia elliottii* Nutt. (Fabaceae), *Mercurio & Wagamon* 83 (FLAS), 1997, 16, 1, 3, 1, 1; *Mimosa quadrivalvis* L. (Fabaceae), *Rider* 101 (FLAS), 1999, 14, 1, 3, 1, 1; *Senna obtusifolia* (L.) H.S. Irwin & Barneby (Fabaceae), *Mercurio & Wagamon* 77 (FLAS), 1997, 16, 1, 3, 1, 1; *Sesbania punicea* (Cav.) Benth. (Fabaceae), *Rider* 79 (FLAS), 1999, 14, 1, 3, 1, 1; *Tephrosia florida* (E. Dietr.) C.E. Wood (Fabaceae), *Rider* 252 (FLAS), 2000, 13, 1, 3, 1, 1; *Tephrosia florida* (E. Dietr.) C.E. Wood (Fabaceae), *Mercurio & Wagamon* 82 (FLAS), 1997, 16, 1, 3, 1, 1; *Trifolium repens* L. (Fabaceae), *Rider* 142 (FLAS), 1999, 14, 1, 3, 1, 1; *Trifolium repens* L. (Fabaceae), *Proenza* 436 (FLAS), 2006, 7, 1, 3, 1, 1; *Vachellia farnesiana* (L.) Wight & Arn. (Fabaceae), *Mercurio & Wagamon* 42 (FLAS), 1997, 16, 1, 3, 2, 1; *Acacia pinetorum* F.J. Herm. (Fabaceae), *Rider* 6 (FLAS), 1998, 15, 1, 3, 2, 1; *Vicia acutifolia* Elliott (Fabaceae), *Rider* 59 (FLAS), 1999, 14, 1, 3, 1, 1; *Wisteria frutescens* (L.) Poir. (Fabaceae), *Rider* 73 (FLAS), 1999, 14, 1, 2, 1, 3; *Wisteria sinensis* (Sims) Sweet (Fabaceae), *Rider* 49 (FLAS), 1999, 14, 1, 3, 1, 2; *Quercus acutissima* Carruth. (Fagaceae), *Rider* 366 (FLAS), 2001, 12, 1, 2, 1, 3; *Quercus alba* L. (Fagaceae), *Rider* 164 (FLAS), 1999, 14, 1, 3, 2, 1; *Quercus incana* W. Bartram (Fagaceae), *Rider* 245 (FLAS), 2000, 13, 1, 2, 2, 1; *Quercus laevis* Walter (Fagaceae), *Mercurio & Wagamon* 74 (FLAS), 1997, 16, 1, 2, 2, 1; *Quercus laurifolia* Michx. (Fagaceae), *Mercurio & Wagamon* 87 (FLAS), 1997, 16, 1, 2, 2, 1; *Quercus margaretiae* (Ashe) Small (Fagaceae), *Mercurio & Wagamon* 68 (FLAS), 1997, 16, 1, 1, 1, 2; *Quercus marilandica* Münchh. (Fagaceae), *Rider* 244 (FLAS), 2000, 13, 1, 3, 1, 1; *Quercus marilandica* Münchh. (Fagaceae), *Mercurio & Wagamon* 132 (FLAS), 1997, 16, 0, 0, 1, 2; *Quercus marilandica* Münchh. (Fagaceae), *Rider* 288 (FLAS), 2000, 13, 1, 2, 2, 1; *Quercus michauxii* Nutt. (Fagaceae), *Mercurio & Wagamon* 151 (FLAS), 1997, 16, 1, 2, 1, 3; *Quercus minima* (Sarg.) Small (Fagaceae), *Rider* 339 (FLAS), 2000, 13, 1, 1, 1, 1; *Quercus nigra* L. (Fagaceae), *Mercurio & Wagamon* 84 (FLAS), 1997, 16, 0, 1, 1, 2; *Quercus shumardii* Buckley (Fagaceae), *Rider* 176 (FLAS), 1999, 14, 1, 3, 2, 1; *Quercus* L. (Fagaceae), *Mercurio & Wagamon* 139 (FLAS), 1997, 16, 1, 3, 1, 1; *Quercus virginiana* Mill. (Fagaceae), *Rider* 183 (FLAS), 2000, 13, 1, 3, 2, 1; *Gelsemium sempervirens* (L.) J. St.-Hil. (Gelsemiaceae), *Rider* 13 (FLAS), 1999, 14, 0, 0, 0, 0; *Sabatia decandra* (Walter) R.M. Harper (Gentianaceae), *Rider* 152 (FLAS), 1999, 14, 1, 3, 1, 1; *Sabatia decandra* (Walter) R.M. Harper (Gentianaceae), *Mercurio & Wagamon* 92 (FLAS), 1997, 16, 1, 3, 1, 1; *Sabatia brevifolia* Raf. (Gentianaceae), *Rider* 162 (FLAS), 1999, 14, 1, 3, 1, 1; *Sabatia campanulata* (L.) Torr. (Gentianaceae), *Rider* 119 (FLAS), 1999, 14, 1, 3, 1, 1; *Sabatia dodecandra* (L.) Britton, Sterns & Poggenb. (Gentianaceae), *Rider* 131 (FLAS), 1999, 14, 1, 3, 1, 1; *Geranium carolinianum* L. (Geraniaceae), *Rider* 358 (FLAS), 2001, 12, 1, 3, 2, 1; *Lachnanthes caroliniana* (Lam.) Dandy (Haemodoraceae), *Rider* 144 (FLAS), 1999, 14, 1, 3, 2, 1; *Lachnanthes caroliniana* (Lam.) Dandy (Haemodoraceae), *Mercurio & Wagamon* 148 (FLAS), 1997, 16, 1, 3, 1, 1;

- Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), *Rider 391* (FLAS), 2002, 11, 0, 1, 2, 3; *Proserpinaca pectinata* Lam. (Haloragaceae), *Proenza 431* (FLAS), 2006, 7, 1, 3, 1, 1; *Najas guadalupensis* (Spreng.) Magnus (Hydrocharitaceae), *Proenza 416* (FLAS), 2005, 8, 1, 2, 1, 3; *Hypericum cistifolium* Lam. (Hypericaceae), *Mercurio & Wagamon 99* (FLAS), 1997, 16, 1, 1, 1; *Hypericum cistifolium* Lam. (Hypericaceae), *Rider 290* (FLAS), 2000, 13, 1, 3, 1, 1; *Hypericum fasciculatum* Lam. (Hypericaceae), *Rider 199* (FLAS), 2000, 13, 1, 3, 1, 1; *Hypericum gentianoides* (L.) Britton, Sterns & Poggenb. (Hypericaceae), *Rider 159* (FLAS), 1999, 14, 1, 3, 1, 2; *Hypericum hypericoides* (L.) Crantz (Hypericaceae), *Rider 302* (FLAS), 2000, 13, 1, 3, 1, 1; *Hypericum microsepalum* (Torr. & A. Gray) A. Gray ex S. Watson (Hypericaceae), *Rider 198* (FLAS), 2000, 13, 1, 3, 1, 1; *Hypericum setosum* L. (Hypericaceae), *Rider 287* (FLAS), 2000, 13, 1, 3, 2, 1; *Hypericum tetrapetalum* Lam. (Hypericaceae), *Rider 196* (FLAS), 2000, 13, 1, 3, 1, 1; *Hypoxis juncea* Sm. (Hypoxidaceae), *Rider 200* (FLAS), 2000, 13, 1, 3, 1, 2; *Hypoxis juncea* Sm. (Hypoxidaceae), *Proenza 424* (FLAS), 2006, 7, 1, 3, 1, 2; *Iris virginica* L. (Iridaceae), *Rider 40* (FLAS), 1999, 14, 1, 3, 1, 2; *Sisyrinchium atlanticum* E.P. Bicknell (Iridaceae), *Rider 56* (FLAS), 1999, 14, 1, 3, 1, 2; *Sisyrinchium rosulatum* E.P. Bicknell (Iridaceae), *Rider 223* (FLAS), 2000, 13, 1, 3, 1, 2; *Itea virginica* L. (Iteaceae), *Mercurio & Wagamon 63* (FLAS), 1997, 16, 1, 3, 2, 1; *Carya glabra* (Mill.) Sweet (Juglandaceae), *Rider 370* (FLAS), 2001, 12, 1, 3, 1, 1; *Carya illinoiensis* (Wangenh.) K. Koch (Juglandaceae), *Rider 78* (FLAS), 1999, 14, 1, 2, 1, 3; *Juncus effusus* L. (Juncaceae), *Rider 21* (FLAS), 1999, 14, 1, 3, 1, 2; *Juncus polycratus* Michx. (Juncaceae), *Rider 266* (FLAS), 2000, 13, 1, 3, 1, 2; *Juncus* L. (Juncaceae), *Mercurio & Wagamon 91* (FLAS), 1997, 16, 0, 1, 1, 2; *Juncus* L. (Juncaceae), *Mercurio & Wagamon 111* (FLAS), 1997, 16, 1, 2, 1, 3; *Callicarpa americana* L. (Lamiaceae), *Mercurio & Wagamon 40* (FLAS), 1997, 16, 1, 3, 1, 1; *Hyptis alata* Shinners (Lamiaceae), *Rider 141* (FLAS), 1999, 14, 1, 3, 1, 1; *Hyptis mutabilis* (Rich.) Briq. (Lamiaceae), *Mercurio & Wagamon 27* (FLAS), 1997, 16, 1, 3, 1, 1; *Hyptis mutabilis* (Rich.) Briq. (Lamiaceae), *Rider 326* (FLAS), 2000, 13, 1, 3, 1, 1; *Physostegia purpurea* (Walter) S.F. Blake (Lamiaceae), *Rider 100* (FLAS), 1999, 14, 1, 3, 1, 1; *Physostegia* Benth. (Lamiaceae), *Rider 112* (FLAS), 1999, 14, 1, 3, 1, 1; *Rosmarinus officinalis* L. (Lamiaceae), *Rider 397* (FLAS), 2002, 11, 1, 1, 1; *Salvia lyrata* L. (Lamiaceae), *Rider 207* (FLAS), 2000, 13, 1, 3, 2, 1; *Salvia lyrata* L. (Lamiaceae), *Rider 64* (FLAS), 1999, 14, 0, 0, 0; *Scutellaria arenicola* Small (Lamiaceae), *Rider 98* (FLAS), 1999, 14, 1, 3, 1, 1; *Teucrium canadense* L. (Lamiaceae), *Rider 135* (FLAS), 1999, 14, 1, 3, 1, 1; *Teucrium canadense* L. (Lamiaceae), *Mercurio & Wagamon 75* (FLAS), 1997, 16, 1, 3, 1, 1; *Trichostema dichotomum* L. (Lamiaceae), *Rider 335* (FLAS), 2000, 13, 1, 3, 1, 1; *Cinnamomum camphora* (L.) J. Presl (Lauraceae), *Rider 75* (FLAS), 1999, 14, 1, 3, 2, 1; *Persea borbonia* (L.) Spreng. (Lauraceae), *Rider 383* (FLAS), 2001, 12, 1, 3, 0, 0; *Persea borbonia* (L.) Spreng. (Lauraceae), *Rider 219* (FLAS), 2000, 13, 1, 3, 1, 1; *Persea palustris* (Raf.) Sarg. (Lauraceae), *Rider 396* (FLAS), 2002, 11, 1, 3, 1, 1; *Sassafras albidum* (Nutt.) Nees (Lauraceae), *Rider 140* (FLAS), 1999, 14, 1, 3, 0, 0; *Pinguicula caerulea* Walter (Lentibulariaceae), *Rider 201* (FLAS), 2000, 13, 1, 3, 1, 1; *Pinguicula lutea* Walter (Lentibulariaceae), *Rider 45* (FLAS), 1999, 14, 1, 3, 1, 1; *Pinguicula pumila* Michx. (Lentibulariaceae), *Rider 10* (FLAS), 1999, 14, 1, 3, 1, 1; *Pinguicula pumila* Michx. (Lentibulariaceae), *Proenza 439* (FLAS), 2006, 7, 1, 3, 1, 1; *Pinguicula pumila* Michx. (Lentibulariaceae), *Rider 394* (FLAS), 2002, 11, 0, 0, 0, 0; *Utricularia gibba* L. (Lentibulariaceae), *Rider 375* (FLAS), 2001, 12, 1, 3, 1, 1; *Utricularia breviscapa* Wright ex Griseb. (Lentibulariaceae), *Rider 22* (FLAS), 1999, 14, 0, 1, 1, 1; *Utricularia subulata* L. (Lentibulariaceae), *Rider 47* (FLAS), 1999, 14, 1, 3, 1, 1; *Lilium catesbeiae* Walter (Liliaceae), *Rider 163* (FLAS), 1999, 14, 0, 0, 0, 1; *Lycopodiella alopecuroides* (L.) Cranfill (Lycopodiaceae), *Rider 387* (FLAS), 2002, 11, 1, 3, 1, 1; *Lycopodiella alopecuroides* (L.) Cranfill (Lycopodiaceae), *Mercurio & Wagamon 95* (FLAS), 1997, 16, 1, 3, 1, 1; *Lythrum lineare* L. (Lythraceae), *Mercurio & Wagamon 120* (FLAS), 1997, 16, 0, 1, 1, 2; *Magnolia grandiflora* L. (Magnoliaceae), *Rider 123* (FLAS), 1999, 14, 1, 1, 1; *Magnolia virginiana* L. (Magnoliaceae), *Rider 107* (FLAS), 1999, 14, 1, 3, 1, 1; *Hibiscus aculeatus* Walter (Malvaceae), *Rider 125* (FLAS), 1999, 14, 0, 1, 1, 2; *Hibiscus moscheutos* L. (Malvaceae), *Rider 114* (FLAS), 1999, 14, 1, 2, 2, 1; *Kosteletzkyia pentacarpa* (L.) Ledeb. (Malvaceae), *Rider 138* (FLAS), 1999, 14, 1, 3, 1, 1; *Pavonia hastata* Cav. (Malvaceae), *Rider 149* (FLAS), 1999, 14, 1, 3, 2, 1; *Zigadenus densus* (Desr.) Fernald (Melanthiaceae), *Rider 86* (FLAS), 1999, 14, 1, 3, 1, 2; *Rhexia alifanus* Walter (Melastomataceae), *Rider 118* (FLAS), 1999, 14, 0, 0, 0; *Rhexia cubensis* Griseb. (Melastomataceae), *Rider 279* (FLAS), 2000, 13, 1, 0, 1, 2; *Rhexia lutea* Walter (Melastomataceae), *Rider 365* (FLAS), 2001, 12, 0, 0, 0, 0; *Rhexia mariana* L. (Melastomataceae), *Rider 121* (FLAS), 1999, 14, 1, 1, 1; *Rhexia nashii* Small (Melastomataceae), *Rider 284* (FLAS), 2000, 13, 1, 2, 1, 3; *Rhexia petiolata* Walter (Melastomataceae), *Rider 313* (FLAS), 2000, 13, 1, 1, 1, 1; *Limnobium spongia* (Bosc) Rich. ex Steud. (Menyanthaceae), *Mercurio & Wagamon 88* (FLAS), 1997, 16, 0, 0, 1, 2; *Morus rubra* L. (Moraceae), *Mercurio & Wagamon 135* (FLAS), 1997, 16, 1, 3, 1, 1; *Musa acuminata* Colla (Musaceae), *Rider 278* (FLAS), 2000, 13, 0, 1, 1, 2; *Morella cerifera* (L.) Small (Myricaceae), *Rider 7* (FLAS), 1998, 15, 1, 3, 2, 1; *Myrica heterophylla* Raf. (Myricaceae), *Rider 392* (FLAS), 2002, 11, 1, 1, 1, 1; *Aletris lutea* Small (Nartheciaceae), *Rider 97* (FLAS), 1999, 14, 1, 2, 1, 3; *Aletris obovata* Nash ex Small (Nartheciaceae), *Rider 106* (FLAS), 1999, 14, 0, 0, 0, 1; *Nymphaea odorata* Aiton (Nymphaeaceae), *Rider 373* (FLAS), 2001, 12, 1, 1, 1, 1; *Nyssa ogeche* W. Bartram ex Marshall (Nyssaceae), *Mercurio & Wagamon 58* (FLAS), 1997, 16, 0, 1, 2, 3; *Nyssa sylvatica* Marshall (Nyssaceae), *Rider 220* (FLAS), 2000, 13, 0, 0, 0, 1; *Chionanthus virginicus* L. (Oleaceae), *Rider 68* (FLAS), 1999, 14, 1, 3, 1, 1; *Fraxinus caroliniana* Mill. (Oleaceae), *Rider 72* (FLAS), 1999, 14, 1, 3, 1, 1; *Fraxinus caroliniana* Mill. (Oleaceae), *Mercurio & Wagamon 24* (FLAS), 1997, 16, 1, 3, 2, 1; *Fraxinus pennsylvanica* Marshall (Oleaceae), *Mercurio & Wagamon 158* (FLAS), 1997, 16, 1, 3, 1, 1; *Ligustrum japonicum* Thunb. (Oleaceae), *Rider 214* (FLAS), 2000, 13, 1, 3, 2, 1; *Ludwigia leptocarpa* (Nutt.) H. Hara (Onagraceae), *Proenza 417* (FLAS), 2005, 8, 1, 3, 2, 1; *Ludwigia maritima* R.M. Harper (Onagraceae), *Rider 280* (FLAS), 2000, 13, 1, 3, 1, 1; *Ludwigia peruviana* (L.) H. Hara (Onagraceae), *Rider 171* (FLAS), 1999, 14, 1, 2, 1, 3; *Ludwigia* L. (Onagraceae), *Mercurio & Wagamon 100* (FLAS), 1997, 16, 0, 1, 1, 1; *Ludwigia virgata* Michx. (Onagraceae), *Rider 128* (FLAS), 1999, 14, 1, 1, 1; *Oenothera laciniata* Hill (Onagraceae), *Rider 65* (FLAS), 1999, 14, 1, 1, 1, 1; *Calopogon multiflorus* Lindl. (Orchidaceae),

- Rider 360 (FLAS), 2001, 12, 1, 3, 1, 2; *Calopogon pallidus* Chapm. (Orchidaceae), Rider 362 (FLAS), 2001, 12, 1, 3, 1, 2; *Calopogon tuberosus* (L.) Britton, Sterns & Poggenp. (Orchidaceae), Rider 99 (FLAS), 1999, 14, 1, 3, 1, 2; *Cleistes diversiflora* (L.) Ames (Orchidaceae), Rider 102 (FLAS), 1999, 14, 1, 3, 2, 1; *Cantharis virens* Cossner (Orchidaceae), Rider 353 (FLAS), 2001, 12, 1, 3, 1, 2; *Platanthera ciliaris* (L.) Lindl. (Orchidaceae), Rider 158 (FLAS), 1999, 14, 1, 3, 2, 1; *Spiranthes praecox* (Walter) S. Watson (Orchidaceae), Rider 67 (FLAS), 1999, 14, 1, 3, 2, 1; *Spiranthes vernalis* Engelm. & A. Gray (Orchidaceae), Rider 91 (FLAS), 1999, 14, 1, 3, 1, 2; *Zeuxine straminea* (L.) Schltr. (Orchidaceae), Rider 11 (FLAS), 1999, 14, 1, 3, 1, 2; *Agalinis aphylla* (Nutt.) Raf. (Orobanchaceae), Rider 347 (FLAS), 2000, 13, 1, 3, 2, 1; *Agalinis fasciata* (Elliott) Raf. (Orobanchaceae), Rider 342 (FLAS), 2000, 13, 1, 3, 2, 1; *Agalinis fasciata* (Elliott) Raf. (Orobanchaceae), Rider 169 (FLAS), 1999, 14, 1, 3, 2, 1; *Agalinis setacea* (J.P. Gmel.) Raf. (Orobanchaceae), Rider 349 (FLAS), 2000, 13, 1, 3, 1, 1; *Oxalis corniculata* L. (Oxalidaceae), Rider 208 (FLAS), 2000, 13, 0, 1, 2, 3; *Phyllanthus urinaria* L. (Phyllanthaceae), Rider 291 (FLAS), 2000, 13, 0, 1, 1, 1; *Physalacria americana* L. (Phytolaccaceae), *Mercruis* & *Wagaman* 41 (FLAS), 1997, 16, 1, 3, 1, 1; *Pinus clausa* (Chapm. ex Engelm.) Sarg. (Pinaceae), *Mercruis* & *Wagaman* 69 (FLAS), 1997, 16, 0, 0, 0, 0; *Pinus elliptica* Engelm. (Pinaceae), *Mercruis* & *Wagaman* 145 (FLAS), 1997, 16, 0, 0, 0, 1; *Pinus glabra* Walter (Pinaceae), Rider 43 (FLAS), 1999, 14, 0, 1, 1, 2; *Pinus palustris* Mill. (Pinaceae), Rider 356 (FLAS), 2001, 12, 1, 3, 1, 2; *Pinus strobus* Michx. (Pinaceae), Rider 384 (FLAS), 2001, 12, 1, 3, 1, 2; *Pinus taeda* L. (Pinaceae), Rider 33 (FLAS), 1999, 14, 1, 3, 1, 2; *Bacopa caroliniana* (Walter) B.L. Rob. (Plantaginaceae), Rider 145 (FLAS), 1999, 14, 1, 3, 1, 1; *Bacopa monnieri* (L.) Wettst. (Plantaginaceae), *Mercruis* & *Wagaman* 102 (FLAS), 1997, 16, 1, 3, 1, 1; *Linaria canadensis* (L.) Dum. Cours. (Plantaginaceae), Rider 29 (FLAS), 1999, 14, 1, 3, 2, 1; *Mecardonia acuminata* (Walter) Small (Plantaginaceae), Rider 71 (FLAS), 1999, 14, 1, 3, 1, 3; *Mecardonia acuminata* (Walter) Small (Plantaginaceae), *Poenza* 438 (FLAS), 2006, 7, 1, 3, 1, 1; *Plantago lanceolata* L. (Plantaginaceae), Rider 212 (FLAS), 2000, 13, 1, 3, 1, 0; *Plantago major* L. (Plantaginaceae), Rider 41 (FLAS), 1999, 14, 1, 3, 1, 1; *Plantago virginica* L. (Plantaginaceae), Rider 205 (FLAS), 2000, 13, 1, 2, 3, 1; *Platynnis occidentalis* L. (Platanaceae), Rider 77 (FLAS), 1999, 14, 1, 2, 1, 3; *Andropogon glomeratus* (Walter) Britton, Steens & Poggenp. (Poaceae), Rider 178 (FLAS), 1999, 14, 1, 3, 1, 2; *Arundinaria gigantea* (Walter) Muhl. (Poaceae), Rider 197 (FLAS), 2000, 13, 1, 3, 1, 2; *Arundinaria gigantea* (Walter) Muhl. (Poaceae), Rider 194 (FLAS), 2000, 13, 1, 3, 1, 2; *Arundo donax* L. (Poaceae), *Poenza* 425 (FLAS), 2005, 8, 1, 3, 1, 2; *Chamaanthium laeve* (L.) H.O. Yates (Poaceae), *Mercruis* & *Wagaman* 115 (FLAS), 1997, 16, 1, 3, 1, 2; *Chamaanthium laeve* (L.) H.O. Yates (Poaceae), *Mercruis* & *Wagaman* 137 (FLAS), 1997, 16, 1, 3, 1, 1; *Chamaanthium nitidum* (Baldwin ex Elliott) H.O. Yates (Poaceae), *Mercruis* & *Wagaman* 112 (FLAS), 1997, 16, 1, 3, 1, 1; *Cleodium jamaicense* Crantz (Poaceae), *Mercruis* & *Wagaman* 26a (FLAS), 1997, 16, 1, 3, 1, 2; *Crenim arachnatum* (Walter) Alph. Wood (Poaceae), Rider 94 (FLAS), 1999, 14, 1, 3, 1, 2; *Crenim arachnatum* (Walter) Alph. Wood (Poaceae), *Mercruis* & *Wagaman* 78 (FLAS), 1997, 16, 1, 3, 1, 2; *Dichanthelium commutatum* (Schult.) Gould (Poaceae), *Mercruis* & *Wagaman* 107 (FLAS), 1997, 16, 1, 3, 1, 1; *Dichanthelium spathocarpum* var. *floridanum* (Vasey) Davidse (Poaceae), *Mercruis* & *Wagaman* 106 (FLAS), 1997, 16, 1, 3, 1, 1; *Dichanthelium umbrosum* (Elliott) Gould & C.A. Clark (Poaceae), *Poenza* 426 (FLAS), 2005, 8, 1, 3, 1, 2; *Dichanthelium umbrosum* (Elliott) Gould & C.A. Clark (Poaceae), *Mercruis* & *Wagaman* 117 (FLAS), 1997, 16, 1, 3, 1, 1; *Dichanthelium scabriulum* (Elliott) Gould & C.A. Clark (Poaceae), *Mercruis* & *Wagaman* 116 (FLAS), 1997, 16, 1, 3, 1, 1; *Dichanthelium dichotomum* var. *unciphyllum* (Trin.) Davidse (Poaceae), *Poenza* 434 (FLAS), 2006, 7, 1, 3, 1, 1; *Digitaria ciliaris* (Retz.) Koeler (Poaceae), Rider 307 (FLAS), 2000, 13, 1, 3, 1, 2; *Imperata cylindrica* (L.) Rausch (Poaceae), *Poenza* 430 (FLAS), 2006, 7, 1, 3, 1, 2; *Lolium perenne* L. (Poaceae), Rider 230 (FLAS), 2000, 13, 1, 3, 1, 2; *Paspalum notatum* Alain ex Flugge (Poaceae), Rider 227 (FLAS), 2000, 13, 1, 3, 1, 2; *Paspalum procne* Walter (Poaceae), Rider 226 (FLAS), 2000, 13, 1, 3, 0, 0; *Paspalum urvillei* Steud. (Poaceae), Rider 285 (FLAS), 2000, 13, 1, 3, 1, 2; *Paspalum urvillei* Steud. (Poaceae), *Poenza* 419 (FLAS), 2005, 8, 1, 3, 1, 2; *Cenchrus purpureus* (Schumach.) Morrenne (Poaceae), Rider 272 (FLAS), 2000, 13, 1, 3, 1, 2; *Polygonum monspeliacum* (L.) Desf. (Poaceae), Rider 203 (FLAS), 2000, 13, 1, 3, 1, 2; *Polygonum monspeliacum* (L.) Desf. (Poaceae), *Poenza* 437 (FLAS), 2006, 7, 0, 0, 0, 0; *Saccharum giganteum* (Walter) Pers. (Poaceae), Rider 175 (FLAS), 1999, 14, 1, 3, 1, 2; *Setaria parviflora* (Poir.) Kerguelen (Poaceae), Rider 263 (FLAS), 2000, 13, 1, 3, 1, 2; *Setaria parviflora* (Poir.) Kerguelen (Poaceae), *Mercruis* & *Wagaman* 142 (FLAS), 1997, 16, 1, 3, 1, 1; *Sorghum ellipticum* (C. Mohr) Nash (Poaceae), Rider 337 (FLAS), 2000, 13, 1, 3, 1, 2; *Tripsacum dactyloides* (L.) L. (Poaceae), Rider 242 (FLAS), 2000, 13, 1, 3, 1, 2; *Tripsacum dactyloides* var. *floridum* (Poir. ex Vasey) Beal (Poaceae), Rider 248 (FLAS), 2000, 13, 1, 3, 1, 2; *Zizaniopsis miliacea* (Michx.) Dill & Asch. (Poaceae), Rider 165 (FLAS), 1999, 14, 1, 3, 1, 2; *Zizaniopsis miliacea* (Michx.) Dill & Asch. (Poaceae), Rider 166 (FLAS), 1999, 14, 1, 3, 1, 2; *Phragmites australis* (L.) Benth. (Poaceae), Rider 69 (FLAS), 1999, 14, 0, 0, 0, 0; *Polygonum cruciatum* L. (Polygalaceae), Rider 369 (FLAS), 2001, 12, 0, 0, 0, 0; *Polygonum cyathiforme* Walter (Polygalaceae), Rider 368 (FLAS), 2001, 12, 1, 3, 1, 2; *Polygonum latifolium* L. (Polygalaceae), Rider 51 (FLAS), 1999, 14, 1, 3, 1, 1; *Polygonum latifolium* L. (Polygalaceae), *Mercruis* & *Wagaman* 15 (FLAS), 1997, 16, 1, 3, 1, 1; *Polygonum nigrum* (Michx.) DC. (Polygalaceae), Rider 23 (FLAS), 1999, 14, 0, 1, 1, 1; *Polygonum nigrum* Elliott (Polygalaceae), Rider 146 (FLAS), 1999, 14, 1, 3, 1, 1; *Polygonum nigrum* Elliott (Polygalaceae), Rider 120 (FLAS), 1999, 14, 1, 3, 1, 1; *Polygonum rugosum* Shurley ex Chapman (Polygalaceae), Rider 62 (FLAS), 1999, 14, 0, 1, 1, 1; *Polygonum polypodioides* (L.) E.G. Andrews & Windham (Polypodiaceae), Rider 386 (FLAS), 2002, 11, 1, 3, 1, 2; *Pontederia cordata* L. (Pontederiaceae), Rider 80 (FLAS), 1999, 14, 1, 2, 1, 3; *Samolus floribundus* Kunth (Primulaceae), *Poenza* 435 (FLAS), 2006, 7, 1, 3, 1, 1; *Clematis crispa* L. (Ranunculaceae), *Mercruis* & *Wagaman* 103 (FLAS), 1997, 16, 1, 1, 1, 1; *Clematis crispa* L. (Ranunculaceae), Rider 83 (FLAS), 1999, 14, 1, 3, 1, 1; *Ranunculus bipinnatus* Michx. (Ranunculaceae), Rider 234 (FLAS), 2000, 13, 1, 3, 1, 1; *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae), Rider 187 (FLAS), 2000, 13, 1, 3, 2, 1; *Potentilla pyrifolia* (Lam.) K.R. Robertson & J.B. Phipps (Rosaceae), Rider 185 (FLAS), 2000, 13, 1, 2, 1, 3; *Potentilla pyrifolia* (Lam.) K.R. Robertson & J.B. Phipps (Rosaceae), Rider 27

- (FLAS), 1999, 14, 1, 3, 2, 1; *Prunus americana* Marshall (Rosaceae), *Rider* 352 (FLAS), 2001, 12, 1, 1, 1, 2; *Prunus pensylvanica* L. (Rosaceae), *Rider* 190 (FLAS), 2000, 13, 1, 2, 3, 1; *Prunus pensylvanica* L. (Rosaceae), *Mercurio & Wagaman* 67 (FLAS), 1997, 16, 1, 3, 2, 1; *Pyrus calleryana* Decne. (Rosaceae), *Rider* 354 (FLAS), 2001, 12, 1, 1, 1, 3; *Rosa loevigata* Michx. (Rosaceae), *Rider* 34 (FLAS), 1999, 14, 1, 1, 1, 3; *Rosa palustris* Marshall (Rosaceae), *Rider* 85 (FLAS), 1999, 14, 1, 3, 2, 1; *Rosa palustris* Marshall (Rosaceae), *Rider* 385 (FLAS), 2002, 11, 1, 3, 2, 1; *Rubus cuneifolius* Pusch (Rosaceae), *Rider* 246 (FLAS), 2000, 13, 1, 3, 2, 1; *Rubus trivialis* Michx. (Rosaceae), *Rider* 25 (FLAS), 1999, 14, 1, 2, 1, 3; *Cephaelanthus occidentalis* L. (Rubiaceae), *Mercurio & Wagaman* 35 (FLAS), 1997, 16, 0, 1, 2, 3; *Diodia virginiana* L. (Rubiaceae), *Rider* 316 (FLAS), 2000, 13, 1, 3, 1, 1; *Hedysarum procumbens* (J.E. Gmel.) Fosberg (Rubiaceae), *Rider* 355 (FLAS), 2001, 12, 1, 3, 2, 1; *Mitchella repens* L. (Rubiaceae), *Rider* 181 (FLAS), 2000, 13, 1, 3, 2, 1; *Populus deltoides* W. Bartram ex Marshall (Salicaceae), *Rider* 215 (FLAS), 2000, 13, 1, 3, 2, 1; *Salix babylonica* L. (Salicaceae), *Rider* 277 (FLAS), 2000, 13, 1, 1, 2, 3; *Salix caroliniana* Michx. (Salicaceae), *Mercurio & Wagaman* 118 (FLAS), 1997, 16, 1, 1, 1, 1; *Salix nigra* Marshall (Salicaceae), *Rider* 48 (FLAS), 1999, 14, 1, 3, 2, 1; *Phenadendron leucarpum* (Raf.) Reveal & M.C. Johnson (Santalaceae), *Rider* 350 (FLAS), 2001, 12, 1, 2, 1, 3; *Acer rubrum* L. (Sapindaceae), *Rider* 19 (FLAS), 1999, 14, 1, 3, 1, 2; *Sarracenia minor* Walter (Sarraceniaceae), *Rider* 206 (FLAS), 2000, 13, 1, 3, 2, 1; *Sarracenia minor* Walter (Sarraceniaceae), *Mercurio & Wagaman* 93 (FLAS), 1997, 16, 1, 3, 2, 1; *Saururus cernuus* L. (Saururaceae), *Rider* 96 (FLAS), 1999, 14, 1, 3, 2, 1; *Ilicium floridanum* J. Ellis (Schisandraceae), *Rider* 177 (FLAS), 1999, 14, 1, 3, 2, 1; *Smilax bona-nox* L. (Smilacaceae), *Rider* 390 (FLAS), 2002, 11, 1, 3, 1, 2; *Smilax glauca* Walter (Smilacaceae), *Rider* 251 (FLAS), 2000, 13, 1, 3, 1, 2; *Smilax laurifolia* L. (Smilacaceae), *Rider* 28 (FLAS), 1999, 14, 1, 2, 1, 3; *Smilax* L. (Smilacaceae), *Mercurio & Wagaman* 160 (FLAS), 1997, 16, 1, 3, 1, 1; *Solanum americanum* Mill. (Solanaceae), *Rider* 133 (FLAS), 1999, 14, 1, 3, 1, 3; *Solanum capsicoides* All. (Solanaceae), *Rider* 228 (FLAS), 2000, 13, 1, 3, 2, 1; *Solanum carolinense* L. (Solanaceae), *Rider* 70 (FLAS), 1999, 14, 1, 3, 1, 1; *Solanum lychnoides* L. (Solanaceae), *Rider* 265 (FLAS), 2000, 13, 1, 3, 1, 3; *Polygonatum procumbens* L. (Tetrachondraceae), R.L. Wilbur 68532 (FLAS), 1997, 16, 1, 3, 1, 1; *Gordonia lasianthus* (L.) J. Ellis (Theaceae), *Mercurio & Wagaman* 1 (FLAS), 1997, 16, 1, 2, 1, 3; *Piriqueta cistoides* subsp. *caroliniana* (Walter) Arbo (Turneraceae), *Rider* 237 (FLAS), 2000, 13, 1, 3, 1, 1; *Typha latifolia* L. (Typhaceae), *Rider* 110 (FLAS), 1999, 14, 1, 2, 1, 3; *Ulmus alata* Michx. (Ulmaceae), *Mercurio & Wagaman* 153 (FLAS), 1997, 16, 1, 3, 1, 2; *Ulmus alata* Michx. (Ulmaceae), *Rider* 283 (FLAS), 2000, 13, 1, 3, 2, 1; *Ulmus americana* L. (Ulmaceae), *Mercurio & Wagaman* 134 (FLAS), 1997, 16, 1, 3, 2, 1; *Urtica urens* L. (Urticaceae), *Smith* 01 (FLAS), 1999, 14, 1, 3, 2, 1; *Lantana depressa* Small (Verbenaceae), *Rider* 271 (FLAS), 2000, 13, 1, 3, 1, 1; *Phytolacca acinosa* (L.) Greene (Verbenaceae), *Rider* 224 (FLAS), 2000, 13, 1, 3, 2, 1; *Viola lanceolata* L. (Violaceae), *Rider* 17 (FLAS), 1999, 14, 1, 3, 1, 1; *Viola palmata* L. (Violaceae), *Rider* 186 (FLAS), 2000, 13, 1, 3, 2, 1; *Viola palmata* L. (Violaceae), *Pruett* 428 (FLAS), 2006, 7, 1, 3, 2, 1; *Viola palmata* L. (Violaceae), *Rider* 39 (FLAS), 1999, 14, 1, 3, 2, 1; *Viola primulifolia* L. (Violaceae), *Rider* 24 (FLAS), 1999, 14, 1, 3, 2, 1; *Viola sororia* Willd. (Violaceae), *Rider* 14 (FLAS), 1999, 14, 1, 3, 1, 1; *Viola sororia* Willd. (Violaceae), *Rider* 46 (FLAS), 1999, 14, 1, 3, 2, 1; *Ampelophis arborea* (L.) Koehne (Vitaceae), *Rider* 256 (FLAS), 2000, 13, 1, 3, 2, 1; *Pithecellobium quinquefolium* (L.) Planch. (Fabaceae), *Mercurio & Wagaman* 65 (FLAS), 1997, 16, 0, 1, 2, 3; *Vitis aestivalis* Michx. (Vitaceae), *Rider* 293 (FLAS), 2000, 13, 0, 0, 1, 2; *Vitis rotundifolia* Michx. (Vitaceae), *Rider* 286 (FLAS), 2000, 13, 1, 1, 1, 2; *Vitis rotundifolia* Michx. (Vitaceae), *Rider* 130 (FLAS), 1999, 14, 0, 0, 0, 1; *Vitis rotundifolia* Michx. (Vitaceae), *Rider* 317 (FLAS), 2000, 13, 1, 1, 1, 1; *Vitis rotundifolia* Michx. (Vitaceae), *Mercurio & Wagaman* 17 (FLAS), 1997, 16, 0, 0, 1, 2; *Xyris* L. (Xyridaceae), *Mercurio & Wagaman* 12 (FLAS), 1997, 16, 0, 0, 1, 2; *Zamia pumila* L. (Zamiaceae), *Rider* 184 (FLAS), 2000, 13, 1, 3, 1, 2. Abbott Collection data set from herbarium specimens: *Aleurites philippinensis* (Mart.) Griseb. (Amaranthaceae), Abbott 3230 (FLAS), 1992, 21, 0, 0, 1, 2; *Taxodium pubescens* Mill. (Anacardiaceae), Abbott 10812 (FLAS), 1997, 16, 0, 0, 0, 1; *Eryngium prostratum* Nutt. ex DC. (Apiaceae), Abbott 3634 (FLAS), 1992, 21, 0, 0, 0, 1; *Phlomis capillacea* (Michx.) Raf. (Apiaceae), Abbott 1775 (FLAS), 1992, 21, 0, 0, 1, 2; *Phlomis capillacea* (Michx.) Raf. (Apiaceae), Abbott 1791 (FLAS), 1992, 21, 0, 0, 0, 1; *Tricosperma nelumbi* Nutt. ex DC. (Apiaceae), Abbott 3213 (FLAS), 1992, 21, 1, 1, 2, 3; *Acalypha peruviana* Walter (Apocynaceae), Abbott 3630 (FLAS), 1992, 21, 0, 0, 0, 1; *Ilex coriacea* (Pursh) Chapm. (Araliaceae), Abbott 10792 (FLAS), 1997, 16, 0, 0, 0, 1; *Ilex coriacea* (Pursh) Chapm. (Araliaceae), Abbott 6683 (FLAS), 1994, 19, 0, 0, 0, 1; *Ilex* L. (Araliaceae), Abbott 14188 (FLAS), 2001, 12, 1, 1, 2, 3; *Ilex x meserveae* S.Y. Hu (Araliaceae), Abbott 14187 (FLAS), 2001, 12, 0, 0, 1, 2; *Weinmannia columbiensis* H. Kast. (Araliaceae), Abbott 19630 (FLAS), 2004, 9, 1, 3, 1, 2; *Anemone baldwinii* Hook. (Araucariaceae), Abbott 12936a (FLAS), 1999, 14, 0, 0, 1, 2; *Butilia capitata* (Mart.) Becc. (Arecaceae), Abbott 6702 (FLAS), 1994, 19, 1, 3, 1, 2; *Aiphanes setosa* (Kunth) Jessop (Asparagaceae), Abbott 1828 (FLAS), 1992, 21, 0, 0, 0, 1; *Asplenium platyneuron* (L.) Britton, Sterns & Poggenb. (Aspleniaceae), Abbott 7978 (FLAS), 1994, 19, 0, 0, 0, 1; *Spileotrichum oppositifolium* (Lam.) D'Arcy (Asteraceae), Abbott 3209 (FLAS), 1992, 21, 0, 0, 0, 1; *Ambrosia trifida* L. (Asteraceae), Abbott 14352 (FLAS), 2001, 12, 1, 1, 1, 2; *Coreopsis tinctoria* Nutt. (Asteraceae), Abbott 1774 (FLAS), 1992, 21, 0, 0, 1, 2; *Emilia sonchifolia* Nicolson (Asteraceae), Abbott 1802 (FLAS), 1992, 21, 0, 0, 0, 1; *Gomphrena pulchella* (Lam.) Cabrera (Asteraceae), Abbott 1805 (FLAS), 1992, 21, 0, 0, 0, 1; *Selinia austromexicana* (Just.) Sweet (Asteraceae), Abbott 6694 (FLAS), 1994, 19, 0, 0, 0, 1; *Mikania scandens* (L.) Willd. (Asteraceae), Abbott 3207 (FLAS), 1992, 21, 0, 0, 0, 1; *Palafuria fayi* A. Gray (Asteraceae), Abbott 17794 (FLAS), 2003, 10, 1, 3, 1, 1; *Palafuria fayi* A. Gray (Asteraceae), Abbott 17793 (FLAS), 2003, 10, 0, 0, 1, 2; *Pluchea baccharis* (Mill.) Pruski (Asteraceae), Abbott 3632 (FLAS), 1992, 21, 0, 0, 0, 1; *Nandina domestica* Thunb. (Berberidaceae), Abbott 11368 (FLAS), 1998, 15, 0, 0, 0, 1; *Tecoma capensis* (Thunb.) Lindl. (Bignoniaceae), Abbott 6704 (FLAS), 1994, 19, 0, 0, 1, 2; *Woodwardia areolata* (L.) T. Moore (Blechnaceae), Abbott 7984 (FLAS), 1994, 19, 0, 0, 0, 1; *Lepidium virginicum* L. (Brassicaceae), Abbott 1798 (FLAS), 1992, 21, 0, 0, 0, 1; *Raphanus raphanistrum* L. (Brassicaceae), Abbott 6692 (FLAS), 1994, 19, 0, 0, 1, 1; *Tillandsia recurvata* (L.) L.

- (Bromeliaceae), Abbott 1837 (FLAS), 1992, 21, 0, 0, 0, 0; *Epi-*
phyllum egyptiacum (DC.) Haw. (Cactaceae), Abbott 6703 (FLAS), 1994, 19, 0, 0, 0, 0; *Lobelia brevifolia* Nutt. ex A. DC. (Campanulaceae), Abbott 10793 (FLAS), 1997, 16, 1, 1, 2, 3; *Triodanis perfoliata* (L.) Nieuwl. (Campanulaceae), Abbott 6681 (FLAS), 1994, 19, 1, 1, 1, 2; *Triodanis perfoliata* (L.) Nieuwl. (Campanulaceae), Abbott 6693 (FLAS), 1994, 19, 1, 1, 1, 1; *Wahlenbergia marginata* (Thunb.) A. DC. (Campanulaceae), Abbott 6679 (FLAS), 1994, 19, 0, 0, 0, 1; *Wahlenbergia marginata* (Thunb.) A. DC. (Campanulaceae), Abbott 10811 (FLAS), 1997, 16, 0, 0, 0, 1; *Humulus lupulus* L. (Cannabaceae), Abbott 13191 (FLAS), 2000, 13, 1, 1, 1, 2; *Lonicera fragrantissima* Lindl. & Paxton (Caprifoliaceae), Abbott 11364 (FLAS), 1998, 15, 0, 0, 1, 2; *Pennycastis americana* (Nutt.) Fenzl ex Walp. (Caryophyllaceae), Abbott 3655 (FLAS), 1992, 21, 1, 3, 1, 2; *Peronychia errecta* (Chapm.) Shinners (Caryophyllaceae), Abbott 10806 (FLAS), 1997, 16, 0, 0, 1, 2; *Maytenus Molinae* (Celastraceae), Abbott 19618 (FLAS), 2004, 9, 1, 3, 2, 1; *Lechea* L. (Cistaceae), Abbott 3637 (FLAS), 1992, 21, 0, 0, 0, 1; *Lechea* L. (Cistaceae), Abbott 17804 (FLAS), 2003, 10, 0, 1, 1, 1; *Lechea* L. (Cistaceae), Abbott 17799 (FLAS), 2003, 10, 0, 0, 0, 1; *Lechea* tenuifolia Michx. (Cistaceae), Abbott 14380 (FLAS), 2001, 12, 0, 0, 1, 2; *Polygonia tenuifolia* Turr. & A. Gray (Cleomaceae), Abbott 16807 (FLAS), 1997, 16, 0, 0, 1, 2; *Clethra alnifolia* L. (Clethraceae), Abbott 10794 (FLAS), 1997, 16, 0, 0, 1, 2; *Laguncularia racemosa* (L.) C.E. Gaertn. (Combretaceae), Abbott 17792 (FLAS), 2003, 10, 0, 0, 0, 1; *Merremia dissecta* (Jacq.) Hallier f. (Convolvulaceae), Abbott 3793 (FLAS), 1992, 21, 0, 0, 1, 2; *Melothria pendula* L. (Cucurbitaceae), Abbott 3794 (FLAS), 1992, 21, 0, 0, 1, 2; *Juniperus virginiana* L. (Cupressaceae), Abbott 1823 (FLAS), 1992, 21, 0, 0, 1, 2; *Metasequoia glyptostroboides* Hu & W.C. Cheng (Cupressaceae), Abbott 11367 (FLAS), 1998, 15, 0, 0, 0, 1; *Cycas circinalis* L. (Cycadaceae), Abbott 10896 (FLAS), 1997, 16, 0, 0, 1, 2; *Bulbostylis* Kunth (Cyperaceae), Abbott 17797 (FLAS), 2003, 10, 0, 0, 1, 2; *Carex cephalophora* Muhl. ex Willd. (Cyperaceae), Abbott 6700 (FLAS), 1994, 19, 1, 1, 1, 1; *Carex festucacea* Schlecht ex Willd. (Cyperaceae), Abbott 6701 (FLAS), 1994, 19, 0, 1, 1, 1; *Carex lupuliniformis* Sartwell ex Dewey (Cyperaceae), Abbott 3211 (FLAS), 1992, 21, 0, 0, 0, 1; *Carex* L. (Cyperaceae), Abbott 3647 (FLAS), 1992, 21, 0, 0, 1, 2; *Cyperus croceus* Vahl (Cyperaceae), Abbott 14370 (FLAS), 2001, 12, 1, 1, 1, 2; *Cyperus distinctus* Steud. (Cyperaceae), Abbott 3620 (FLAS), 1992, 21, 0, 0, 0, 1; *Cyperus filicinus* Vahl (Cyperaceae), Abbott 10810 (FLAS), 1997, 16, 0, 0, 0, 1; *Cyperus iria* L. (Cyperaceae), Abbott 3212 (FLAS), 1992, 21, 0, 0, 0, 1; *Pyrus polynychia* (Rothb.) P. Beauvois (Cyperaceae), Abbott 3644 (FLAS), 1992, 21, 0, 0, 0, 1; *Cyperus surinamensis* Rothb. (Cyperaceae), Abbott 3678 (FLAS), 1992, 21, 0, 0, 0, 1; *Eleocharis radicans* (Poir.) Kunth (Cyperaceae), Abbott 14846 (FLAS), 2002, 11, 1, 1, 1, 2; *Fimbristylis* Vahl (Cyperaceae), Abbott 3625 (FLAS), 1992, 21, 0, 0, 0, 1; *Fuirena squarrosa* Michx. (Cyperaceae), Abbott 3658 (FLAS), 1992, 21, 0, 0, 1, 2; *Kyllinga brevifolia* Rorth. (Cyperaceae), Abbott 3614 (FLAS), 1992, 21, 0, 0, 0, 1; *Rhynchospora colorata* (L.) H. Pfeiff. (Cyperaceae), Abbott 3628 (FLAS), 1992, 21, 0, 0, 0, 1; *Rhynchospora colorata* (L.) H. Pfeiff. (Cyperaceae), Abbott 1790 (FLAS), 1992, 21, 0, 0, 0, 1; *Rhynchospora inexpansa* (Michx.) Vahl (Cyperaceae), Abbott 14374 (FLAS), 2001, 12, 0, 0, 1, 2; *Rhynchospora* Vahl (Cyperaceae), Abbott 3717 (FLAS), 1992, 21, 0, 0, 0, 1; *Rhynchospora* Vahl (Cyperaceae), Abbott 3623 (FLAS), 1992, 21, 0, 0, 0, 1; *Rhynchospora* Vahl (Cyperaceae), Abbott 3648 (FLAS), 1992, 21, 0, 0, 0, 1; *Clifnia monophylla* (Lam.) Brinton ex Sang. (Cyrtaceae), Abbott 10797 (FLAS), 1997, 16, 0, 0, 0, 1; *Clifnia monophylla* (Lam.) Brinton ex Sang. (Cyrtaceae), Abbott 6686 (FLAS), 1994, 19, 0, 0, 1, 2; *Diospyros virginiana* L. (Ebenaceae), Abbott 3217 (FLAS), 1992, 21, 0, 0, 0, 1; *Chimaphila umbellata* (L.) Presl (Ericaceae), Abbott 11371 (FLAS), 1998, 15, 0, 0, 1, 2; *Gaultheria dumosa* (Andrews) A. Gray (Ericaceae), Abbott 6682 (FLAS), 1994, 19, 0, 0, 0, 1; *Gaultheria dumosa* (Andrews) A. Gray (Ericaceae), Abbott 6685 (FLAS), 1994, 19, 0, 0, 0, 1; *Gaultheria frondosa* (L.) Torr. & A. Gray (Ericaceae), Abbott 6684 (FLAS), 1994, 19, 0, 0, 0, 1; *Leucanthus racemosus* (L.) A. Gray (Ericaceae), Abbott 6687 (FLAS), 1994, 19, 0, 1, 2, 3; *Rhododendron* L. (Ericaceae), Abbott 6688 (FLAS), 1994, 19, 0, 1, 1, 2; *Vaccinium arboreum* Marshall (Ericaceae), Abbott 6596 (FLAS), 1994, 19, 0, 0, 0, 1; *Vaccinium myrsinites* Lam. (Ericaceae), Abbott 6691 (FLAS), 1994, 19, 0, 0, 0, 1; *Zenobia pulverulenta* (W. Bartram ex Willd.) Pollard (Ericaceae), Abbott 14375 (FLAS), 2001, 12, 0, 0, 0, 1; *Eriocaulon compressum* Lam. (Eriocaulaceae), Abbott 10787 (FLAS), 1997, 16, 0, 0, 0, 1; *Lachnocalyx anceps* (Walpers) Morong (Eriocaulaceae), Abbott 3612 (FLAS), 1992, 21, 0, 0, 0, 1; *Syngonanthus flavidulus* (Michx.) Ruhland (Eriocaulaceae), Abbott 6677 (FLAS), 1994, 19, 0, 0, 0, 1; *Erythroxylum coca* Lam. (Erythroxylaceae), Abbott 14376 (FLAS), 2001, 12, 0, 0, 1, 2; *Acalypha gracilens* A. Gray (Euphorbiaceae), Abbott 3788 (FLAS), 1992, 21, 0, 0, 0, 1; *Euphorbia hypericifolia* L. (Euphorbiaceae), Abbott 1768 (FLAS), 1992, 21, 0, 0, 0, 1; *Cnidocalyx wrenii* (L.) Arthur (Euphorbiaceae), Abbott 3796 (FLAS), 1992, 21, 0, 0, 1, 2; *Crotalaria micrantha* G.L. Webster (Euphorbiaceae), Abbott 3639 (FLAS), 1992, 21, 0, 0, 0, 1; *Euphorbia cyathophora* Murray (Euphorbiaceae), Abbott 6705 (FLAS), 1994, 19, 1, 1, 1, 2; *Euphorbia floridana* Chapm. (Euphorbiaceae), Abbott 10808 (FLAS), 1997, 16, 0, 0, 0, 1; *Sapium sebiferum* (L.) Roxb. (Euphorbiaceae), Abbott 3801 (FLAS), 1992, 21, 0, 0, 1, 2; *Amorpha fruticosa* L. (Fabaceae), Abbott 3206 (FLAS), 1992, 21, 0, 0, 0, 1; *Dalbergia erianthophyllum* (L.) Taub. (Fabaceae), Abbott 17791 (FLAS), 2003, 10, 1, 1, 1, 2; *Desmanthus* Willd. (Fabaceae), Abbott 3218 (FLAS), 1992, 21, 1, 1, 2, 3; *Lupinus perennis* L. (Fabaceae), Abbott 10814 (FLAS), 1997, 16, 0, 1, 2, 3; *Pongamia pinnata* (L.) Pierre (Fabaceae), Abbott 17902 (FLAS), 2003, 10, 0, 0, 1, 2; *Rhynchospora cyathidea* (Bergot.) Wilbur (Fabaceae), Abbott 10815 (FLAS), 1997, 16, 0, 0, 0, 1; *Vicia acutifolia* Elliott (Fabaceae), Abbott 6695 (FLAS), 1994, 19, 0, 0, 1, 2; *Quercus pumila* Walp. (Fagaceae), Abbott 10802 (FLAS), 1997, 16, 0, 0, 0, 1; *Quercus* L. (Fagaceae), Abbott 17802 (FLAS), 2003, 10, 0, 0, 1, 2; *Sabicea compundata* (L.) Torr. (Gentianaceae), Abbott 3633 (FLAS), 1992, 21, 0, 0, 0, 0; *Sabicea grandiflora* (A. Gray) Small (Gentianaceae), Abbott 3643 (FLAS), 1992, 21, 0, 0, 0, 1; *Sabicea grandiflora* (A. Gray) Small (Gentianaceae), Abbott 3639 (FLAS), 1992, 21, 0, 0, 0, 0; *Lachnanthes caroliniana* (Lam.) Dandy (Haemodoraceae), Abbott 10795 (FLAS), 1997, 16, 1, 1, 1, 1; *Lachnanthes caroliniana* (Lam.) Dandy (Haemodoraceae), Abbott 3633 (FLAS), 1992, 21, 0, 0, 0, 1; *Primula palustris* L. (Hedysaraceae), Abbott 1767 (FLAS), 1992, 21, 0, 0, 0, 1; *Egeria densa* Planch. (Hydrocharitaceae), Abbott 3789 (FLAS), 1992, 21, 0, 0, 1, 2; *Hypericum hypericoides* (L.) Crantz (Hypericaceae), Abbott 6689 (FLAS), 1994, 19, 0, 1, 1, 2; *Hypericum suffruticous* W.P. Adams & N. Robson (Hyper-

- caceae), Abbott 3622 (FLAS), 1992, 21, 0, 0, 0, 1; *Hypericum temperatum* Lam. (Hypericaceae), Abbott 3660 (FLAS), 1992, 21, 0, 0, 0, 1; *Crocos vernum* Hill (Iridaceae), Abbott 11369 (FLAS), 1998, 15, 0, 0, 0, 1; *Iseos flaccida* A. Braun (Isotaceae), Abbott 18460 (FLAS), 2003, 10, 0, 0, 0; *Carya floridana* Sarg. (Juglandaceae), Abbott 17801 (FLAS), 2003, 10, 0, 0, 2, 3; *Juncus megaphyllos* M.A. Curtis (Juncaceae), Abbott 1786 (FLAS), 1992, 21, 0, 0, 0, 1; *Calamintha ashii* (Weber) Shinners (Lamiaceae), Abbott 10805 (FLAS), 1997, 16, 1, 1, 2, 3; *Calamintha dentata* Chapman (Lamiaceae), Abbott 10804 (FLAS), 1997, 16, 0, 0, 1, 2; *Conradina* A. Gray (Lamiaceae), Abbott 17796 (FLAS), 2003, 10, 1, 1, 1, 1; (Lauraceae), Abbott 19143 (FLAS), 2004, 9, 1, 1, 1, 1; *Liriodendron tulipifera* (Planch.) Tid. (Lauraceae), Abbott 10796 (FLAS), 1997, 16, 0, 0, 1, 2; *Lindernia grandiflora* (Michx.) Pennell (Linderniaceae), Abbott 3214 (FLAS), 1992, 21, 0, 0, 0, 1; *Lindernia grandiflora* Nutt. (Linderniaceae), Abbott 3641 (FLAS), 1992, 21, 0, 0, 0, 1; *Mimulus glomeratus* (Chapm.) Shinners (Linderniaceae), Abbott 3611 (FLAS), 1992, 21, 0, 0, 0, 1; *Eucnide Zucc.* (Loasaceae), Abbott 19852 (FLAS), 2005, 8, 1, 1, 1, 2; *Mitrula petiolata* (Walter ex J.R. Gmel.) Torr. & A. Gray (Loganiaceae), Abbott 3616 (FLAS), 1992, 21, 0, 0, 0, 1; *Lythrum alatum* Pursh (Lythraceae), Abbott 3631 (FLAS), 1992, 21, 0, 0, 0, 1; *Gomphium hirtum* L. (Malvaceae), Abbott 3216 (FLAS), 1992, 21, 0, 0, 0, 1; *Melia azedarach* L. (Meliaceae), Abbott 3800 (FLAS), 1992, 21, 0, 0, 1, 2; *Corolla caroliniana* (L.) DC. (Meliaceae), Abbott 14382 (FLAS), 2001, 12, 0, 0, 1, 2; *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae), Abbott 3787 (FLAS), 1992, 21, 0, 0, 0, 1; *Lophiola americana* (Pursh) A. Wood (Nartheciaceae), Abbott 10798 (FLAS), 1997, 16, 0, 0, 0, 1; *Osmunda americanus* (L.) Benth. & Hook. f. ex A. Gray (Osmundaceae), Abbott 17795 (FLAS), 2003, 10, 0, 1, 1, 1; *Ludwigia peploides* (Kunth) P.H. Raven (Onagraceae), Abbott 3201 (FLAS), 1992, 21, 0, 1, 1, 1; *Oenothera biennis* Nutt. (Onagraceae), Abbott 17803 (FLAS), 2003, 10, 0, 0, 0, 1; *Ophioglossum undulatum* L. f. (*Ophioglossaceae*), Abbott 19180 (FLAS), 2004, 9, 0, 0, 1, 2; *Habenaria repens* Nutt. (Oncidaceae), Abbott 3651 (FLAS), 1992, 21, 0, 0, 0, 1; *Burkiera americana* L. (Orobanchaceae), Abbott 3624 (FLAS), 1992, 21, 0, 0, 0, 1; *Pauflou pallida* L. (Passifloraceae), Abbott 18103 (FLAS), 2003, 10, 1, 1, 1, 1; *Petrea cordata* Lundell (Petrenaceae), Abbott 19767 (FLAS), 2005, 8, 0, 0, 1, 2; *Astragalus jacobinensis* (Müll. Arg.) G.L. Webster (Phyllanthaceae), Abbott 16262 (FLAS), 1995, 18, 0, 0, 1, 2; *Bacopa caroliniana* (Walter) B.L. Rob. (Plantaginaceae), Abbott 3652 (FLAS), 1992, 21, 1, 1, 2, 3; *Gratiola hispida* (Benth. ex Lindl.) Pollard (Plantaginaceae), Abbott 3644 (FLAS), 1992, 21, 0, 0, 0, 1; *Linaria canadensis* (L.) Dum. Cours. (Plantaginaceae), Abbott 1794 (FLAS), 1992, 21, 0, 0, 0, 1; *Plantago virginica* L. (Plantaginaceae), Abbott 1806 (FLAS), 1992, 21, 0, 0, 0, 1; *Scoparia dulcis* L. (Plantaginaceae), Abbott 3636 (FLAS), 1992, 21, 0, 0, 0, 1; *Scoparia dulcis* L. (Plantaginaceae), Abbott 1771 (FLAS), 1992, 21, 0, 0, 0, 1; *Andropogon virginicus* L. (Poaceae), Abbott 3613 (FLAS), 1992, 21, 0, 0, 0, 1; *Cenchrus intertus* M.A. Curtis (Poaceae), Abbott 1818 (FLAS), 1992, 21, 0, 0, 0, 1; *Cynodon dactylon* (L.) Pers. (Poaceae), Abbott 1819 (FLAS), 1992, 21, 0, 0, 1, 2; *Dianthus barbatus* (Trin.) Freckmann & Lelong (Poaceae), Abbott 17805 (FLAS), 2003, 10, 1, 1, 2, 3; *Eryngium* Wolf (Poaceae), Abbott 3633 (FLAS), 1992, 21, 0, 0, 0, 1; *Eustachys* Desv. (Poaceae), Abbott 1821 (FLAS), 1992, 21, 0, 0, 1, 2; *Eustachys* Desv. (Poaceae), Abbott 3626 (FLAS), 1992, 21, 0, 0, 0, 1; *Colostachys longifolia* subsp. *rigida* (Bosc ex Nees) Soreng (Poaceae), Abbott 3629 (FLAS), 1992, 21, 0, 0, 0, 1; *Paspalum setigerum* Michx. (Poaceae), Abbott 3649 (FLAS), 1992, 21, 0, 0, 0, 1; *Paspalum L.* (Poaceae), Abbott 3615 (FLAS), 1992, 21, 0, 0, 0, 1; *Paspalum L.* (Poaceae), Abbott 3647 (FLAS), 1992, 21, 0, 0, 0, 1; *Paspalum urvillei* Steud. (Poaceae), Abbott 3619 (FLAS), 1992, 21, 0, 0, 0, 1; *Paspalum vaginatum* Sw. (Poaceae), Abbott 3627 (FLAS), 1992, 21, 0, 0, 0, 1; *Sacciolepis striata* (L.) Nash (Poaceae), Abbott 3621 (FLAS), 1992, 21, 0, 0, 0, 1; *Setaria parviflora* (Poir.) Kerguélen (Poaceae), Abbott 3215 (FLAS), 1992, 21, 0, 0, 0, 1; *Polygonum boykinii* Nutt. (Polygonaceae), Abbott 14371 (FLAS), 2001, 12, 0, 0, 0, 1; *Polygonum incarnatum* L. (Polygonaceae), Abbott 14379 (FLAS), 2001, 12, 0, 0, 1, 2; *Polygonum luteum* L. (Polygonaceae), Abbott 6678 (FLAS), 1994, 19, 0, 0, 0, 1; *Polygonum multiflorum* Torr. & A. Gray (Polygonaceae), Abbott 14373 (FLAS), 2001, 12, 0, 0, 1, 2; *Polygonum polygaloides* Walter (Polygonaceae), Abbott 14372 (FLAS), 2001, 12, 0, 0, 0, 1; *Polygonum verticillatum* L. (Polygonaceae), Abbott 14378 (FLAS), 2001, 12, 0, 0, 0, 1; *Anemone leprosa* Hook. & Arn. (Polygonaceae), Abbott 3786 (FLAS), 1992, 21, 0, 0, 0, 1; *Eriogonum tomentosum* Michx. (Polyponaceae), Abbott 10803 (FLAS), 1997, 16, 0, 0, 0, 1; *Ranunculus baldwinii* Baldwin (Polygonaceae), Abbott 6553 (FLAS), 1994, 19, 0, 0, 0, 1; *Oldenlandia uniformis* L. (Rubiaceae), Abbott 3654 (FLAS), 1992, 21, 0, 0, 0, 1; *Richardia brasiliensis* Gomes (Rubiaceae), Abbott 3802 (FLAS), 1992, 21, 1, 2, 1, 3; *Richardia brasiliensis* Gomes (Rubiaceae), Abbott 1795 (FLAS), 1992, 21, 0, 0, 0, 1; *Richardia scabra* L. (Rubiaceae), Abbott 3803 (FLAS), 1992, 21, 1, 1, 1, 2; *Spermacoce remota* Lam. (Rubiaceae), Abbott 1769 (FLAS), 1992, 21, 0, 0, 1, 2; *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae), Abbott 6711 (FLAS), 1994, 19, 1, 3, 1, 2; *Acer saccharinum* L. (Sapindaceae), Abbott 11366 (FLAS), 1998, 15, 0, 0, 1, 2; *Cardeispermum halicacabum* L. (Sapindaceae), Abbott 3219 (FLAS), 1992, 21, 0, 1, 2, 3; *Eucalyptus japonica* (Thunb. ex Roem. & Schult.) Dippel (Staphyleaceae), Abbott 14377 (FLAS), 2001, 12, 0, 0, 0, 1, 2; *Syrinx americanus* Lam. (Syringaceae), Abbott 6696 (FLAS), 1994, 19, 0, 0, 1, 2; *Polypteron procumbens* L. (Tetrachondraceae), Abbott 3642 (FLAS), 1992, 21, 1, 2, 1, 3; *Phyla nodiflora* (L.) Greene (Verbenaceae), Abbott 3635 (FLAS), 1992, 21, 0, 0, 0, 1; *Hybanthus* Jacq. (Violaceae), Abbott 19766 (FLAS), 2005, 8, 1, 3, 2, 1; *Viola pedata* L. (Violaceae), Abbott 14381 (FLAS), 2001, 12, 0, 0, 1, 2; *Ximenia americana* L. (Ximeniaceae), Abbott 17806 (FLAS), 2003, 10, 0, 0, 1, 2.
- B) Herbarium samples for age discrimination. *Artemisia* (Asteraceae) data set from herbarium specimens: *Artemisia incana* (W. Bartram) Exell, *Antoni-Dewald s.n.* (FLAS), 1984, 29, 1, 3, 1, 2; *A. incana*, *Arnold s.n.* (FLAS), 1941, 72, 0, 0, 1, 2; *A. incana*, *Bailey 35* (FLAS), 1991, 22, 0, 1, 1, 2; *A. incana*, *Buckner 340* (FLAS), 1991, 22, 0, 1, 2, 3; *A. incana*, *Carr 5352* (FLAS), 2001, 12, 0, 1, 1, 1; *A. incana*, *Cary E2* (FLAS), 1966, 47, 0, 1, 1, 2; *A. incana*, *Cooley & Eaton 6591* (FLAS), 1959, 54, 0, 0, 0, 1; *A. incana*, *D'Arey 1503* (FLAS), 1967, 46, 0, 0, 1, 2; *A. incana*, *D'Arey 1549* (FLAS), 1967, 46, 0, 0, 0, 1; *A. incana*, *DeLaney 3071c* (FLAS), 1998, 15, 0, 1, 1, 2; *A. incana*, *Eatley 765* (FLAS), 1988, 25, 1, 3, 1, 2; *A. incana*, *Equithus 33* (FLAS), 1988, 25, 1, 3, 1, 2; *A. incana*, *Haring & Hoffmann 4* (FLAS), 2007, 6, 1, 3, 2, 1; *A. incana*, *Herring 1160* (FLAS), 1993, 20, 1, 3, 2, 1; *A. incana*, *Herring 648* (FLAS), 1992, 21, 1, 1, 1, 2; *A. incana*, *Hornby s.n.* (FLAS), 1989, 24, 0, 1, 1, 2;

- A. incana*, Hume s.n. (FLAS), 1937, 76, 0, 0, 0, 1; *A. incana*, Kral 2277 (FLAS), 1956, 57, 0, 0, 0, 1; *A. incana*, Kral 2711 (FLAS), 1956, 57, 0, 0, 1, 2; *A. incana*, Layne s.n. (FLAS), 1893, 120, 0, 0, 0, 1; *A. incana*, Major 3035 (FLAS), 2008, 5, 1, 3, 1, 1; *A. incana*, Martin 1003 (FLAS), 1977, 36, 0, 0, 0, 1; *A. incana*, Martin 1009 (FLAS), 1977, 36, 0, 1, 1, 2; *A. incana*, Merrill s.n. (FLAS), 1925, 88, 0, 0, 0, 1; *A. incana*, Penney 1479 (FLAS), 2002, 11, 0, 0, 1, 2; *A. incana*, Shear 1602 (FLAS), 1985, 28, 0, 1, 1, 2; *A. incana*, Shear 1603 (FLAS), 1985, 28, 1, 3, 1, 2; *A. incana*, Tan 443 (FLAS), 1990, 23, 0, 0, 1, 2; *A. incana*, Viers 1 (FLAS), 1993, 20, 1, 3, 1, 2; *A. incana*, Ward 1232 (FLAS), 1959, 54, 0, 0, 0, 1; *A. incana*, Ward 3442 (FLAS), 1963, 50, 0, 0, 0, 1; *A. incana*, Ward 4502 (FLAS), 1965, 48, 0, 0, 0, 1; *A. incana*, Ward 4557 (FLAS), 1965, 48, 1, 0, 0, 1; *A. incana*, West & Arnold s.n. (FLAS), 1941, 72, 0, 0, 0, 1; *A. incana*, West & Arnold s.n. (FLAS), 1946, 67, 0, 0, 0, 1; *A. incana*, West & Arnold s.n. (FLAS), 1949, 73, 0, 0, 0, 1; *A. incana*, West s.n. (FLAS), 1939, 74, 0, 0, 0, 1; *A. incana*, Wiggins 1972 (FLAS), 1965, 48, 0, 1, 1, 2; *A. longifolia*, Arnold & West s.n. (FLAS), 1946, 67, 0, 1, 1, 2; *A. longifolia*, Arnold & West s.n. (FLAS), 1937, 76, 0, 0, 0, 1; *A. longifolia*, Arnold & West s.n. (FLAS), 1935, 78, 0, 1, 2, 3; *A. longifolia*, Arnold s.n. (FLAS), 1931, 82, 0, 0, 1, 2; *A. longifolia*, Beckner 1253 (FLAS), 1966, 47, 0, 1, 2, 3; *A. longifolia*, Bryan s.n. (FLAS), 1985, 28, 0, 1, 1, 2; *A. longifolia*, Gulledge 313 (FLAS), 1997, 16, 1, 3, 1, 3; *A. longifolia*, Herring 728 (FLAS), 1992, 21, 0, 1, 1, 2; *A. longifolia*, Kobayashi 59 (FLAS), 2001, 12, 1, 3, 2, 1; *A. longifolia*, Kral s.n. (FLAS), 1933, 80, 0, 0, 0, 1; *A. longifolia*, Martin 1042 (FLAS), 1977, 36, 0, 1, 1, 1; *A. longifolia*, Martin 1128 (FLAS), 1977, 36, 0, 1, 1, 2; *A. longifolia*, Orzell & Bridges 20040 (FLAS), 1992, 21, 1, 3, 2, 1; *A. longifolia*, Penney 1116 (FLAS), 1998, 15, 1, 3, 2, 1; *A. longifolia*, Shear 974 (FLAS), 1982, 31, 0, 1, 1, 2; *A. longifolia*, Smith 1505 (FLAS), 1967, 46, 0, 1, 2, 3; *A. longifolia*, Smith 1720 (FLAS), 1976, 37, 0, 1, 1, 2; *A. longifolia*, Tan 608 (FLAS), 1990, 23, 1, 2, 2, 1; *A. longifolia*, Viers 50 (FLAS), 1993, 20, 1, 3, 2, 1; *A. longifolia*, Weber s.n. (FLAS), 1927, 86, 0, 1, 2, 3; *A. longifolia*, West & Arnold s.n. (FLAS), 1941, 72, 0, 1, 1, 1; *A. longifolia*, Wiggins 19748 (FLAS), 1965, 48, 0, 1, 1, 2; *A. longifolia*, Wilmer s.n. (FLAS), 1945, 68, 0, 0, 0; *A. obovata* (Willd.) Nash, Baltzell 964 (FLAS), 1969, 44, 0, 1, 1, 2; *A. obovata*, Baltzell 10686 (FLAS), 1979, 34, 0, 1, 1, 2; *A. obovata*, Baltzell 8269 (FLAS), 1976, 37, 0, 1, 1, 1; *A. obovata*, Beckner 2325 (FLAS), 1969, 44, 0, 1, 1, 1; *A. obovata*, Bradley 1622 (FLAS), 1998, 15, 1, 3, 2, 1; *A. obovata*, Bouz 14757 (FLAS), 1945, 68, 0, 0, 1, 2; *A. obovata*, Christian 1677 (FLAS), 1987, 26, 0, 1, 1, 1; *A. obovata*, Christian 381 (FLAS), 1986, 27, 1, 3, 1, 1; *A. obovata*, Christian 477 (FLAS), 1986, 27, 0, 1, 1, 1; *A. obovata*, Christian 527 (FLAS), 1986, 27, 0, 1, 1, 1; *A. obovata*, Christian 599 (FLAS), 1986, 27, 0, 1, 1, 3; *A. obovata*, Christian 890 (FLAS), 1986, 27, 0, 1, 1, 1; *A. obovata*, Courard s.n. (FLAS), 1963, 50, 0, 1, 1, 2; *A. obovata*, Judd 2776 (FLAS), 1980, 33, 1, 3, 1, 1; *A. obovata*, Kral 2444 (FLAS), 1956, 57, 0, 1, 1, 2; *A. obovata*, Martin & Cooper 430 (FLAS), 1975, 38, 0, 0, 1, 1; *A. obovata*, Martin & Cooper 461 (FLAS), 1975, 38, 0, 1, 1, 1; *A. obovata*, Orzell & Bridges 16657 (FLAS), 1991, 22, 1, 3, 2, 1; *A. obovata*, Ward 29 (FLAS), 1960, 53, 0, 1, 1, 2; *A. obovata*, Wilson & Merrill s.n. (FLAS), 1939, 74, 0, 1, 2, 3; *A. obovata*, Weber s.n. (FLAS), 1936, 77, 0, 0, 1, 2; *A. obovata*, West & Arnold s.n. (FLAS), 1946, 67, 0, 0, 1, 2; *A. obovata*, Zerba s.n. (FLAS), 1988, 25, 1, 3, 1, 1; *A. obovata*, Zone 36 (FLAS), 1982, 31, 1, 3, 1, 1; *A. parviflora* (Michx.) Dunal, Bickner s.n. (FLAS), 1977, 36, 0, 1, 2, 2; *A. parviflora*, Ford & West s.n. (FLAS), 1959, 54, 0, 1, 2, 2; *A. parviflora*, Herring 381 (FLAS), 1991, 22, 1, 3, 2, 1; *A. parviflora*, Howard s.n. (FLAS), 2005, 8, 1, 3, 2, 1; *A. parviflora*, Judd 3296 (FLAS), 1983, 30, 0, 1, 1, 2; *A. parviflora*, Malone 65 (FLAS), 1979, 34, 0, 1, 1, 1; *A. parviflora*, Morris 50 (FLAS), 1991, 22, 0, 1, 1, 1; *A. parviflora*, Tan 716 (FLAS), 1990, 23, 1, 3, 2, 1; *A. parviflora*, Thomas 110781 (FLAS), 1989, 24, 0, 0, 0, 0; *A. pygmaea* (W. Bartram) Dunal, Amoenus 638 (FLAS), 1991, 22, 1, 3, 1, 2; *A. pygmaea*, Baltzell 8407 (FLAS), 1976, 37, 0, 0, 0, 1; *A. pygmaea*, Beckner 1196 (FLAS), 1966, 47, 0, 1, 2, 3; *A. pygmaea*, Curtis 6129 (FLAS), 1898, 115, 0, 0, 0, 1; *A. pygmaea*, Easley 763 (FLAS), 1988, 25, 1, 3, 2, 1; *A. pygmaea*, Hornby s.n. (FLAS), 1989, 24, 1, 1, 2, 3; *A. pygmaea*, Matthew & Scanlon 117 (FLAS), 2000, 13, 1, 3, 1, 1; *A. pygmaea*, Orzell & Bridges 13878 (FLAS), 1990, 23, 1, 1, 1; *A. pygmaea*, Orzell & Bridges 199909 (FLAS), 1992, 21, 1, 3, 2, 1; *A. pygmaea*, West s.n. (FLAS), 1928, 85, 0, 0, 1, 2; *A. reticulata* Steyermark ex Chapm., Arnold & West s.n. (FLAS), 1935, 78, 0, 0, 0, 1; *A. reticulata*, Baltzell 3935 (FLAS), 1972, 41, 0, 1, 2, 3; *A. reticulata*, Baltzell 5803 (FLAS), 1974, 39, 0, 1, 2, 3; *A. reticulata*, Beckner 1739 (FLAS), 1967, 46, 0, 1, 2, 3; *A. reticulata*, Christmas 1678 (FLAS), 1987, 26, 0, 1, 2, 3; *A. reticulata*, Christmas 1722 (FLAS), 1987, 26, 0, 1, 2, 3; *A. reticulata*, Christmas 351 (FLAS), 1986, 27, 0, 1, 2, 3; *A. reticulata*, Cox LWR36 (FLAS), 2002, 11, 1, 3, 2, 1; *A. reticulata*, Easley 764 (FLAS), 1988, 25, 0, 1, 1, 2; *A. reticulata*, Goldman 2768 (FLAS), 2004, 9, 1, 3, 1, 2; *A. reticulata*, Hume & West s.n. (FLAS), 1936, 77, 0, 0, 0, 2; *A. reticulata*, Kobayashi 285 (FLAS), 2001, 12, 1, 3, 2, 1; *A. reticulata*, Kral 2068 (FLAS), 1956, 57, 0, 0, 1, 2; *A. reticulata*, Kral 2124 (FLAS), 1956, 57, 0, 1, 2, 3; *A. reticulata*, Loupeich 377 (FLAS), 1978, 35, 0, 0, 1, 2; *A. reticulata*, Scanlon 182 (FLAS), 2001, 12, 1, 3, 2, 1; *A. reticulata*, Scanlon 322 (FLAS), 2002, 11, 0, 1, 2, 3; *A. reticulata*, van Hook & Wargo 872 (FLAS), 1997, 16, 0, 1, 2, 3; *A. reticulata*, Ward 8-70 (FLAS), 1961, 52, 0, 1, 1, 1; *A. reticulata*, Zamiefer 712 (FLAS), 1999, 14, 1, 3, 2, 1; *A. tetramera* Small, Kral 2517 (FLAS), 1956, 57, 0, 1, 1, 1; *A. tetramera*, Orzell & Bridges 16912 (FLAS), 1991, 22, 1, 3, 2, 1; *A. triloba* (L.) Dunal, Abbott 1935 (FLAS), 1992, 21, 1, 2, 1, 3; *A. triloba*, Christian 1844 (FLAS), 1960, 53, 0, 1, 1, 2; *Mikania* (Asteraceae) data set from herbarium specimens: *Mikania* Willd., Aymard 6231 (FLAS), 1987, 24, 0, 0, 0, 1; *Mikania* Willd., Cox 79897 (MO), 1997, 14, 1, 3, 1, 1; *Mikania* Willd., Haukebach 71975 (MO), 2001, 10, 1, 1, 1, 1; *Mikania* Willd., Huysse 1811 (MO), 2005, 6, 1, 3, 1, 1; *Mikania* Willd., Lewis 578 (MO), 1993, 18, 1, 3, 1, 0; *Mikania* Willd., Morris 1841 (MO), 1996, 15, 1, 3, 2, 1; *Mikania* Willd., Pruski 4043 (MO), 2006, 5, 1, 1, 1, 1; *Mikania* Willd., Ritter R3099 (MO), 1976, 35, 0, 0, 0, 1; *Mikania* Willd., Ritter R3137 (MO), 1976, 35, 1, 1, 2, 2; *Mikania* australis R.M. King & H. Rob., Harley H50729 (MO), 1992, 19, 1, 1, 1, 1; *M. australis* (DC.) Macbride, Pedersen 13884 (MO), 1984, 27, 1, 3, 1, 1; *M. argentea* DC., Wauan 8627 (MO), 1992, 19, 1, 1, 1, 1; *M. argentea*, Zandie 7341 (MO), 1988, 23, 1, 1, 1, 1; *M. banisteriae* DC., Acero-Do-Rio 7596 (MO), 1995, 16, 0, 0, 1, 1; *M. banisteriae*, Bernaldo 1121 (MO), 1987, 24, 0, 1, 1, 2; *M. banisteriae*, Dryer 1736 (MO), 1978, 33, 0, 0, 0, 1; *M. banisteriae*, Galdeira 2418 (MO), 1996, 15, 0, 0, 0, 1; *M. banisteriae*, Kremser

- 3065 (MO), 1988, 23, 1, 1, 1; *M. banisteriae*, *Roxas* 2326 (MO), 2004, 7, 1, 1, 1; *M. banisteriae*, *Silva* 489 (MO), 1988, 23, 1, 1, 1; *M. baturifolia* DC., *Lewis* 7167 (MO), 1968, 43, 0, 1, 2, 3; *M. burchellii* Baker, *Hatschbach* 46842 (MO), 1983, 28, 0, 0, 0, 1; *M. campanulata* Gardner, *Lam* 10233 (MO), 1994, 17, 1, 1, 1, 1; *M. capensis* DC., *Banda* 3457 (MO), 1989, 22, 0, 0, 0, 1; *M. capensis*, *Chapman* 9249 (MO), 1988, 23, 0, 0, 1, 1; *M. capensis*, *Chikwui* 168 (MO), 1989, 22, 0, 1, 2, 3; *M. capensis*, *Gentry* 11396 (MO), 1974, 37, 0, 0, 1, 1; *M. capensis*, *Goldsblatt* 12526 (MO), 2004, 7, 1, 3, 1, 1; *M. capensis*, *Lowry* 4305 (MO), 1987, 24, 0, 1, 1, 2; *M. chenopodiifolia* Willd., *Croat* 53482 (MO), 1981, 30, 0, 0, 0, 1; *M. chenopodiifolia*, *Fenzl* 2139 (MO), 2005, 6, 1, 3, 1, 1; *M. chenopodiifolia*, *Fenzl* 958 (MO), 2001, 10, 1, 1, 1; *M. chenopodiifolia*, *Jacques-Georges* 16463 (MO), 1958, 53, 0, 0, 0, 1; *M. chenopodiifolia*, *Kocher* 22388 (MO), 1999, 12, 1, 3, 1, 1; *M. chenopodiifolia*, *Merville* 1356 (MO), 1996, 15, 1, 3, 1, 1; *M. chenopodiifolia* (C.D. Adams) W.C. Holmes & McDaniel, *Adam* 30009 (MO), 1975, 36, 0, 0, 0, 1; *M. chenopodiifolia*, *Jacques-Georges* 25130 (MO), 1969, 42, 0, 1, 1, 2; *M. chlorolepis* Baker, *Hans* 9332 (MO), 1905, 106, 0, 0, 0, 1; *M. chlorolepis*, *Zuluaga* 5537 (MO), 1996, 15, 0, 1, 1, 1; *M. congesta* DC., *King* 10285 (MO), 1992, 19, 1, 1, 2, 3; *M. congesta*, *Liebm* 32923 (MO), 1982, 29, 0, 0, 1, 2; *M. congesta*, *Nee* 44166 (MO), 1993, 18, 1, 1, 1, 1; *M. congesta*, *Ram* SAN 48 (MO), 1992, 19, 1, 1, 2; *M. congesta*, *Sovit* 4586 (MO), 1991, 20, 1, 1, 1, 1; *M. condana* (Burm. f.) B.L. Rob., *Adams* 30066 (MO), 1975, 36, 1, 1, 2, 3; *M. condana*, *Baylis* 10174 (MO), 1985, 26, 0, 1, 1, 1; *M. condana*, *Cedra* 10 (MO), 1983, 28, 0, 0, 1, 2; *M. condana*, *Carvalho* 3686 (MO), 1988, 23, 1, 3, 1, 1; *M. condana*, *Carvalho* 4167 (MO), 1989, 22, 1, 1, 1, 1; *M. condana*, *Chikwui* 183 (MO), 1989, 22, 0, 0, 1, 2; *M. condana*, *Kennedy* 38 (MO), 1984, 27, 0, 0, 0, 0; *M. condana*, *Korren* 14 (MO), 1971, 40, 0, 1, 1, 1; *M. condana*, *Phillips* 1529 (MO), 1976, 35, 0, 1, 2, 3; *M. condana*, *Pfe* 2656 (MO), 1981, 30, 0, 0, 0, 0; *M. condana*, *Rusakurindore* 1129 (MO), 1985, 28, 0, 0, 1, 2; *M. condana*, *Tawakali* 1555 (MO), 1989, 22, 1, 1, 2, 3; *M. cordifolia* (L. f.) Willd., *Alipi* 1139 (MO), 1998, 13, 1, 3, 1, 1; *M. cordifolia*, *Annuo-M* 2838 (MO), 2006, 5, 1, 3, 1, 2; *M. cordifolia*, *Apuard* 5246 (FLAS), 1987, 24, 0, 0, 0, 1; *M. cordifolia*, *Cristobal* 2248 (MO), 1992, 19, 1, 1, 1, 1; *M. cordifolia*, *Croat* 50966 (MO), 1980, 31, 1, 3, 1, 1; *M. cordifolia*, *Fisher-Marrow* 805 (FLAS), 1981, 30, 0, 0, 1, 2; *M. cordifolia*, *Jimenes* 64 (FLAS), 2000, 11, 0, 1, 2, 3; *M. cordifolia*, *King* 10463 (MO), 1993, 18, 1, 1, 1, 1; *M. cordifolia*, *Liemer* 1534 (MO), 1974, 37, 0, 0, 1; *M. cordifolia*, *Linnex* 598 (MO), 2006, 5, 1, 3, 2, 1; *M. cordifolia*, *Lott* 3571 (FLAS), 1991, 20, 1, 1, 1, 2; *M. cordifolia*, *Miller* 5930 (MO), 1991, 20, 0, 1, 1, 1; *M. cordifolia*, *Nee* 48888 (MO), 1998, 13, 1, 3, 1, 1; *M. cordifolia*, *Schinini* 6875 (MO), 1973, 38, 0, 1, 2, 3; *M. cordifolia*, *Toledo* 13201 (MO), 1995, 16, 1, 3, 1, 1; *M. cordifolia*, *Velazquez* 27623 (MO), 1991, 20, 1, 1, 1, 2; *M. cordifolia*, *Watum* 11565 (MO), 1996, 15, 1, 3, 1, 1; *M. cordifolia*, *Watum* 5597 (MO), 1989, 22, 0, 0, 0, 1; *M. cordifolia*, *Zamani* 28005 (MO), 1983, 28, 0, 0, 0, 1; *M. cordifolia*, *Zardini* 2722 (MO), 1987, 24, 1, 3, 1, 2; *M. decumbens* Malme, *Davide* 111014 (MO), 1976, 35, 0, 0, 0, 1; *M. decumbens*, *Watum* 1396 (MO), 2002, 9, 1, 3, 1, 1; *M. decumbens*, *Pederen* 15003 (MO), 1988, 23, 0, 1, 1, 1; *M. decumbens*, *Pederen* 5053 (MO), 1959, 52, 0, 0, 1, 2; *M. elliptica* DC., *Thomae* 9716 (MO), 1993, 18, 0, 0, 0; *M. erythrophyllum* W.C. Holmes, *Wright* 46 (MO), 1982, 29, 0, 1, 1, 2; *M. fragilis* Urb., *King* 10625 (MO), 1995, 16, 0, 0, 0, 1; *M. fulva* (Hook. & Arn.) Baker, *Pederen* 13731 (MO), 1984, 27, 0, 1, 2, 3; *M. glomerata* Spreng., *Zardini* 6769 (MO), 1988, 23, 0, 0, 0, 1; *M. goyazensis* (B.L. Rob.) R.M. King & H. Rob., *King* 8280 (MO), 1980, 31, 0, 0, 0, 1; *M. guava* Bonpl., *Acosta* 92 (MO), 1999, 12, 1, 3, 1, 1; *M. guava*, *Jaramillo* 200 (MO), 1994, 17, 1, 1, 1, 1; *M. guava*, *McDaniel* 23735 (MO), 1980, 31, 0, 0, 0, 1; *M. guava*, *McDaniel* 692 (MO), 1982, 29, 1, 2, 1, 3; *M. guava*, *McPherson* 20723 (MO), 2008, 3, 1, 3, 1, 2; *M. guava*, *Rinach* V 5619 (MO), 1981, 30, 0, 0, 1, 2; *M. hirsutissima* DC., *Krapovickas* 44153 (MO), 1992, 19, 1, 1, 1, 1; *M. hirsutissima*, *Pederen* 15025 (MO), 1988, 23, 1, 1, 1, 2; *M. hirsutissima*, *Pederen* 6553 (MO), 1962, 49, 1, 1, 1, 1; *M. hirsutissima*, *Vascon* 863 (MO), 1987, 24, 1, 1, 1, 2; *M. hispidissima* Dušén, *Hatschbach* 54800 (MO), 1990, 21, 1, 1, 1, 1; *M. hookeriana* DC., *Mari* 2102 (MO), 1974, 37, 0, 1, 1, 1; *M. hookeriana*, *Nee* 7019 (MO), 1973, 38, 0, 0, 0, 1; *M. hookeriana*, *Rinach* V 5597 (MO), 1981, 30, 1, 1, 1; *M. hookeriana*, *Rosa* 12639 (MO), 1995, 16, 1, 3, 1, 1; *M. hookeriana*, *Villa* 417 (MO), 2000, 11, 0, 0, 1, 1; *M. hookeriana*, *Webster* 23197 (MO), 1978, 33, 0, 1, 1, 1; *M. houstoniae* (L.) B.L. Rob., *Martinez* S. 8647 (MO), 1984, 27, 1, 3, 1, 1; *M. houstoniae* Hook. & Arn., *Rosato* 4540 (MO), 1988, 23, 0, 1, 2, 3; *M. involucrata*, *Watum* 10309 (MO), 1994, 17, 1, 3, 1, 1; *M. involucrata*, *Watum* 1559 (MO), 2002, 9, 1, 3, 1, 1; *M. invigata* Sch. Bip. ex Baker, *Romera* 4311 (MO), 1997, 14, 1, 3, 1, 1; *M. leucandra* Benth., *Croat* 13493 (MO), 1971, 40, 0, 0, 0, 1; *M. lepidophylla* Urb., *Zamani* 42928 (MO), 1989, 22, 0, 0, 0, 1; *M. ligustrifolia* DC., *Hatschbach* 24054 (MO), 1970, 41, 1, 3, 1, 1; *M. lindleyana*, *Vargas* 23947 (MO), 1997, 14, 1, 3, 1, 1; *M. lindleyana*, *Zardini* 5697 (MO), 1988, 23, 1, 3, 1, 2; *M. lindleyana*, *Zardini* 6485 (MO), 1988, 23, 1, 3, 1, 2; *M. lundiana* DC., *Hatschbach* 34322 (MO), 1974, 37, 0, 0, 1, 1; *M. micrantha* Kunze, *Anderson* SN (NA), 2011, 0, 1, 3, 0, 0; *M. micrantha*, *Aymard* 4491 (FLAS), 1986, 25, 0, 0, 0, 0; *M. micrantha*, *Aymard* 5351 (FLAS), 1987, 24, 0, 0, 0, 0; *M. micrantha*, *Bennet* 7539 (FLAS), 2004, 7, 0, 0, 0, 0; *M. micrantha*, *Berthelot* 1687 (MO), 2006, 5, 1, 1, 1, 2; *M. micrantha*, *Calvino* 26148 (MO), 1974, 37, 0, 1, 1, 1; *M. micrantha*, *Carter* 5184 (MO), 1986, 25, 0, 0, 0, 1; *M. micrantha*, *Castillo* M. 1604 (MO), 1992, 19, 0, 0, 0, 0; *M. micrantha*, *Correa* 414 (MO), 1967, 44, 0, 0, 0, 1; *M. micrantha*, *Degener* 15084 (MO), 1941, 70, 0, 0, 0, 1; *M. micrantha*, *Fortunato* 6838 (MO), 2000, 11, 1, 1, 1, 1; *M. micrantha*, *Garcia* 5352 (MO), 1993, 18, 0, 0, 0, 1; *M. micrantha*, *Gentry* 15160 (MO), 1975, 36, 0, 0, 0, 1; *M. micrantha*, *Haber* 10362 (MO), 1990, 21, 1, 1, 1, 1; *M. micrantha*, *Haber* 3720 (MO), 1985, 26, 0, 0, 0, 1; *M. micrantha*, *Hahn* 2327 (MO), 1984, 27, 0, 0, 0, 1; *M. micrantha*, *Hammel* 6162 (MO), 1980, 31, 1, 1, 1, 2; *M. micrantha*, *Harling* 26818 (MO), 1993, 18, 0, 0, 1, 1; *M. micrantha*, *Higgins* 94 (MO), 1994, 17, 0, 0, 0, 1; *M. micrantha*, *Haus* 1810 (MO), 1994, 17, 1, 1, 1, 1; *M. micrantha*, *Jaramillo* 266 (MO), 1994, 17, 0, 0, 0, 1; *M. micrantha*, *King* 10646 (MO), 1996, 15, 0, 0, 0, 1; *M. micrantha*, *King* 12106 (MO), 2002, 9, 1, 3, 1, 2; *M. micrantha*, *Kuning* 47019 (MO), 1983, 28, 0, 0, 1; *M. micrantha*, *Liemer* 487 (MO), 1973, 38, 0, 0, 0, 1; *M. micrantha*, *Luna* 19901 (MO), 1988, 23, 0, 3, 1, 2; *M. micrantha*, *Madrikian* 106 (MO), 1989, 22, 0, 0, 0, 1; *M. micrantha*, *Martinez* S. 31777 (MO), 1999, 12, 1, 3, 1, 1; *M. micrantha*, *Mason* 3412 (MO), 1980, 31, 0, 0, 0, 1; *M. micrantha*, *McDan-*

- et al* 17521 (MO), 1973, 38, 0, 1, 2, 3; *M. micrantha*, McPherson 6618 (MO), 1984, 27, 0, 0, 0, 1; *M. micrantha*, Melan 12122 (MO), 1997, 14, 1, 3, 1, 2; *M. micrantha*, Moreno 5309 (MO), 1980, 31, 0, 0, 0, 1; *M. micrantha*, Morrou 219 (MO), 1992, 19, 1, 1, 1, 1; *M. micrantha*, NA (MO), 2007, 4, 1, 3, 1, 1; *M. micrantha*, Nez 29308 (MO), 1984, 27, 0, 0, 0, 1; *M. micrantha*, Novelo R. 2249 (MO), 1998, 13, 1, 3, 1, 2; *M. micrantha*, Overholt MM04 (NA), 2011, 0, 1, 3, 2, 1; *M. micrantha*, Rio 90 (MO), 1984, 27, 0, 0, 0, 1; *M. micrantha*, Rousseau 187 (MO), 1982, 29, 0, 0, 0, 1; *M. micrantha*, Rav SAN297 (MO), 1992, 19, 1, 3, 1, 2; *M. micrantha*, Sandou 1485 (MO), 1993, 18, 0, 0, 0, 0; *M. micrantha*, Schmitz 24080 (MO), 1984, 27, 0, 0, 1, 2; *M. micrantha*, Schwarzel 221 (MO), 1981, 30, 0, 0, 0, 1; *M. micrantha*, Steury 6046 (MO), 1981, 30, 0, 0, 0, 1; *M. micrantha*, Villegas 24739 (MO), 1997, 14, 0, 0, 0, 1; *M. micrantha*, Villacorta 899 (MO), 1991, 20, 0, 0, 1, 1; *M. micrantha*, Wason 8397 (MO), 1992, 19, 1, 1, 2, 2; *M. micrantha*, Williams 1131 (MO), 1990, 21, 1, 1, 1, 1; *M. micrantha*, Zuk 3226 (MO), 1987, 24, 1, 1, 1, 1; *M. microcephala* DC., Heringer 18210 (MO), 1981, 30, 0, 0, 1, 2; *M. micropent* DC., Bidgood 4456 (MO), 2000, 11, 1, 3, 1, 1; *M. micropent*, Gobbo 362 (MO), 1999, 12, 1, 3, 1, 1; *M. micropent*, McDaniell 23283 (FLAS), 1980, 31, 0, 0, 0, 1; *M. maclellanii* DC., Benthall 10587 (MO), 1998, 13, 1, 3, 1, 1; *M. maclellanii*, Reid 1112 (MO), 1986, 25, 0, 0, 1, 2; *M. nitida* (DC.) R.M. King & H. Rob., Hind 60 (MO), 1992, 19, 0, 0, 1, 2; *M. numularia* DC., Nakajima 1885 (MO), 1996, 15, 1, 1, 1, 1; *M. oblongifolia* DC., Condeiro 1984 (MO), 2002, 9, 1, 3, 1, 1; *M. oblongifolia*, Kummerow 1784 (MO), 1982, 29, 1, 1, 1, 2; *M. oblongifolia*, Kummerow 3228 (MO), 1990, 21, 1, 3, 1, 1; *M. oblongifolia*, Nakajima 1320 (MO), 1995, 16, 1, 1, 1, 1; *M. oblongifolia*, Pachano 439 (MO), 2002, 9, 1, 3, 2, 1; *M. obsoleta* (Vell.) G.M. Barroso, Thomas 10453 (MO), 1994, 17, 1, 1, 1, 1; *M. obsoleta* DC., Hatchbach 51216 (MO), 1987, 24, 0, 0, 1, 1; *M. odontiniformis* Urb., Acevedo-Rodríguez 7056 (MO), 1994, 17, 0, 0, 0, 0; *M. odontiniformis*, Acevedo 9489 (MO), 1995, 16, 1, 3, 1, 1; *M. odontiniformis*, Haenke 9486 (MO), 1981, 30, 0, 0, 1, 1; *M. officinalis* Mart., Duen 2.n. (MO), 1914, 97, 0, 0, 1, 2; *M. officinalis*, Harley 27208 (MO), 1988, 23, 1, 3, 1, 1; *M. officinalis*, Harley H50721 (MO), 1992, 19, 1, 3, 1, 1; *M. officinalis*, Heringer 3429 (MO), 1980, 31, 0, 1, 1, 1; *M. officinalis*, Miranda 408 (MO), 2002, 9, 0, 0, 0, 1; *M. officinalis*, Phileax 3041 (MO), 1967, 44, 0, 0, 1, 2; *M. officinalis*, Romero 4590 (MO), 1997, 14, 1, 3, 2, 1; *M. officinalis*, Stannard H51776 (MO), 1992, 19, 1, 3, 1, 2; *M. pacchypylia* Urb., Acevedo 6221 (MO), 1993, 18, 1, 1, 1, 1; *M. pacchypylia*, Taylor 10899 (MO), 1992, 19, 1, 3, 1, 1; *M. palustris* (Gaertner) R.M. King & H. Rob., Irwin 32022 (MO), 1971, 40, 1, 1, 1, 1; *M. paniculata* DC., Hatchbach 51214 (MO), 1987, 24, 0, 0, 1, 2; *M. paniculata*, Hatchbach s.n. (MO), 1984, 27, 0, 1, 1, 1; *M. papillata* Klatt, Valeur 280 (MO), 1929, 82, 0, 0, 0, 1; *M. papillata*, Zamou 32452 (MO), 1984, 27, 0, 0, 0, 0; *M. paranaensis* Dušén, Wawan 1420 (MO), 2002, 9, 1, 3, 1, 1; *M. parodii* Cabrera, Pedersen 6940 (MO), 1964, 47, 0, 0, 1, 2; *M. parviflora* (Aubl.) H. Karst., Burnham 1770 (MO), 1998, 13, 1, 1, 1, 1; *M. parviflora*, Duarte 2687 (MO), 1950, 61, 0, 0, 1, 2; *M. parviflora*, McDaniell 24775 (MO), 1981, 30, 0, 0, 1, 2; *M. periplacifolia* Hoek & Ann., Cowan 4076 (MO), 1983, 28, 1, 3, 1, 1; *M. periplacifolia*, Fortunato 5957 (MO), 1998, 15, 1, 1, 1, 2; *M. periplacifolia*, Menela 5193 (MO), 1993, 18, 1, 1, 2, 3; *M. periplacifolia*, Zandui 36712 (MO), 1993, 18, 1, 3, 1, 1; *M. phaeocarpa* Mart., Irwin 29085 (MO), 1971, 40, 0, 0, 0, 1; *M. phaeocarpa*, Pinoni H51326 (MO), 1992, 19, 1, 1, 1, 2; *M. pohlii* (Baker) R.M. King & H. Rob., King 8320 (MO), 1980, 31, 0, 0, 0, 1; *M. porosa* Urb., Grimes 3234 (MO), 1992, 19, 1, 3, 2, 1; *M. products* Urb. & Ekman, Zamoni 19202 (MO), 1982, 29, 0, 3, 1, 1; *M. pseudorimachii* W.C. Holmes & McDaniell, Zamoni 13276 (MO), 1989, 22, 1, 1, 2, 3; *M. pulsatichya* DC., Crast 73274 (MO), 1992, 19, 1, 3, 1, 1; *M. pulsatichya*, Dwyer 7799 (MO), 1967, 44, 0, 0, 0, 1; *M. pulsatichya*, Heringer 7013 (MO), 1981, 30, 0, 0, 1, 2; *M. pulsatichya*, Nez 6679 (MO), 1973, 38, 0, 0, 0, 1; *M. pulsatichya*, Pereira 301 (MO), 1982, 29, 0, 1, 2, 3; *M. pulsatichya*, Ritter 1641 (MO), 1995, 16, 1, 3, 2, 1; *M. pulsatichya*, Smith 6295 (MO), 1984, 27, 0, 1, 1, 2; *M. pulsatichya*, Solomon 11719 (MO), 1984, 27, 0, 0, 1, 2; *M. purpureum* (Baker) R.M. King & H. Rob., Romero 1632 (MO), 1995, 16, 0, 1, 1, 2; *M. pyramidata* Doen. Sm., Lues 407 (MO), 1988, 23, 1, 1, 1, 1; *M. ramuliflora* Gardner, Pennington 327 (MO), 1982, 29, 0, 1, 1, 2; *M. ramuliflora*, Romero 3555 (MO), 1996, 15, 1, 3, 1, 1; *M. sagittifera* B.L. Rob., Long 706 (MO), 1987, 24, 1, 3, 1, 1; *M. sagittifera*, Remmier 5533 (MO), 1996, 15, 1, 3, 2, 1; *M. sagittifera*, Sitemi 1322 (MO), 2000, 11, 1, 1, 2, 3; *M. scandens* (L.) Willd., Abies 9448 (FLAS), 1996, 15, 1, 1, 1, 2; *M. scandens*, Alder 19323 (FLAS), 1956, 57, 0, 0, 0, 1; *M. scandens*, Amoros 463 (FLAS), 1993, 18, 1, 3, 1, 1; *M. scandens*, Anderson 12360 (FLAS), 1989, 22, 1, 3, 1, 2; *M. scandens*, Arnold s.n. (FLAS), 1940, 73, 0, 0, 0, 1; *M. scandens*, Baltzell 7267 (FLAS), 1975, 38, 0, 0, 0, 1; *M. scandens*, Beirne s.n. (FLAS), 2004, 7, 1, 3, 1, 2; *M. scandens*, Blomquist 5283 (FLAS), 1932, 81, 0, 0, 0, 1; *M. scandens*, Brat 15026 (FLAS), 1945, 68, 0, 0, 0, 1; *M. scandens*, Bredder 20637 (MO), 1971, 40, 0, 0, 0, 1; *M. scandens*, Bridges 23854 (MO), 1972, 39, 0, 0, 1, 2; *M. scandens*, Bridges 24190 (FLAS), 1995, 16, 1, 1, 1, 1; *M. scandens*, Brumback 628-32 (FLAS), 1932, 81, 0, 0, 0, 0; *M. scandens*, Bryson 4919 (FLAS), 1986, 25, 0, 1, 2, 3; *M. scandens*, Christman 16 (FLAS), 1964, 49, 0, 0, 0, 1; *M. scandens*, Craighead s.n. (FLAS), 1955, 58, 0, 0, 0, 1; *M. scandens*, Craighead s.n. (FLAS), 1956, 57, 0, 0, 0, 1; *M. scandens*, Cheager 264 (FLAS), 1963, 50, 0, 0, 0, 1; *M. scandens*, Crast 28936 (MO), 1973, 38, 0, 1, 1, 1; *M. scandens*, Cubanetti s.n. (FLAS), 1998, 15, 0, 0, 0, 1; *M. scandens*, D'Any 2165 (FLAS), 1967, 46, 0, 0, 0, 1; *M. scandens*, D'Any 8230 (MO), 1975, 36, 0, 0, 0, 1; *M. scandens*, Davis s.n. (FLAS), 1941, 72, 0, 0, 0, 1; *M. scandens*, Davis 157 (FLAS), 1978, 35, 0, 0, 0, 1; *M. scandens*, Easley 564 (FLAS), 1986, 27, 0, 0, 0, 1; *M. scandens*, Edwards 81 (FLAS), 2001, 10, 1, 3, 1, 1; *M. scandens*, Fleming 417 (FLAS), 1978, 35, 0, 0, 0, 1; *M. scandens*, Flórez 2 (FLAS), 1985, 26, 1, 1, 1, 2; *M. scandens*, Ford 182 (FLAS), 1934, 79, 0, 0, 0, 1; *M. scandens*, Ford 3127 (FLAS), 1954, 59, 0, 0, 0, 1; *M. scandens*, Fried 00-046 (FLAS), 2000, 11, 1, 3, 1, 1; *M. scandens*, Garland 204 (FLAS), 1983, 28, 1, 3, 1, 1; *M. scandens*, Godfrey 50059 (FLAS), 1949, 64, 0, 0, 0, 1; *M. scandens*, Gallego 112 (FLAS), 1996, 15, 1, 3, 1, 2; *M. scandens*, Hernandez M. 6828 (MO), 1981, 30, 0, 1, 1, 1; *M. scandens*, Herring 1621 (FLAS), 2002, 9, 1, 1, 1, 1; *M. scandens*, Herring 460 (FLAS), 1991, 20, 1, 1, 1, 2; *M. scandens*, Herring 70 (FLAS), 1990, 21, 0, 0, 0, 1; *M. scandens*, Hood 2386 (FLAS), 1949, 64, 0, 0, 0, 1; *M. scandens*, Hood 421 (FLAS), 1947, 66,

- 0, 0, 0, 1; *M. scandens*, *Ionta* 106 (FLAS), 2001, 10, 1, 3, 1, 1; *M. scandens*, *Jacomo* 793 (FLAS), 2011, 0, 1, 3, 0, 0; *M. scandens*, *King* 10593 (MO), 1993, 18, 1, 3, 1, 2; *M. scandens*, *Kirkman* 413 (FLAS), 1975, 36, 0, 0, 1, 2; *M. scandens*, *Krem* 1202 (FLAS), 1982, 29, 0, 0, 1, 2; *M. scandens*, *Ledin s.n.* (FLAS), 1946, 67, 0, 0, 0, 1; *M. scandens*, *Longbottom* 7992 (FLAS), 2006, 5, 1, 3, 2, 1; *M. scandens*, *Nes* 52873 (MO), 2004, 7, 1, 3, 1, 1; *M. scandens*, *Nelson* 24215 (FLAS), 2003, 8, 1, 1, 1, 1; *M. scandens*, *Newbig* 14-2003 (FLAS), 2003, 8, 1, 3, 1, 1; *M. scandens*, *Nouvel* 193 (MO), 1975, 36, 0, 0, 0, 1; *M. scandens*, *Orzell & Bridges* L2556 (FLAS), 1989, 22, 1, 3, 1, 2; *M. scandens*, *Rakotonandava* 419 (MO), 1995, 16, 0, 0, 0, 0; *M. scandens*, *Rogers* 682 (FLAS), 1986, 25, 0, 0, 1, 2; *M. scandens*, *Savio* 12 (FLAS), 2001, 10, 1, 3, 1, 2; *M. scandens*, *Scazon* 35 (FLAS), 2000, 11, 1, 1, 1, 1; *M. scandens*, *Schaez* 1457 (MO), 1987, 24, 1, 1, 1, 1; *M. scandens*, *Scull s.n.* (FLAS), 1937, 76, 0, 0, 0, 1; *M. scandens*, *Sherman* 266 (MO), 1991, 20, 1, 3, 2, 1; *M. scandens*, *Small* 6993 (FLAS), 1915, 98, 0, 0, 0, 1; *M. scandens*, *Small* 8240 (FLAS), 1917, 96, 0, 0, 0, 1; *M. scandens*, *Smith* 1731 (FLAS), 1967, 46, 0, 0, 0, 1; *M. scandens*, *Smith* 3831 (MO), 2003, 8, 1, 3, 1, 1; *M. scandens*, *Smith* 731 (FLAS), 1961, 52, 0, 0, 0, 1; *M. scandens*, *Smur* s.n. (FLAS), 2006, 5, 1, 3, 1; *M. scandens*, *Thomas* 145589 (MO), 1995, 16, 1, 3, 2, 1; *M. scandens*, *Tilley* 2382 (FLAS), 2001, 10, 1, 3, 1, 2; *M. scandens*, *Tilley* 2607 (FLAS), 2001, 10, 1, 3, 1, 2; *M. scandens*, *van Hoek* 712 (FLAS), 1996, 15, 0, 0, 1, 1; *M. scandens*, *van Montfrans* 95 (FLAS), 1978, 33, 0, 0, 1, 2; *M. scandens*, *Vincent* 573 (FLAS), 1976, 37, 0, 0, 1, 1; *M. scandens*, *Weber* s.n. (FLAS), 1935, 78, 0, 0, 0, 1; *M. scandens*, *West & Arnold* s.n. (FLAS), 1940, 73, 0, 0, 0, 1; *M. scandens*, *Wichterman* 41 (FLAS), 1971, 42, 0, 0, 0, 1; *M. scandens*, *Windler et al.* 4133 (FLAS), 1972, 41, 0, 0, 0, 1; *M. scandens*, *Wunderlin* 8547 (MO), 1979, 32, 0, 0, 0, 1; *M. schenckii* Hieron., *Iruia* 28933 (MO), 1971, 40, 0, 0, 0, 1; *M. semifolia* DC., *Romero* 4057 (MO), 1997, 14, 1, 3, 1, 1; *M. semifolia*, *Silva* 1074 (MO), 1992, 19, 0, 1, 1, 1; *M. semifolia*, *Silva* 579 (MO), 1989, 22, 1, 3, 1, 1; *M. sepiaria* Sch. Bip. ex Baker, *Cordaia* 1439 (MO), 1997, 14, 0, 1, 1, 1; *M. similicina* DC., *Heringer* 1915 (MO), 1979, 32, 0, 0, 1, 2; *M. strobilifera* Britton, *Liegier* 32692 (MO), 1982, 29, 0, 0, 1, 2; *M. strobilifera* Hieron., *Jorgensen* 1563 (MO), 1995, 16, 1, 3, 2, 1; *M. strobilifera*, *Jorgensen* 92370 (MO), 1990, 21, 1, 3, 1, 1; *M. strobilifera* Klaat, *Craat* 79917 (MO), 1997, 14, 1, 3, 2, 1; *M. strobilifera*, *Gentry* 5782 (MO), 1972, 39, 0, 0, 0, 1; *M. strobilifera*, *Haber* 10068 (MO), 1990, 21, 0, 0, 0, 1; *M. strobilifera*, *Tipaz* 1687 (MO), 1992, 19, 0, 1, 1, 1; *M. strobilifera*, *van der Werf* 12184 (MO), 1991, 20, 1, 1, 1, 1; *M. thapsoides* DC., *Romero* 4873 (MO), 1998, 13, 0, 0, 1, 1; *M. thapsoides*, *Zardini* 17159 (MO), 1989, 22, 0, 0, 0, 0; *M. tessellata* B.L. Rob., *Harms* 299 (MO), 1992, 19, 1, 1, 1; *M. tessellata*, *Manriquez* 3330 (MO), 1989, 22, 0, 1, 2, 3; *M. tenuipinnata* B.L. Rob., *Pedersen* 5975 (MO), 1961, 50, 0, 1, 1, 2; *M. trachylepis*, *Robinson* 1984 (MO), 1981, 30, 0, 1, 1, 1; *M. trachylepis*, *Robinson* 1989 (MO), 1989, 22, 1, 0, 0, 0; *M. tenuifolia* Hook. & Arn., *Caldera* 26189 (MO), 1975, 36, 1, 3, 1, 1; *M. tenuifolia* Hieron., *Pedersen* 13921 (MO), 1984, 27, 1, 3, 1, 2; *M. tenuifolia*, *Soria* 4209 (MO), 1990, 21, 1, 1, 1, 1; *M. tenuifolia* B.L. Rob., *Hanchbach* 26593 (MO), 1971, 40, 0, 1, 2, 3; *M. tenuifolia* DC., *Poerada* A. 3949 (MO), 1985, 26, 0, 0, 1, 2; *M. tenuifolia*, *Rodriguez* 4273 (MO), 1999, 12, 1, 3, 1, 1; *M. tenuifolia* Sch. Bip. ex Baker, *Nakajima* 1914 (MO), 1996, 15, 1, 1, 1, 1. Polygalaceae data set from herbarium specimens: *Acanthocladus guayaquilensis* B. Eriksen & B. Stahl, *Valecende* 1703 (MO), 1982, 30, 0, 0, 0, 1; *Arenaria acuminata* (Willd.) J.E.B. Pastore & J.R. Abbott, *Anaujo* 1198 (MO), 2004, 8, 1, 3, 1, 0; *A. acuminata*, *Anaujo* 1370 (MO), 2004, 8, 0, 0, 0, 0; *A. acuminata*, *Freira* 17 (MO), 2006, 7, 1, 1, 1, 1; *Arenaria Raf.*, *Purpur* 355 (MO), 2008, 5, 1, 3, 1, 2; *Arenaria Raf.*, *Sessens* 33039 (MO), 2012, 1, 1, 3, 1, 1; *Arenaria Raf.*, *Villarsel* 746 (MO), 2006, 7, 0, 0, 0, 0; *Arenaria violacea* (Aubl.) J.E.B. Pastore & J.R. Abbott, *Abbott* 25297 (FLAS), 2010, 2, 1, 3, 1, 1; *Arenaria violacea*, *Montes* 9582 (MO), 2003, 10, 0, 0, 0, 0; *Arenaria violacea*, *Raubik* 191 (MO), 1977, 35, 0, 0, 0, 1; *Arenaria apiculata* (Oliv.) Stapf, *Joungkind* 8275 (MO), 2008, 4, 1, 3, 1, 1; *Bredemeyera* Willd., *Pomida* 818 (MO), 2008, 4, 0, 1, 1, 1; *Bredemeyera colletisoides* (Phil.) Chodat, *Fortunatu* 5561 (MO), 1997, 16, 0, 0, 0, 1; *B. densiflora* A.W. Benn., *Chaque* 215 (MO), 2005, 7, 1, 3, 1, 1; *B. floribunda* Willd., *Chaque* 94 (MO), 2005, 7, 1, 3, 1, 1; *B. lucida* (Benth.) Klotzsch ex Hassk., *Steuwe* 32594 (MO), 2012, 1, 1, 3, 1, 2; *Caamembeca* J.E.B. Pastore, *Tenn* 3131 (MO), 2008, 5, 1, 1, 2, 3; *Caamembeca* J.E.B. Pastore, *Tenn* 3607 (MO), 2009, 4, 1, 1, 2, 3; *Caamembeca spectabilis* (DC.) J.E.B. Pastore, *Brummit* 19367 (MO), 1996, 16, 0, 1, 1, 1; *Carpolechia* G. Don, *Aleid* 689 (MO), 1999, 13, 1, 3, 1, 1; *Carpolechia* G. Don, *Fatu* 465 (MO), 1999, 13, 1, 1, 1, 1; *Carpolechia* G. Don, *Hizza* 175 (MO), 1998, 14, 0, 1, 1, 2; *Carpolechia* G. Don, *Kayombo* 5143 (MO), 2005, 7, 1, 3, 1, 1; *Carpolechia* G. Don, *Kludkens* 2599 (MO), 2005, 7, 1, 3, 1, 1; *Carpolechia* G. Don, *Salla* 326 (MO), 1999, 13, 0, 1, 1, 1; *Carpolechia* G. Don, *Sioni* 798 (MO), 1999, 13, 0, 1, 1, 1; *Carpolechia* alba G. Don, *White* 1057 (MO), 1993, 20, 0, 0, 1, 0; *Comaperma ericinum* DC., *Hedobas* 79 (MO), 1989, 23, 1, 3, 1, 1; *C. ericinum* DC., *Johow* 5280 (MO), 1997, 15, 1, 1, 1; *C. pallidum* Pedley, *Latz* 11703 (MO), 1990, 22, 0, 1, 1, 1; *C. secundum* Banks ex DC., *Harwood* 942 (MO), 2000, 12, 1, 3, 1, 1; *C. secundum*, *Rüter* 686 (MO), 2001, 11, 0, 1, 2, 3; *Epirixanthes elongata* Blume, *Hui* 17814 (TAIE), 2012, 1, 1, 2, 1; *Epirixanthes elongata*, *Shih-Wen Chang* s.n. (NA), 2012, 1, 1, 3, 2, 1; *Epirixanthes elongata*, *Tam HK43266* (HK), 2012, 1, 1, 3, 2, 1; *Gymnaspuria* (Wight & Arn.) Benth. & Hook. f., *Hanchbach* 46915 (MO), 1983, 29, 0, 0, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Montes* 9519 (MO), 2003, 10, 0, 0, 0, 0; *Hebecarpa* (Chodat) J.R. Abbott, *Blandia* 107 (MO), 2007, 5, 0, 0, 0, 1; *Hebecarpa* (Chodat) J.R. Abbott, *Davida* 35213 (MO), 1994, 19, 0, 0, 0, 1; *Hebecarpa* (Chodat) J.R. Abbott, *Garcia* 1973 (MO), 2000, 13, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Holt* EB074 (MO), 2010, 3, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Martinez* 23914 (MO), 1988, 25, 1, 1, 2, 3; *Hebecarpa* (Chodat) J.R. Abbott, *Momles* 9744 (MO), 2003, 10, 0, 0, 0, 1; *Hebecarpa* (Chodat) J.R. Abbott, *Steuwe* 27975 (MO), 2009, 4, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Steuwe* 30253 (MO), 2010, 3, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Steuwe* 30843 (MO), 2010, 3, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Steuwe* 33071 (MO), 2012, 1, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Steuwe* 33209 (MO), 2012, 1, 1, 3, 1, 2; *Hebecarpa* (Chodat) J.R. Abbott, *Zamani* 1517 (MO), 1988, 24, 1, 3, 1, 2; *Hebecarpa barbeyana* (Chodat) J.R. Abbott, *Luz* 5701 (MO), 2006, 7, 1, 3, 1, 2; *Hebecarpa cabrerae* (Chodat) Paiva, *Carvalho* 2772 (MO), 1986, 27, 0, 0, 0; *Mannina Ruiz & Pav.*, *Armejo* 3834 (MO), 2007, 5, 1, 3, 1, 3; *Mannina Ruiz & Pav.*, *Anaujo* 3870 (MO), 2007, 5, 1,

- 3, 2, 1; *Momma Ruiz & Pav.*, *Arroyo P. 4802* (MO), 2010, 3, 1, 3, 1, 2; *Momma Ruiz & Pav.*, *Fruit 4001* (MO), 2007, 6, 1, 1, 1, 2; *Momma Ruiz & Pav.*, *Fruit 4044* (MO), 2007, 6, 1, 3, 1, 2; *Momma Ruiz & Pav.*, *Fuentes 11540* (MO), 2007, 5, 1, 1, 1, 1; *Momma Ruiz & Pav.*, *Fuentes 9689* (MO), 2005, 7, 1, 3, 1, 1; *Momma Ruiz & Pav.*, *Llally 1691* (MO), 2009, 4, 1, 3, 1, 2; *Momma Ruiz & Pav.*, *Maldonado 2031* (MO), 2002, 10, 1, 3, 1, 1; *Momma Ruiz & Pav.*, *Pemaranda 624* (MO), 2008, 5, 1, 3, 1, 2; *Momma Ruiz & Pav.*, *Tenaz 2312* (MO), 2008, 5, 1, 1, 1, 2; *Momma Ruiz & Pav.*, *Tenaz 3542* (MO), 2009, 4, 1, 1, 1, 1; *Momma Ruiz & Pav.*, *Tenaz 3578* (MO), 2009, 4, 1, 1, 1, 1; *Momma Ruiz & Pav.*, *Ullas 1312* (MO), 2003, 10, 1, 1, 1, 1; *Momma Ruiz & Pav.*, *Ullas 1377* (MO), 2003, 10, 1, 1, 1, 1; *Momma arborea* Chodat, *Linnaea 1467* (MO), 2008, 5, 0, 0, 0, 1; *M. bridgemanii* Chodat, *Arrojo 3096* (MO), 2005, 7, 1, 3, 1, 1; *M. ciliolata* Sessé & Moc. ex DC., *Garcia 114* (MO), 2007, 5, 1, 3, 1, 1; *M. ciliolata*, *Valentina 84* (MO), 2010, 3, 1, 3, 1, 2; *M. crepitans* Chodat, *Aguilar 4516* (MO), 1996, 16, 0, 0, 0, 0; *M. berzagii* Chodat, *Carrasco 278* (MO), 2004, 8, 1, 3, 1, 1; *M. berzagii*, *Luzen 13600* (MO), 1990, 22, 1, 3, 1, 2; *M. lechleriana* Chodat, *Fernandez 1950* (MO), 2003, 9, 1, 3, 2, 1; *M. lechleriana* Chodat, *NA* (MO), 2002, 10, 1, 1, 3, 2; *M. longifolia* DC., *Mayfield s.n.* (MO), 2003, 9, 1, 2, 1, 3; *M. longifolia* DC., *Rodriguez 5517* (MO), 1999, 13, 0, 0, 1, 2; *M. macrodonta* Chodat, *Solomon 5999* (MO), 1981, 31, 1, 3, 1, 2; *M. pseudopilosa* Ferreyra, *Cerd 32010* (MO), 1998, 14, 1, 3, 1, 2; *M. pseudopilosa*, *Cerd 57510* (MO), 2006, 6, 0, 1, 1, 1; *M. rubyi* Chodat, *River 1801* (MO), 1995, 17, 1, 3, 1, 1; *M. salicifolia* Ruiz & Pav., *Fuentes 6708* (MO), 2004, 8, 1, 3, 2, 1; *M. salicifolia*, *Liberman 1280* (MO), 1986, 26, 1, 3, 2, 1; *M. saprogenes* Donn. Sm., *Martinez 383* (MO), 1995, 17, 0, 0, 0, 1; *M. weddelliana* Chodat, *Schmit 117* (MO), 1991, 21, 1, 3, 1, 1; *Moutabes Aubl.*, *Montenegro 11223* (MO), 2005, 8, 0, 0, 1; *Moutabes seubata* (Ruiz & Pav.) Poepp. & Endl., *Chiquimaco 1221* (MO), 2008, 5, 0, 0, 0, 1; *M. aculeata*, *Huamantupa 7867* (MO), 2006, 6, 1, 1, 1, 1; *Muraltia DC.*, *Leontine 22969* (MO), 1985, 27, 0, 1, 2, 3; *Muraltia harveyana* Levyns, *Goldblatt 11666* (MO), 2001, 12, 1, 1, 1, 2; *Muraltia heisteria* (L.) DC., *Madren s.n.* (MO), 2006, 6, 1, 3, 1, 2; *M. heisteria*, *Symon 15213* (MO), 1994, 18, 0, 1, 2, 3; *M. spinosa* (L.) E. Forst & J.C. Manning, *Goldblatt 11669* (MO), 2001, 12, 0, 0, 1, 2; *Polygala L.*, *Ar. Mingui 3617* (MO), 2003, 10, 1, 3, 1, 2; *Polygala L.*, *Anaga 4095* (MO), 2008, 4, 1, 3, 1, 2; *Polygala L.*, *Aratua-Munakanni 3951* (MO), 2007, 6, 1, 3, 1, 2; *Polygala L.*, *Arroyo P. 5050* (MO), 2010, 3, 1, 3, 1, 2; *Polygala L.*, *Burley 2810* (MO), 1989, 23, 0, 1, 1, 1; *Polygala L.*, *Carrasco 876* (MO), 2003, 10, 1, 1, 1, 1; *Polygala L.*, *Carretero 876* (MO), 2003, 9, 1, 3, 2, 1; *Polygala L.*, *Criollo 2972* (MO), 2009, 4, 1, 3, 1, 2; *Polygala L.*, *Clarke 70-03* (MO), 1991, 21, 1, 1, 2, 3; *Polygala L.*, *Croat 53261* (MO), 1981, 31, 0, 0, 0, 1; *Polygala L.*, *Croat 96287* (MO), 2006, 7, 1, 3, 2, 1; *Polygala L.*, *Dold 3516* (MO), 1999, 13, 0, 0, 1, 2; *Polygala L.*, *Elias 108* (MO), 1999, 13, 1, 3, 2, 1; *Polygala L.*, *Festo 1960* (MO), 2005, 7, 1, 3, 2, 1; *Polygala L.*, *Festo 492* (MO), 2000, 12, 1, 3, 1, 2; *Polygala L.*, *Festa 79* (MO), 1998, 14, 0, 1, 1, 1; *Polygala L.*, *Gereau 3118* (MO), 1989, 23, 0, 0, 0, 1; *Polygala L.*, *Gereau 3527* (MO), 1991, 21, 0, 0, 1, 1; *Polygala L.*, *Gereau 4281* (MO), 1991, 21, 0, 0, 0, 1; *Polygala L.*, *Gereau 4354* (MO), 1991, 21, 0, 0, 1, 1; *Polygala L.*, *Gereau 4441* (MO), 1991, 21, 0, 0, 1, 1; *Polygala L.*, *Gereau 5075* (MO), 1992, 20, 0, 1, 2, 3; *Polygala L.*, *Gobba 263* (MO), 1999, 13, 0, 1, 2, 3; *Polygala L.*, *Gobba 301* (MO), 1999, 13, 0, 1, 2; *Polygala L.*, *Gobba 26781* (MO), 2005, 8, 1, 1, 1, 1; *Polygala L.*, *Gobba 26884* (MO), 2005, 8, 0, 1, 1, 1; *Polygala L.*, *Gobba 28364* (MO), 2005, 8, 1, 1, 1, 2; *Polygala L.*, *Gobba 28386* (MO), 2005, 8, 0, 1, 1, 1; *Polygala L.*, *Gobba 28820* (MO), 2005, 8, 0, 1, 1, 2; *Polygala L.*, *Gutierrez 1258* (MO), 2005, 8, 1, 1, 1, 1; *Polygala L.*, *Gutierrez 1258* (MO), 2005, 7, 1, 3, 2, 1; *Polygala L.*, *Harder 2511* (MO), 1995, 17, 1, 1, 1, 1; *Polygala L.*, *Harder 2758* (MO), 1995, 17, 1, 1, 1, 1; *Polygala L.*, *Harder 3094* (MO), 1994, 18, 0, 0, 0, 1; *Polygala L.*, *Harder 3262* (MO), 1994, 18, 1, 1, 1, 1; *Polygala L.*, *Harder 3269* (MO), 1994, 18, 1, 3, 1, 1; *Polygala L.*, *Harder 3491* (MO), 1996, 16, 0, 1, 1, 1; *Polygala L.*, *Harder 3623* (MO), 1996, 16, 1, 1, 1, 3; *Polygala L.*, *Harder 3629* (MO), 1996, 16, 0, 0, 0, 0; *Polygala L.*, *Harder 3630* (MO), 1996, 16, 1, 2, 1, 3; *Polygala L.*, *Harder 3826* (MO), 1997, 15, 0, 0, 0, 0; *Polygala L.*, *Harder 3833* (MO), 1997, 15, 1, 2, 1, 1; *Polygala L.*, *Jimenez 771* (MO), 2007, 5, 1, 3, 1, 2; *Polygala L.*, *Kayombo 2158* (MO), 1999, 13, 0, 0, 0, 0; *Polygala L.*, *Kayombo 2341* (MO), 1999, 13, 1, 3, 1, 2; *Polygala L.*, *Kayombo 2743* (MO), 1999, 13, 1, 1, 1, 1; *Polygala L.*, *Kayombo 443* (MO), 1989, 23, 0, 0, 0, 0; *Polygala L.*, *Kayombo 456* (MO), 1989, 23, 0, 1, 2, 3; *Polygala L.*, *Kayombo 4814* (MO), 2004, 8, 0, 0, 1; *Polygala L.*, *Kucher 24310* (MO), 2001, 11, 1, 3, 1, 2; *Polygala L.*, *Kucher 24335* (MO), 2001, 11, 0, 0, 0, 1; *Polygala L.*, *Kucher 24495* (MO), 2001, 11, 1, 3, 1, 2; *Polygala L.*, *Kucher 25045* (MO), 2002, 10, 1, 1, 1, 2; *Polygala L.*, *Kucher 25136* (MO), 2002, 10, 1, 3, 1, 2; *Polygala L.*, *Li Heng 8909* (MO), 1997, 16, 1, 2, 1, 3; *Polygala L.*, *Lovett 151* (MO), 1991, 21, 1, 1, 2, 3; *Polygala L.*, *Lovett 1691* (MO), 1987, 25, 0, 0, 0, 1; *Polygala L.*, *Lovett 1748* (MO), 1987, 25, 0, 0, 1, 2; *Polygala L.*, *Lovett 237* (MO), 1991, 21, 1, 1, 1, 1; *Polygala L.*, *Lovett 2397* (MO), 1987, 25, 1, 1, 1, 3; *Polygala L.*, *Lovett 29* (MO), 1991, 21, 1, 3, 1, 2; *Polygala L.*, *Lovett 3426* (MO), 1989, 23, 0, 0, 1, 2; *Polygala L.*, *Lovett 3465* (MO), 1989, 23, 0, 0, 1, 2; *Polygala L.*, *Lovett 3884* (MO), 1990, 22, 0, 0, 1, 2; *Polygala L.*, *Lovett 402* (MO), 1991, 21, 0, 1, 2, 3; *Polygala L.*, *Lovett 4149* (MO), 1990, 22, 0, 1, 2, 3; *Polygala L.*, *Lovett 4177* (MO), 1990, 22, 0, 1, 2, 3; *Polygala L.*, *Lovett 4211* (MO), 1990, 22, 0, 1, 2, 3; *Polygala L.*, *Lovett 4417* (MO), 1990, 22, 0, 1, 2, 3; *Polygala L.*, *Lovett 4855* (MO), 1997, 15, 0, 0, 0, 1; *Polygala L.*, *Lovett 5842* (MO), 2002, 10, 1, 3, 2, 1; *Polygala L.*, *Lovett II 4855* (MO), 1997, 16, 0, 0, 0, 0; *Polygala L.*, *Luke 10946* (MO), 2005, 7, 1, 3, 1, 1; *Polygala L.*, *Lauwaika 370* (MO), 1996, 16, 1, 1, 1, 1; *Polygala L.*, *Lauwaika 585* (MO), 1997, 15, 1, 3, 2, 1; *Polygala L.*, *Lauwaika 591* (MO), 1997, 15, 0, 0, 0, 0; *Polygala L.*, *MacBryde 612* (MO), 1971, 42, 0, 0, 0, 1; *Polygala L.*, *Mwangoaka 1624* (MO), 2000, 12, 1, 3, 2, 1; *Polygala L.*, *Mwangoaka 2510* (MO), 2001, 11, 1, 1, 2, 3; *Polygala L.*, *Mwangoaka 3855* (MO), 2005, 7, 1, 3, 2, 1; *Polygala L.*, *Mwangoaka 395* (MO), 1999, 13, 1, 1, 1, 1; *Polygala L.*, *Mwangoaka 507* (MO), 1999, 13, 1, 3, 1, 1; *Polygala L.*, *Mwangoaka 5174* (MO), 2007, 6, 1, 1, 1, 2; *Polygala L.*, *Mwangoaka 820* (MO), 1999, 13, 1, 3, 1, 1; *Polygala L.*, *Mwangoaka 929* (MO), 1999, 13, 0, 0, 0, 1; *Polygala L.*, *Nauer 106* (MO), 1995, 17, 1, 3, 1, 2; *Polygala L.*, *O'Neill 25* (MO), 1996, 16, 1, 1, 1, 1; *Polygala L.*, *Piranga 1238* (MO), 2005, 7, 1, 3, 1, 0; *Polygala L.*, *Ponzi 374* (MO), 2008, 5, 1, 3, 1, 1; *Polygala L.*, *Rakotovao 390*

- (MO), 2007, 5, 0, 0, 0; *Polygala L.*, *Salle 203* (MO), 1999, 13, 1, 3, 2, 1; *Polygala L.*, *Salle 76* (MO), 1998, 14, 0, 0, 1, 2; *Polygala L.*, *Sant'Ana 942* (MO), 2000, 13, 1, 1, 2, 3; *Polygala L.*, *Scharr 3569* (MO), 1993, 19, 1, 1, 1, 1; *Polygala L.*, *Schott 4059* (MO), 2003, 9, 1, 3, 2, 1; *Polygala L.*, *Schmidt 1316* (MO), 1994, 18, 1, 2, 1, 3; *Polygala L.*, *Schmidt 2348* (MO), 1997, 15, 0, 0, 1; *Polygala L.*, *Schmidt 2446* (MO), 1997, 15, 1, 3, 2, 1; *Polygala L.*, *Schmidt 2448* (MO), 1997, 15, 1, 1, 2, 2; *Polygala L.*, *Schmidt 2514* (MO), 1997, 15, 1, 3, 1, 2; *Polygala L.*, *Schmidt 2543* (MO), 1997, 15, 1, 3, 1, 2; *Polygala L.*, *Schmidt 12* (MO), 1998, 14, 0, 0, 1, 2; *Polygala L.*, *Simon 441* (MO), 2000, 12, 0, 1, 1, 2; *Polygala L.*, *Simon 503* (MO), 2000, 12, 0, 0, 1, 2; *Polygala L.*, *Simon 685* (MO), 2001, 11, 0, 1, 1, 2; *Polygala L.*, *Sitomi 1023* (MO), 1999, 13, 1, 2, 1, 3; *Polygala L.*, *Sitomi 1209* (MO), 2000, 12, 1, 1, 1, 1; *Polygala L.*, *Smeky 600* (MO), 2010, 3, 1, 3, 1, 2; *Polygala L.*, *Sugang WS-1916* (MO), 2008, 4, 0, 0, 0, 0; *Polygala L.*, *Sugang WS-2192* (MO), 2008, 4, 1, 3, 1, 1; *Polygala L.*, *Tenam 2984* (MO), 2008, 4, 1, 3, 1, 2; *Polygala L.*, *Tenam 995* (MO), 2007, 5, 1, 3, 2, 1; *Polygala L.*, *Tenam 996* (MO), 2007, 5, 1, 2, 1, 1; *Polygala L.*, *Tep-Jongseuen DAT161* (MO), 1999, 13, 1, 3, 1, 2; *Polygala L.*, *Ternes 410* (MO), 2009, 4, 1, 3, 2, 1; *Polygala L.*, *van der Werff 14411* (MO), 1997, 16, 0, 0, 0, 0; *Polygala L.*, *van der Werff 20124* (MO), 2005, 8, 0, 0, 1, 2; *Polygala L.*, *van Valkenburg 2922* (MO), 2004, 9, 1, 3, 1, 2; *Polygala L.*, *Villalobos 308* (MO), 2005, 7, 1, 3, 2, 1; *Polygala L.*, *Xian Gaoguan 14629* (MO), 2002, 11, 1, 1, 1, 1; *Polygala L.*, *Zhu 2317* (MO), 1999, 14, 0, 0, 0, 1; *Polygala L.*, *Zimba 1068* (MO), 1997, 15, 1, 3, 1, 2; *Polygala L.*, *Zimba 661* (MO), 1995, 17, 0, 0, 0; *Polygala L.*, *Zimba 795* (MO), 1996, 16, 0, 0, 0; *Polygala engleri* Chodat, *Anquier 2765* (MO), 1972, 41, 0, 0, 0, 1; *P. engleri*, *Anquier 3397* (MO), 1974, 39, 0, 0, 0, 1; *P. engleri*, *Ewango 2153* (MO), 1999, 14, 0, 0, 0, 1; *P. major* Jacq., *Bard 2001-58* (MO), 2001, 12, 0, 0, 0, 1; *P. rupestris* Pourr., *Delennay 1351* (MO), 1974, 39, 0, 0, 0, 1; *P. umbonata* Craib, *Macmillan 01-558* (MO), 2001, 11, 0, 0, 0, 1; *P. umbonata*, *Sankhamethawee 260* (MO), 2001, 11, 0, 0, 0, 1; *P. abyssinica* R. Br. ex Fresen., *de Wildt 227* (MO), 1969, 44, 0, 0, 0, 1; *P. acicularis* Oliv., *Walter 1213* (MO), 2003, 10, 0, 1, 1, 1; *P. acicularis*, *Walter 1898* (MO), 2007, 5, 1, 1, 1, 2; *P. acicularis*, *White 1368* (MO), 1995, 18, 0, 1, 1, 1; *P. adenocarpus* Exell, *Patel 7107* (MO), 2005, 7, 1, 1, 1, 1; *P. africana* Chodat, *Bidgood 6950* (MO), 2008, 5, 1, 3, 1, 2; *P. albida* (A.W. Benn.) Grandaona, *Zardini 7390* (MO), 1988, 24, 0, 0, 1, 2; *P. albida* Schinz, *Harter 3937* (MO), 1997, 15, 0, 0, 0, 1; *P. albida*, *Leeuwenberg 10206* (MO), 1972, 41, 0, 0, 0, 1; *P. albida*, *Leeuwenberg 8455* (MO), 1971, 42, 0, 0, 0, 1; *P. albida*, *Zimba 1066* (MO), 1997, 15, 0, 0, 1, 2; *P. amurensis* Czern., *Polotschek s.n.* (MO), 1989, 24, 0, 0, 0, 1; *P. arenaria* Oliv., *McPherson 16912* (MO), 1997, 16, 0, 0, 0, 1; *P. arenaria*, *White 1432* (MO), 1995, 18, 0, 0, 0, 1; *P. anilioides* Buch.-Ham. ex D. Don, *KEKE 945* (MO), 1989, 23, 1, 2, 1, 3; *P. apiculoides* Kunth, *Annals 1493* (MO), 2004, 8, 1, 3, 2, 1; *P. chinensis* L., *Grevillea 182* (MO), 2001, 11, 0, 0, 0, 0; *P. compressa* H. Perrier, *Gauzier 5216* (MO), 2007, 6, 1, 3, 2, 1; *P. compressa*, *Leandri 4578* (MO), 1960, 53, 0, 0, 0, 0; *P. compressa*, *Ranamajatava 32* (MO), 2008, 5, 1, 1, 1, 1; *P. cristata* P. Taylor, *Jongkind 7420* (MO), 2006, 6, 1, 3, 2, 1; *P. darwiniensis* A.W. Benn., *Clarke 79-11* (MO), 1991, 21, 0, 1, 1, 1; *P. didyma* C.Y. Wu, *Gaoligong Shan Biodiversity Survey 23811* (MO), 2005, 7, 1, 3, 2, 1; *P. didyma*, *GSBS 19513* (MO), 2004, 9, 1, 1, 2, 2; *P. eriopetala* DC., *Bidgood 6123* (MO), 2006, 6, 1, 3, 1, 1; *P. fallax* Hemsl., *Deng Kuiwei s.n.* (MO), 1993, 20, 0, 0, 0, 0; *P. fallax*, *Long Chuan 4270* (MO), 2004, 9, 0, 0, 1, 1; *P. fernandeziana* Paiva, *Leeuwenberg 10187* (MO), 1972, 41, 0, 0, 0, 1; *P. gilleriana* E.M.A. Petit, *White 1299* (MO), 1995, 18, 1, 1, 1, 2; *P. grandiflora* Baill., *Bauer 18875* (MO), 1964, 49, 0, 0, 0, 1; *P. grandiflora*, *Humbert 28188* (MO), 1955, 58, 0, 0, 0, 0; *P. grossiana* Baill., *Ibrahim 11818* (MO), 2010, 3, 1, 3, 1, 2; *P. hildebrandii* Baill., *Gautier 2522* (MO), 1994, 18, 0, 0, 1, 2; *P. hildebrandii*, *Gautier 5370* (MO), 2009, 4, 1, 1, 1, 1; *P. humbertii* H. Perrier, *Randriambololana 80* (MO), 1994, 18, 0, 0, 0, 0; *P. irwinii* Wurdack, *Mae 8714* (MO), 1999, 13, 0, 1, 1, 1; *P. japonica* Hoouai., *Li Ji-Dong 512* (MO), 2004, 9, 0, 0, 0, 1; *P. japonica*, *Long Chuan 4243* (MO), 2004, 9, 0, 0, 0, 1; *P. kuharicae* Schinz, *Bester 9254* (MO), 2009, 4, 1, 3, 1, 1; *P. laingii* Kunth, *Cteor 101604* (MO), 2011, 2, 1, 3, 1, 2; *P. longifolia* Poir., *Sankhamethawee 252* (MO), 2001, 11, 0, 1, 1, 2; *P. lutea* L., *Arnold s.n.* (FLAS), 1937, 76, 0, 0, 0, 0; *P. lutea*, *Arnold s.n.* (FLAS), 1932, 81, 0, 0, 0, 0; *P. lutea*, *Brinkley 12* (FLAS), 1931, 82, 0, 0, 0, 1; *P. lutea*, *Claus 188* (FLAS), 1967, 46, 0, 0, 0, 1; *P. lutea*, *D'Arcy 1538* (FLAS), 1967, 46, 0, 0, 0, 1; *P. lutea*, *Dunstan 20230* (FLAS), 1956, 57, 0, 0, 0, 0; *P. lutea*, *Earle s.n.* (FLAS), 1889, 124, 0, 0, 0, 0; *P. lutea*, *Evers s.n.* (FLAS), 1936, 77, 0, 0, 0, 1; *P. lutea*, *Hood 1677* (FLAS), 1949, 64, 0, 0, 0, 1; *P. lutea*, *Hood 366* (FLAS), 1947, 66, 0, 0, 0, 1; *P. lutea*, *Jasperney s.n.* (FLAS), 1893, 120, 0, 0, 0, 0; *P. lutea*, *Jones 15952* (FLAS), 1969, 44, 0, 0, 0, 1; *P. lutea*, *Ker s.n.* (FLAS), 1933, 80, 0, 0, 0, 0; *P. lutea*, *Kmt 7767* (FLAS), 1958, 55, 0, 0, 0, 1; *P. lutea*, *Loucks & White s.n.* (FLAS), 1927, 86, 0, 0, 0, 0; *P. lutea*, *Melvin 3589* (FLAS), 1956, 57, 0, 0, 0, 1; *P. lutea*, *Moore & Lawrence 748* (FLAS), 1949, 64, 0, 0, 0, 1; *P. lutea*, *Murrill s.n.* (FLAS), 1941, 72, 0, 0, 0, 1; *P. lutea*, *Rudford 44855* (FLAS), 1966, 47, 0, 0, 0, 1; *P. lutea*, *Rau s.n.* (FLAS), 1885, 128, 0, 0, 0, 1; *P. lutea*, *Rau s.n.* (FLAS), 1884, 129, 0, 0, 0, 1; *P. lutea*, *Rolfi 291* (FLAS), 1893, 120, 0, 0, 0, 0; *P. lutea*, *Scull s.n.* (FLAS), 1937, 76, 0, 0, 0, 1; *P. lutea*, *Shippy s.n.* (FLAS), 1937, 76, 0, 0, 0, 1; *P. lutea*, *Smith & Hedger 964* (FLAS), 1939, 74, 0, 0, 0, 1; *P. lutea*, *Smith & Myint 287* (FLAS), 1961, 52, 0, 0, 0, 1; *P. lutea*, *Smith & Myint 494* (FLAS), 1961, 52, 0, 0, 0, 1; *P. lutea*, *Smith s.n.* (FLAS), 1961, 52, 0, 0, 0, 0; *P. lutea*, *Ward & Smith 2545* (FLAS), 1961, 52, 0, 0, 0, 1; *P. lutea*, *Ward 1276* (FLAS), 1959, 54, 0, 0, 0, 1; *P. lutea*, *Ward 6229* (FLAS), 1967, 46, 0, 0, 0, 1; *P. lutea*, *West & Arnold s.n.* (FLAS), 1940, 73, 0, 0, 0, 1; *P. lutea*, *West & Arnold s.n.* (FLAS), 1940, 73, 0, 0, 0, 1; *P. lutea*, *West s.n.* (FLAS), 1942, 71, 0, 0, 0, 1; *P. lutea*, *West s.n.* (FLAS), 1926, 87, 0, 0, 0, 0; *P. lutea*, *Wigg 19514* (FLAS), 1965, 48, 0, 0, 0, 1; *P. mandarinii* Chodat, *Vergu 247* (MO), 2005, 7, 1, 3, 1, 2; *P. melilotoides* Chodat, *Bidgood 6894* (MO), 2008, 5, 1, 1, 1, 2; *P. nimbaleensis* Giseke, *Bidgood 5349* (MO), 2006, 6, 1, 3, 1, 1; *P. oblongata* (Britton) S.F. Blake, *Michelangeli 1454* (NA), 2009, 3, 1, 3, 1, 2; *P. paniculata* L., *Gaudichia 1467* (MO), 2010, 3, 1, 3, 1, 2; *P. paniculata*, *Labat 3901* (MO), 2006, 6, 1, 3, 1, 2; *P. peplis* Baill., *Cremers 3707* (MO), 1975, 38, 0, 0, 0, 1; *P. paniculifolia* DC., *Maxwell 98-1083* (MO), 1998, 14, 0, 0, 0, 0; *P. petitiiana* A. Rich., *Chapman 9013* (MO), 1988, 24, 0, 1, 2, 3; *P. producta* N.E. Br., *Sungur 684* (MO), 1983, 30, 0, 0, 0, 1; *P. rugosa* Hochst. & Steud., *de Wildt 4930* (MO), 1969, 44, 0, 0, 0, 1; *P. rugosa*, *de Wildt 4930* (MO), 1969, 44, 0, 0, 0, 1; *P. seneca var. latifolia* Torn. & A. Gray, *Eur 9369* (MO), 2006, 6, 1, 3, 1, 1; *P. serpyllifolia* Hose, *Delessain 1074* (MO), 1973,

- 40, 0, 0, 0, 1; *P. sibirica* L., Baufford 38265 (MO), 2007, 5, 1, 3, 1, 2; *P. sibirica*, Duan Lin-Dong 679 (MO), 2004, 9, 0, 0, 0, 1; *P. sibirica*, Li Ji-Dong GJ (MO), 2004, 9, 0, 0, 1, 1; *P. sibirica*, Song Jie 113-02 (MO), 2008, 5, 1, 3, 1, 2; *P. subappennina* S.K. Chen, Xiao Jian-xi 5594 (MO), 2003, 10, 0, 0, 0, 1; *P. subappennina* S.K. Chen, Zhu 2144 (MO), 1999, 14, 0, 0, 0, 1; *P. trichophlopha* Chodat, Maxwell 99-300 (MO), 1999, 13, 1, 3, 1, 2; *P. ussuriensis* Griseb., Bidgad 5336 (MO), 2006, 6, 1, 3, 1, 1; *P. variabilis* Kunch., Cesar 101601 (MO), 2011, 2, 1, 3, 2, 1; *P. verticillata* L., Abbott 25336 (FLAS), 2010, 2, 1, 3, 1, 2; *P. virgata* var. *decoya* (Sond.) Harv., Juresson 1114 (MO), 1984, 29, 0, 0, 0, 1; *P. virgata* var. *decoya*, Musangka 5485 (MO), 2007, 5, 1, 3, 1, 1; *P. vulgaris* L., Lewis 15901 (MO), 2004, 9, 0, 0, 0, 1; *P. vulgaris*, Pignal 1763 (MO), 2001, 12, 0, 0, 0, 1; *P. welwitschii* Chodat, Bidgad 8112 (MO), 2009, 4, 0, 0, 0, 1; *P. welwitschii*, Kami 1312 (MO), 2011, 2, 1, 1, 1, 1; *Polygonoides chaenophyllum* (L.) O.Schwarz, Andrew s.n. (MO), 2007, 6, 1, 1, 1, 2; *Pteromomma herbacea* (DC.) B. Erikson, Leegaard 19731B (MO), 1999, 15, 1, 3, 1, 1; *Rhinostrophi* (S.E.Blaeke) J.R.Abbott, Wilder 10-148 (MO), 2010, 3, 1, 3, 1, 2; *Rhinostrophi* *cormata* (Kellogg) J.R.Abbott, Ahart 13921 (MO), 2007, 6, 0, 0, 0, 1; *R. subspinosa* (S. Watson) J.R.Abbott, Tiehm 16221 (MO), 2010, 3, 1, 3, 1, 2; *Salomandra* Lour., Grajmanu 157 (MO), 2001, 11, 0, 1, 1, 1; *S. canariensis*, Nothia 9760524 (MO), 1997, 15, 1, 3, 1, 1; *S. canariensis*, Nothia 9830009 (MO), 1998, 14, 1, 3, 1, 2; *Securidaca* L., Erom 156 (MO), 2005, 7, 1, 1, 1, 1; *Securidaca* L., Fuentes 7916 (MO), 2005, 7, 1, 3, 1, 1; *Securidaca* L., Gereau 3854 (MO), 1991, 21, 0, 0, 1, 1; *Securidaca* L., Gereau 4347 (MO), 1991, 21, 0, 0, 1, 2; *Securidaca* L., Jardim 1717 (MO), 1998, 15, 0, 0, 0, 1; *Securidaca* L., Wu Su-Geng 2615 (MO), 2011, 2, 1, 3, 1, 1; *Securidaca* *inappendiculata* Hassk., Fan 11382 (NA), 2012, 1, 1, 3, 1, 2; *S. inappendiculata*, Fan 11383 (NA), 2012, 1, 1, 3, 1, 2; *S. oblonga* Schlecht., Pena-Chucarre 2883 (MO), 2007, 6, 1, 1, 1, 1; *S. welwitschii* Oliv., de Koning 7064 (MO), 1976, 57, 0, 0, 0, 0; *S. yaoshanensis* K.S. Hao, Fan 11400 (NA), 2012, 1, 1, 3, 1, 2; *S. yaoshanensis*, Fan 11401 (NA), 2012, 1, 1, 3, 1, 2; *S. yaoshanensis*, Fan 11402 (NA), 2012, 1, 1, 3, 1, 2. *Portulace* (*Portulacaceae*) data set from herbarium specimen: *Portulaca* *amilla* Spgr., Abbott 22102 (FLAS), 2006, 7, 1, 1, 1, 1; *P. amilla*, Andrew s.n. (FLAS), 2003, 10, 1, 3, 2, 1; *P. amilla*, Bhan s.n. (FLAS), 2006, 7, 1, 3, 1, 1; *P. amilla*, Burkhalter 10684 (FLAS), 1987, 26, 0, 0, 0; *P. amilla*, Creager 503 (FLAS), 1965, 48, 0, 0, 0, 0; *P. amilla*, Eastley 1024 (FLAS), 1990, 23, 0, 0, 0, 0; *P. amilla*, Eastley 631 (FLAS), 1981, 32, 0, 0, 1, 1; *P. amilla*, Ghobbi 4385 (FLAS), 1976, 37, 0, 0, 0, 0; *P. amilla*, Godfrey 79803 (FLAS), 1982, 31, 0, 0, 0, 0; *P. amilla*, Godfrey 82428 (FLAS), 1987, 26, 0, 0, 1, 1; *P. amilla*, Guada 903 (FLAS), 1988, 25, 1, 1, 1, 1; *P. amilla*, Judd s.n. (FLAS), 1979, 34, 0, 0, 1, 1; *P. amilla*, Martin 186 (FLAS), 2002, 11, 0, 0, 1, 1; *P. amilla*, Notis 179 (FLAS), 2001, 12, 0, 0, 1, 1; *P. amilla*, Penney 1229 (FLAS), 1998, 15, 0, 0, 1, 1; *P. amilla*, Riley 198 (FLAS), 2001, 12, 1, 3, 1, 1; *P. amilla*, Scudder 1066 (FLAS), 1975, 38, 0, 0, 0, 1; *P. amilla*, Schaffer 952 (FLAS), 1973, 40, 0, 0, 0, 1; *P. amilla*, Shuey 2129 (FLAS), 1978, 35, 1, 1, 1, 1; *P. amilla*, Wilson 2 (FLAS), 1976, 37, 0, 0, 0, 0; *P. amilla* L., Baltzell 11235 (FLAS), 1980, 33, 0, 0, 0, 0; *P. amilla*, Baltzell 5619 (FLAS), 1973, 40, 0, 0, 0, 0; *P. amilla*, Burgis s.n. (FLAS), 1947, 66, 0, 0, 0, 0; *P. amilla*, Cooley 2568 (FLAS), 1954, 59, 0, 0, 0, 0; *P. amilla*, Craighead s.n. (FLAS), 1956, 57, 0, 0, 0, 0; *P. amilla*,
- Craeger 424 (FLAS), 1965, 48, 0, 0, 0, 1; *P. amilla*, D'Arcy 2942 (FLAS), 1968, 45, 0, 0, 0, 0; *P. amilla*, Greenberg 195 (FLAS), 1991, 22, 1, 3, 2, 1; *P. amilla*, Gunter 148 (FLAS), 1988, 25, 0, 0, 0, 1; *P. amilla*, Hoffman 11 (FLAS), 1963, 50, 0, 0, 0, 0; *P. amilla*, Howard 20851 (FLAS), 1992, 21, 0, 0, 0, 1; *P. amilla*, Lange 1514 (FLAS), 2012, 1, 1, 3, 1, 1; *P. amilla* Martin 76 (FLAS), 2000, 13, 0, 0, 0, 1; *P. amilla*, McCas 10866 (FLAS), 1969, 44, 0, 0, 0, 1; *P. amilla*, subach 288 (FLAS), 1961, 52, 0, 0, 0, 0; *P. amilla*, Porter-Utley 952 (FLAS), 1995, 18, 0, 0, 0, 1; *P. amilla*, Small 5471 (FLAS), 1915, 98, 0, 0, 0, 1; *P. amilla*, Smith 1648 (FLAS), 1967, 46, 0, 0, 0, 0; *P. amilla*, Smith 1872 (FLAS), 1967, 46, 0, 0, 0, 0; *P. amilla*, Wier s.n. (FLAS), 1932, 81, 0, 0, 0, 1. *Hibiscus* (*Malvaceae*) data set from herbarium specimen: *Hibiscus* *agathophylloides* (Kurz) Mere, Hisp HLF7330 (MO), 2008, 3, 1, 1, 1; *Cienfuegosia humbertiana* (Hochr.) Fryxell, Phillipian 335 (MO), 1992, 19, 1, 3, 2, 1; *Bombicidiondendron vidaliianum* (Nees) Mert. & Rolfe, Margraff 106605 (FLAS), 1949, 64, 0, 0, 0, 1; *Hibiscus* L., Ardingoer 248 (MO), 1972, 39, 0, 0, 1, 1; *Hibiscus* L., Craven 6650 (MO), 1981, 30, 1, 3, 1, 2; *Hibiscus* L., Le 7044 (MO), 1977, 34, 1, 2, 1, 3; *Hibiscus* L., Lorance 291 (MO), 1979, 32, 1, 1, 1; *Hibiscus* L., Wilson 96-J (MO), 1996, 15, 1, 2, 1, 3; *Hibiscus* *aper* Hook. E, Gilir 6228 (FLAS), 1968, 45, 0, 0, 0, 1; *H. aper*, Weber s.n. (FLAS), 1966, 47, 0, 1, 0, 1; *H. aper*, Wilms s.n. (FLAS), 1943, 70, 0, 0, 0, 1; *H. aper* Garcke, Eichler 22299 (MO), 1977, 34, 1, 3, 1, 2; *H. aper*, Stevans 15084 (MO), 1935, 76, 1, 1, 2, 3; *H. dimidiata* Schneck, Strackwick 3478 (FLAS), 1981, 32, 0, 0, 1, 1; *H. aper* Fryxell, Holm-Nielsen 2218 (MO), 1973, 38, 0, 0, 0, 0; *H. fallax* Craven, E.D.Wilson & Fryxell, Leach 4662 (MO), 2001, 10, 0, 0, 0, 0; *H. faulkneriae* Vollesen, Mboe 69 (MC), 2000, 11, 0, 0, 0, 0; *H. ferrugineus* Cav., Geyeri 3143 (MC), 1989, 22, 1, 3, 1, 2; *H. ferrugineus*, Lorren 3357 (MO), 1982, 22, 1, 1, 1, 1; *H. ferrugineus*, Phillipian 3942 (MO), 1992, 1, 1, 3, 1, 2; *H. florifolium* Ulbr., Elia 6 (MO), 1999, 12, 1, 3, 1, 1; *H. florifolium*, Friis 3354 (MO), 1982, 29, 1, 3, 1, 2; *H. florifolium*, Gereau 6414 (MO), 2000, 11, 1, 3, 1, 2; *H. florifolium* Gisek Seydel 2588 (MO), 1961, 50, 0, 2, 1, 3; *H. fragrans* Rusby, Cuern 973 (MO), 2000, 11, 0, 0, 0, 0; *H. furcellata* De Balsell 8351 (FLAS), 1976, 37, 0, 0, 0, 1; *H. furcellata*, De Balsell 8351 (FLAS), 1967, 46, 0, 0, 0, 0; *H. furcellata*, Curtis 1 (FLAS), 1896, 117, 0, 0, 0, 1; *H. furcellata*, D'Arcy 20 (FLAS), 1968, 45, 0, 0, 0, 1; *H. furcellata*, Delaney 1 (FLAS), 1984, 29, 0, 0, 0, 1; *H. furcellata*, Germany s.n. (FLA), 2003, 10, 0, 0, 0, 1; *H. furcellata*, Godfrey 65652 (FLA), 1964, 49, 0, 0, 0, 1; *H. furcellata*, Head 4310 (FLAS), 1956, 62, 0, 0, 0, 1; *H. furcellata*, McCart 11062 (FLAS), 1969, 9, 0, 0, 0, 1; *H. furcellata*, Perkins s.n. (FLAS), 1937, 76, 0, 0, 1; *H. furcellata*, Small 9494 (FLAS), 1920, 93, 0, 0, 0, 0; *H. furcellata*, Wunderlin 10188 (FLAS), 1965, 28, 0, 0, 0, 1; *H. gilletii* De Wild., Lemoine 235 (MO), 1996, 15, 0, 0, 1, 2; *H. grandiflora* Baill., Randrianafika 399 (MO), 2004, 7, 1, 3, 1, 1; *H. hamabo* Siebold & Zucc., McFadden s.n. (FLAS), 1959, 1, 0, 0, 0, 1; *H. heterophylla* Vent., Henderson H2995 (MO), 1983, 28, 0, 0, 1, 2; *H. hildebrandtii* Sprague & Hutch., Mungaka 3954 (MO), 2005, 6, 1, 3, 1, 2; *H. hirsuta* L., Meijer 101 (MO), 1975, 36, 0, 0, 0, 0; *H. hirsutissima* Endl., Crossfield 9 (MO), 1978, 33, 0, 0, 1, 2; *H. isaloensis* Hochr. & Humb., Courte 5026 (MO), 1955, 56, 0, 0, 0, 1; *H. kirki* Mass., L. 642 (MO), 1987, 24, 1, 1, 1, 2; *H. kirki*, Maui 1403 (MO).

- 1972, 39, 0, 1, 1, 1; *H. kirkii* Hillebr. ex Wiers, *Wood* 3389 (MO), 1994, 17, 0, 0, 1, 1; *H. krichauffiana* E. Muell., *Cornwall* 295 (MO), 1977, 34, 0, 0, 1, 1; *H. laevigata* Moric. ex Sch., *Breedlove* 47595 (MO), 1980, 31, 1, 1, 2; *H. laevigata*, *Jones* 178 (MO), 1986, 25, 1, 1, 1, 2; *H. laxiflora* A. St.-Hil., *Heringer* 2617 (MO), 1979, 32, 0, 0, 1; *H. lobata* (Murray) Kunze, *Bardot-Vincentou* 1628 (MO), 2007, 4, 1, 3, 0, 0; *H. lobata*, *Darr* 2728 (MO), 1984, 27, 0, 0, 1, 1; *H. lobata*, *Harder* 3650 (MO), 1996, 15, 1, 3, 1, 2; *H. lobata*, *Marsue* 383 (MO), 1999, 12, 1, 3, 2, 0; *H. lobata*, *Smith* 4108 (MO), 1983, 28, 1, 1, 1, 1; *H. longiflora* Friesell, *Tenorio* L. 20138 (MO), 2001, 10, 1, 3, 1, 1; *H. longiflora*, *Tenorio* L. 21310 (MO), 2001, 10, 1, 3, 2, 1; *H. ludwigii* Eckl. & Zeyh., *Ellenberg* 1083 (MO), 1994, 17, 1, 1, 1, 1; *H. ludwigii*, *Kucher* 24706 (MO), 2001, 10, 1, 3, 1, 0; *H. lunatifolius* Willd., *Kayembe* 2595 (MO), 1999, 12, 1, 1, 1, 1; *H. lunatifolius*, *Mollet* 161 (MO), 2000, 11, 1, 3, 2, 1; *H. macrognathus* Baill., *Rallionne* 332 (MO), 2003, 8, 0, 0, 0, 0; *H. mandarinum* Humbert ex Hochr., *Phillipson* 3978 (MO), 1992, 19, 1, 3, 2, 1; *H. mandarinum* Zucc., *Ward* 5712 (FLAS), 1966, 47, 0, 0, 1; *H. mandarinum*, *Watson* s.n. (FLAS), 1938, 75, 0, 0, 0, 1; *H. mastersianus* Hilc., *Harder* 2851 (MO), 1995, 16, 0, 0, 0, 1; *H. mastersianus*, *Schmidt* 2475 (MO), 1997, 14, 0, 0, 0, 0; *H. meekowii* Gacke, *Matzl* 146 (MO), 1998, 13, 0, 0, 0, 1; *H. meekowii*, *Maaengalong* 725 (MO), 2002, 9, 0, 0, 0, 0; *H. meyeri* Harv., *Hardy* 7156 (MO), 1990, 21, 0, 1, 2, 3; *H. meyeri*, *Hermau* 1236 (MO), 1990, 21, 1, 3, 1, 2; *H. meyeri*, *Kucher* 22642 (MO), 1999, 12, 1, 3, 1, 2; *H. microanthus* L. F., *Davida* 8430 (MO), 1974, 37, 0, 0, 1, 2; *H. microanthus*, *Gobbo* 339 (MO), 1999, 12, 1, 3, 1, 0; *H. microanthus*, *Kindeketia* 1094 (MO), 2001, 10, 1, 3, 1, 1; *H. microanthus*, *Kucher* 23098 (MO), 2000, 11, 1, 1, 1, 1; *H. microanthus*, *McPherson* 14939 (MO), 1990, 21, 1, 1, 1, 1; *H. microanthus*, *Smith* 5700 (MO), 1994, 17, 1, 3, 1, 1; *H. microanthus*, *Welling* 56 (MO), 1996, 15, 1, 2, 1, 3; *H. microcarpus* Gacke, *Makwarela* 30 (MO), 1997, 14, 1, 3, 2, 1; *H. migeodii* Esseil, *Harder* 4034 (MO), 1997, 14, 1, 3, 1, 1; *H. migeodii*, *Lauvillea* 669 (MO), 1997, 14, 1, 3, 2, 1; *H. mutabilis* L., *Bentley* s.n. (FLAS), 1948, 65, 0, 0, 0, 1; *H. mutabilis*, *West* s.n. (FLAS), 1937, 76, 0, 0, 0, 1; *H. mutabilis*, *Williams* s.n. (FLAS), 1929, 84, 0, 0, 0, 1; *H. mustae* N.E. Br., *Leach* 11328 (MO), 1961, 50, 0, 0, 0, 0; *H. nigricalyx* Baker F., *Lauvillea* 2354 (MO), 1996, 15, 0, 0, 0, 0; *H. moldeae*, *Lovett* 3287 (MO), 1989, 22, 0, 0, 0, 1; *H. nyikensis* (Pursh.) Vahl, *La Croix* 4731 (MO), 1987, 24, 1, 2, 1, 3; *H. oxydorpha* Bojer ex Baker, *Harder* 1547 (MO), 1993, 18, 1, 1, 1, 2; *H. palmatus* Forsk., *Balogh* 6187 (MO), 2006, 5, 1, 3, 0, 1; *H. palmatum*, *Germishuizen* 9495 (MO), 1997, 14, 1, 1, 1, 2; *H. panduriformis* Burm. F., *Aub* 1348 (MO), 1971, 40, 1, 3, 0, 1; *H. panduriformis*, *Mast* 1267 (MO), 1974, 37, 0, 0, 1, 2; *H. panduriformis*, *Nuttall* 870 (MO), 1952, 59, 0, 0, 0, 2; *H. parviflora* L.H. Bailey, *Bin* 6533 (MO), 1999, 12, 1, 3, 0, 0; *H. parviflora*, *Min-Xiang* 92346 (MO), 1992, 19, 1, 2, 1, 3; *H. parviflora*, *Zhanghai* 474 (MO), 2002, 9, 1, 3, 1, 2; *H. pedunculata* L. F., *Kemp* 797 (MO), 1977, 34, 0, 0, 1, 1; *H. pedunculata*, *Marques* 2875 (MO), 1976, 35, 0, 0, 1, 1; *H. phoeniceum* Jacq., *Campe* 6107 (MO), 1999, 12, 1, 3, 1, 2; *H. phoeniceum*, *Harman* 3488 (MO), 1970, 41, 0, 0, 1, 2; *H. phoeniceum*, *Severns* 26928 (MO), 2008, 3, 1, 3, 1, 1; *H. phoeniceum*, *Vasquez* 27247 (MO), 2001, 10, 0, 0, 0, 0; *H. physaloides* Guill. & Perr., *Barthelat* 743 (MO), 2002, 9, 1, 1, 1, 1; *H. physaloides*, *Barthelat* 935 (MO), 2002, 9, 1, 3, 1, 1; *H. physaloides*, *de Neve* 3321 (MO), 1984, 27, 0, 0, 1, 1; *H. physaloides*, *Muanglang* 318 (MO), 1999, 12, 1, 1, 0, 0; *H. physaloides*, *Phillipson* 3829 (MO), 1992, 19, 1, 3, 1, 0; *H. physaloides*, *White* 1421 (MO), 1995, 16, 1, 3, 1, 1; *H. plataniifolius* (Willd.) Sweet, *Brumback* 9411 (FLAS), 1978, 35, 0, 0, 0, 1; *H. platycalyx* Mast., *Schmidt* 2365 (MO), 1997, 14, 1, 3, 0, 0; *H. platycalyx*, *Smith* 2945 (MO), 1980, 31, 0, 0, 1, 2; *H. poeppigii* (Spreng.) Garcke, *Carmichael* 5939 (MO), 2000, 11, 1, 3, 1, 2; *H. poeppigii*, *Garcia* 1210 (MO), 1988, 23, 1, 1, 1, 1; *H. poeppigii*, *Tapia* 1250 (MO), 2001, 10, 1, 3, 2, 1; *H. prasterina* R.A. Dyer, *Fryx* 49 (MO), 1991, 20, 1, 3, 1, 2; *H. prasterina*, *Raymond* 117 (MO), 1973, 38, 0, 0, 1, 2; *H. pusilla* Thunb., *Reynier* 8490 (MO), 1978, 33, 0, 0, 1, 2; *H. pusilla*, *Bester* 6469 (MO), 2006, 5, 1, 3, 1, 1; *H. pusilla*, *Fryx* 31 (MO), 1991, 20, 1, 1, 1, 1; *H. pusilla*, *Glen* 2337 (MO), 1991, 20, 1, 1, 2, 1; *H. pusilla*, *Kemp* 1170 (MO), 1977, 36, 0, 0, 1, 1; *H. pycnostemon* Hochr., *Kayomba* 3522 (MO), 2000, 11, 1, 2, 1, 3; *H. pycnostemon*, *Sironi* 1144 (MO), 2008, 11, 1, 3, 1, 2; *H. radiata* Cav., *Griffiths* 1500 (MO), 1982, 29, 0, 0, 0, 0; *H. radiata*, *Guzman* 1122 (MO), 1980, 31, 0, 0, 0; *H. rhodanthus* Güske ex Schinz, *Beyls* 10159 (MO), 1984, 27, 0, 1, 2, 3; *H. rhodanthus*, *Brooks* 186 (MO), 1990, 21, 1, 1, 1, 1; *H. rhodanthus*, *Gerten* 5207 (MO), 1993, 18, 1, 3, 1, 1; *H. rhodanthus*, *Harder* 1204 (MO), 1992, 19, 1, 3, 2, 1; *H. ribifolius* A. Gray, *Harbison* s.n. (FLAS), 1959, 54, 0, 0, 0, 1; *H. rosa-sinensis* L., *Avery* 1669 (FLAS), 1976, 37, 0, 0, 0, 1; *H. rosa-sinensis*, *Dickey* s.n. (FLAS), 1939, 74, 0, 0, 0, 1; *H. rosa-sinensis*, *Hume* s.n. (FLAS), 1949, 64, 0, 0, 0, 1; *H. rosa-sinensis*, *McFarlin* 10420 (FLAS), 1935, 78, 0, 0, 0, 1; *H. rosa-sinensis*, *McFarlin* 8892 (FLAS), 1934, 79, 0, 0, 0, 0; *H. rosa-sinensis*, *Meyer* & *Mazzee* 14305 (FLAS), 1973, 40, 0, 0, 0, 1; *H. rosa-sinensis*, *Meyer* & *Mazzee* 14306 (FLAS), 1973, 40, 0, 0, 0, 0; *H. rosa-sinensis*, *Skene* 963 (FLAS), 1982, 31, 0, 1, 2, 3; *H. rotundifolia* Smith s.n. (FLAS), 1979, 34, 1, 1, 2, 3; *H. rotundifolia* Guill. & Perr., *Fay* 6323 (MO), 1984, 27, 0, 0, 0, 1; *H. rotundifolia*, *Festu* 253 (MO), 1999, 12, 0, 0, 0, 1; *H. sabdariffa* L., *West* s.n. (FLAS), 1943, 70, 0, 0, 0, 1; *H. schinzii* Güske, *Marguer* s.n. (MO), 1974, 37, 0, 0, 1, 1; *H. schinzii*, *Smith* 3285 (MO), 1980, 31, 0, 1, 1, 1; *H. schizopetalus* (Dyer) Hook. F., *McCart* 9367 (FLAS), 1967, 46, 0, 0, 1, 1; *H. schizopetalus*, *Watson* s.n. (FLAS), 1959, 74, 0, 0, 0, 1; *H. scutellii* Baker F., *Gautier* 28802 (MO), 1992, 19, 0, 0, 1, 2; *H. sellii* Güske, *Krapoetcker* 46128 (MO), 1994, 17, 1, 1, 2, 3; *H. sidiformis* Baill., *Harder* 3835 (MO), 1997, 14, 1, 3, 1, 2; *H. sidiformis*, *Retzschke* 753 (MO), 2004, 7, 0, 0, 1, 1; *H. sidiformis*, *Schmidt* 2409 (MO), 1997, 14, 1, 1, 1, 1; *H. sinense* L.H. Bailey, *Lai* 12 (MO), 1996, 15, 1, 3, 1, 1; *H. solandra* L'Héz., *Adams* 15001 (MO), 1983, 28, 0, 0, 1, 2; *H. somalensis* Franch., *Gobbo* 714 (MO), 2000, 11, 1, 3, 1, 2; *H. somalensis*, *Kayomba* 2258 (MO), 1999, 12, 1, 3, 1, 1; *H. squamans* Hochr., *Fay* 5866 (MO), 1983, 28, 0, 0, 0, 1; *H. squamans*, *Madore* 6201 (MO), 1996, 15, 0, 0, 0, 1; *H. stenophyllus* Baker, *Labat* 3658 (MO), 2006, 5, 1, 3, 1, 2; *H. stenophyllus* (Guill. & Perr.) Seiden., *Duvall* 374 (MO), 1999, 12, 1, 3, 2, 1; *H. stroblii* Hook., *Zardini* 57076 (MO), 2001, 10, 1, 1, 1, 1; *H. stroblii*, *Leuz* 13519 (MO), 1993, 18, 1, 3, 1, 2; *H. stroblii*, *Macarochie* 1675 (MO), 1973, 38, 0, 0, 0, 1; *H. stroblii*, *Macarochie* 1692 (MO), 1973, 38, 0, 0, 0, 1; *H. subreniformis* Burret Davy, *Gressner* 799 (MO), 1973, 38, 0, 0, 0, 1; *H. minitressa* L.

- Ching-I Peng* 17869 (MO), 1999, 12, 0, 0, 0, 1; *H. surattensis*, *Milne* 365 (MO), 2000, 11, 0, 0, 0, 0; *H. surattensis*, *Miangua* 1820 (MO), 2001, 10, 0, 0, 0, 0; *H. syriacus* L., *Arnold* s.n. (FLAS), 1936, 77, 0, 0, 0, 1; *H. syriacus*, *Harrison* & R. *Garen* 34 (FLAS), 1975, 38, 0, 0, 0, 1; *H. syriacus*, *Weber* s.n. (FLAS), 1932, 81, 0, 0, 0, 1; *H. thysaniana* Baill., *Noyer* 1026 (MO), 1992, 19, 1, 3, 2, 1; *H. triplum* L., *Gaochang-jong* 20065-277-4 (MO), 2006, 5, 1, 3, 1, 1; *H. triplum*, *Henderson* 95-760 (MO), 1995, 16, 1, 2, 1, 3; *H. triplum*, *Tadeusz* 12038 (MO), 2005, 6, 1, 3, 2, 1; *H. uncinellus* DC., *Gillis* 9511 (FLAS), 1970, 43, 0, 0, 0, 0; *H. urticifolius* A. St.-Hil. & Naudin, *Fernandez* Canar 6181 (MO), 1982, 29, 0, 0, 0, 1; *H. urticifolius* L., *Pierce* 56 (FLAS), 1952, 61, 0, 0, 0, 1; *H. ussuriensis* Sprague, de Wilde 5047 (MO), 1969, 42, 1, 1, 1, 1; *Houttuynia triocularis* F. Muell., *Nordenstam* 1263 (MO), 1989, 22, 0, 0, 0; *H. umbellata* Hochst., *Manjakabery* 110 (MO), 2006, 5, 1, 1, 1, 2; *Humbertia decaryi* (Hochst.) Dorr, *Baum* 385 (MO), 2003, 8, 0, 0, 0, 0; *H. decaryi*, *Capuron* s.n. (MO), 1968, 43, 0, 1, 2, 3; *H. decaryi*, *McPherson* 14942 (MO), 1990, 21, 1, 3, 1, 1; *H. decaryi*, *Phillipson* 2649 (MO), 1987, 24, 0, 1, 2, 3; *H. decaryi*, *Phillipson* 3473 (MO), 1990, 21, 0, 1, 1, 1; *H. quasariibesides* Hochst., *Baum* 389 (MO), 2003, 8, 1, 3, 2, 0; *H. quasariibesides*, *Luckow* 4314 (MO), 1998, 13, 1, 3, 2, 1; *H. quasariibesides*, *McPherson* 17457 (MO), 1998, 13, 0, 0, 1; *Kosteletzkya reflexiflora* Hochst., *Phillipson* 2898 (MO), 1988, 23, 1, 1, 1, 2; *K. retrofractacea* Hochst., *Bender-Vaucouleur* 1555 (MO), 2007, 4, 1, 3, 2, 0; *Malvesticia concinna* Kunth, *Annujo-M.* 874 (MO), 2004, 7, 1, 1, 1, 1; *M. concinna*, *Calligas* 10454 (MO), 1992, 19, 1, 1, 1, 3; *M. concinna*, *Hammel* 4096 (MO), 1978, 33, 0, 0, 0, 1; *M. williamsonii* Ulbr., *Harling* 16693 (MO), 1980, 31, 0, 0, 0, 1; *M. williamsonii*, *McDaniel* 15218 (MO), 1971, 40, 0, 0, 0, 1; *Megistostegium microphyllum* Hochst., *Dorr* 4110 (MO), 1985, 26, 0, 0, 1, 2; *M. microphyllum*, *Dumort* 1245 (MO), 1990, 21, 1, 1, 2, 1; *M. microphyllum*, *McPherson* 14961 (MO), 1990, 21, 1, 1, 1, 1; *M. microphyllum*, *Schott* 2567 (MO), 1990, 21, 1, 1, 1, 1; *M. microphyllum*, *Willing* 71 (MO), 1988, 23, 0, 1, 2, 3; *M. modiolae* (Drake) Hochst., *Kesting* 2242 (MO), 1989, 22, 1, 1, 1, 2; *M. perrieri* Hochst., *Schott* 2971 (MO), 1990, 21, 0, 0, 0, 1; *Pavonia* Cav., *Ash* 2430 (MO), 1974, 37, 0, 0, 1, 2; *Pavonia* Cav., *Frits* 3351 (MO), 1982, 29, 1, 1, 2, 3; *Pavonia almatana* Ulbr., *Harley* 55518 (MO), 2006, 5, 1, 3, 1, 1; *P. angustifolia* Benth., *Philippe* 21398 (MO), 1993, 18, 1, 1, 1, 1; *P. antiaurita* (Standl.) Fryxell, *Flora-France* 3791 (MO), 1994, 17, 1, 1, 1, 1; *P. arborea* Hochst. ex Steud., *Aub* 2557 (MO), 1974, 37, 0, 0, 0, 1; *P. argentina* Gürke, *Krapovickas* 30817 (MO), 1977, 34, 1, 1, 1, 2; *P. aurigloba* Krapov. & Cristóbal, *Krapovickas* 47544 (MO), 1998, 13, 1, 1, 1, 2; *P. aurigloba*, *Quarin* 2908 (MO), 1975, 36, 0, 0, 1, 2; *P. betonicifolia* C. Presl, *Pedersen* 13407 (MO), 1982, 29, 0, 0, 1, 2; *P. burchellii* (DC.) R.A. Dyer, *Boular* 11406 (MO), 1977, 34, 0, 0, 1, 2; *P. burchellii*, *Rasaburindore* 5849 (MO), 2004, 7, 1, 1, 1, 1; *P. burchellii*, *Sousa* 59 (MO), 1999, 12, 1, 3, 1, 1; *P. burchellii*, *Venter* 10222 (MO), 2003, 8, 1, 3, 1, 0; *P. calycina* Ulbr., *Zimba* 1000 (MO), 1997, 14, 1, 3, 1, 2; *P. cancellata* (L.) Cav., *Haber* 8903 (MO), 1988, 23, 0, 1, 1, 1; *P. cancellata*, *Krapovickas* 42905 (MO), 1989, 22, 1, 1, 1, 1; *P. cancellata*, *Short* 152 (MO), 1996, 15, 1, 3, 1, 2; *P. castaneifolia* A. St.-Hil. & Naudin, *Harling* 24230 (MO), 1985, 26, 1, 2, 1, 3; *P. castaneifolia*, *Mayfield* s.n. (MO), 2001, 10, 1, 1, 1, 1; *P. clathrata* Mast., *Balloulli* 4332 (MO), 1989, 22, 1, 3, 1, 2; *P. coccinea* Cav., *Gen-*
- try* 50560 (MO), 1985, 26, 1, 1, 1, 2; *P. columella* Cav., *Chrysanthemum* 8720 (MO), 1987, 24, 1, 2, 1, 3; *P. columella*, *Lesserdy* (MO), 1961, 50, 0, 0, 0, 1; *P. columella*, *Stalmans* 1594 (MO), 1987, 24, 1, 3, 1, 2; *P. columella*, *Stalmans* 316 (MO), 1984, 7, 0, 1, 2, 3; *P. communis* A. St.-Hil., *Zardini* 8578 (MO), 1981, 23, 1, 1, 2, 3; *P. communis* Gürke, *Krapovickas* 43957 (MO), 1992, 19, 1, 1, 1, 1; *P. cryptica* Krapov. & Cristóbal, *Jimenez* 845 (MO), 2008, 3, 1, 3, 0, 0; *P. cymbalaria* A. St.-Hil. & Naudin, *Projecto Venetia* 659 (MO), 1979, 32, 0, 0, 1, 2; *P. dappatata* Turcz., *Foster* 1455 (MO), 1969, 42, 0, 0, 0, 1; *P. dentata* Barn Davy, *Souub* 152 (MO), 1983, 28, 1, 1, 2, 3; *P. denudata* Krapov., *Krapovickas* 41956 (MO), 1988, 23, 0, 0, 1; *P. eurychlamys* Ulbr., *Gutiérrez* 1046 (MO), 2004, 7, 1, 3, 2, 1; *P. flexuosa* Miq., *Krapovickas* 35498 (MO), 1980, 31, 0, 0, 1; *P. flauferuginea* (Porsak), Hepper & J.R.I. Wood, *Liebenberg* 8828 (MO), 1977, 34, 0, 0, 0, 1; *P. flauferuginea*, *Sims* 64 (MO), 2001, 10, 1, 3, 1, 1; *P. formosa* Fryxell, *Apaza* 82 (MO), 2007, 4, 1, 3, 1, 1; *P. frixi* Krapov., *Gutiérrez* 1116 (MO), 2004, 7, 1, 3, 1, 1; *P. fruticosa* (Mill.) Fawc. & Rendle, *Mayfield* s.n. (Sp. ID 585-585-1205) (MO), 2001, 10, 0, 0, 0, 1; *P. fruticosa*, *McDaniel* 23669 (MO), 1980, 31, 1, 3, 1, 1; *P. frutescens* Taylor 11637 (MO), 1994, 17, 1, 3, 1, 2; *P. fruticosa*, *Tec* 1009 (MO), 2007, 4, 1, 1, 1, 1; *P. fruticosa* Krapov., *Tern* 12561 (MO), 1988, 23, 1, 3, 1, 2; *P. gareckiana* Gürke, *Hausbach* 43833 (MO), 1981, 30, 0, 0, 0, 1; *P. gareckiana*, *Villarroel* 2047 (MO), 2008, 3, 1, 1, 1, 1; *P. geminiflora* Moric., *Jansen* & *cobs* 2753 (MO), 1992, 19, 1, 3, 0, 0; *P. glazioviana* Gürke, *Harley* 18936 (MO), 1977, 34, 0, 0, 0, 1; *P. glechomifolia* (Rich.) Gürke, *O'Brien* 194 (MO), 1985, 26, 0, 0, 1, 1; *P. glechomoides* A. St.-Hil., *Cervantes* E. 25 (MO), 2006, 5, 1, 3, 2, 1; *P. gracilis* R.E. Fr., *Ner* 38930 (MO), 1990, 21, 1, 1, 1, 1; *P. grandiflora* A. St.-Hil., *Iruvia* 26532 (MO), 1970, 41, 0, 1, 2, 1; *P. gareckiana* R.E. Fr., *Krapovickas* 22998 (MO), 1973, 38, 0, 1, 2; *P. gareckiana*, *Oliviera* 238 (MO), 1980, 31, 0, 0, 1, 2; *P. harleyi* Krapov., *Marim* 1686 (MO), 1994, 17, 1, 1, 3, 1; *P. hamiltoniana* Chodat, *Krapovickas* 44588 (MO), 1993, 18, 1, 1, 2; *P. hamata* Cav., *Lepisch* 534 (MO), 1991, 20, 1, 1, 2, 3; *P. hamata*, *Villarroel* 1414 (MO), 2007, 4, 1, 3, 1, 1; *P. hebe* Zardini 56658 (MO), 2001, 10, 0, 0, 0, 0; *P. heterotricha* Fryxell, *Ner* 48381 (MO), 1998, 13, 1, 1, 1, 2; *P. hieronymi* Gürke, *Gutiérrez* 755 (MO), 2004, 7, 1, 1, 1, 1; *P. hieronymi* Gürke, *Luzano* 1363-A (MO), 2005, 6, 1, 3, 1, 1; *P. hieronymi* Gürke, *Zardini* 29464 (MO), 1991, 20, 1, 2, 1, 3; *P. humifusa* A. St.-Hil., *Krapovickas* 31548 (MO), 1977, 34, 0, 0, 1, 2; *P. involucrata* R.E. Fr., *Flor* 62 (MO), 2008, 3, 1, 3, 2, 1; *P. involucrata* Krapov., *Fuentes* 3326 (MO), 2001, 10, 1, 1, 1, 1; *P. kilimani* Gürke, *Mwanambi* 16187 (MO), 1992, 19, 1, 1, 2; *P. kilimanjacharica* Gürke, *Simons* 601 (MO), 2000, 11, 1, 3, 2, 1; *P. kotschyana* Hochst. ex Webb, *Wieland* 4495 (MO), 1987, 24, 1, 2, 1; *P. leucophylla* A. St.-Hil., *Soleman* 13715 (MO), 1985, 26, 1, 2, 3; *P. leucophaea* Urb., *Burb* 2530 (MO), 1970, 41, 1, 1, 3; *P. leucantha* Poepp. ex Gürke, *Körning* 47064 (MO), 1981, 28, 0, 0, 1, 2; *P. malacogallii* Fryxell, *Hernandez* 2318 (MO), 1998, 13, 1, 1, 1, 1; *P. malacophylla* (Link & Otto) Garcke, *Nelos* 4685 (MO), 1971, 40, 1, 1, 1, 1; *P. malacophylla*, *Wöh* 3328 (MO), 1996, 15, 1, 1, 1, 1; *P. martii* Colla, *Iraia* 233 (MO), 1969, 42, 0, 1, 2, 3; *P. matogrossensis* R.E. Fr., *Krapovickas* 44410 (MO), 1993, 18, 1, 1, 1, 1; *P. minima* Ekman, *Krapovickas* 14635 (MO), 1969, 42, 0, 0, 1, 2; *P. montis* Fryxell, *Alvarado* C. 728 (MO), 2002, 9, 1, 3, 1, 0; *P. multiflora*

DNA PRESERVATION IN PLANT SPECIMENS

A. St.-Hil., *Kallunki* 588 (MO), 1994, 17, 1, 3, 1, 1; *P. mertii* Kunth, *Ner* 47856 (MO), 1998, 13, 1, 1, 1, 1; *P. merrii* Krapov., *Zardini* 9203 (MO), 1989, 22, 1, 1, 1, 2; *P. mayarensis* Fryxell, *Flora F* 1302 (MO), 1989, 22, 1, 3, 2, 1; *P. apulifolia* S. Moore, *Zardini* 27616 (MO), 1991, 20, 1, 1, 2, 3; *P. acyphylaria* Donn. Sm., *Bailey* 651 (MO), 1973, 38, 0, 0, 1; *P. acyphylaria*, *Liesner* 5062 (MO), 1978, 33, 0, 0, 0, 1; *P. acyphylaria*, *Villars* 6 (MO), 2004, 7, 1, 3, 1, 1; *P. paludicola* Nicolson ex Fryxell, *Prewar* 4104 (MO), 2001, 10, 0, 0, 1, 1; *P. paludicola*, *Wurthington* 21260 (MO), 1992, 19, 1, 3, 2, 1; *P. paniculata* Cav., *Haenckia* 49 (MO), 1988, 23, 1, 3, 1, 2; *P. paniculata*, *Jans* 9 (MO), 1972, 39, 1, 1, 2, 3; *P. paniculata*, *Milanowski* 5 (MO), 1993, 18, 1, 3, 1, 2; *P. paniculata*, *Whiteford* 10126 (MO), 1998, 13, 1, 3, 1, 1; *P. paniculata* Hochst., *Belgiana* 329 (MO), 2005, 6, 1, 1, 1, 1; *P. penduliflora* (Standl.) Standl., *Fernandez* 333 (MO), 1992, 19, 0, 0, 0, 1; *P. persicaria* Giseke, *Dodon* 8795 (MO), 1979, 32, 0, 0, 0, 1; *P. persicaria*, *Haber* 680 (MO), 1984, 27, 0, 0, 1, 1; *P. persicaria*, *Mander* 2759 (MO), 1994, 17, 1, 1, 1, 1; *P. persicaria*, *Ner* 9761 (MO), 1974, 37, 0, 0, 0, 1; *P. plementina* (DC.) Fryxell, *Telles* V. 11842 (MO), 1989, 22, 1, 1, 1, 2; *P. pratincola* Cav., *Youthbed* 737 (MO), 1998, 13, 1, 2, 1, 3; *P. procumbens* Wulp., *Solan* 3760 (MO), 1969, 42, 0, 1, 2, 3; *P. pseudophyllata* Planch. & Linden ex Triana & Planch., *Delgado* 71 (MO), 1999, 12, 0, 1, 1, 1; *P. rimbai* Krapov. & Cristobal, *Serr* 282 (MO), 1999, 12, 1, 3, 2, 1; *P. rimbai* (Aitchav.) Fryxell & Krapov., *Knapenicka* 41933 (MO), 1988, 23, 0, 0, 1, 2; *P. rhinanthus* Scyzyl., *Syed* 4484 (MO), 1967, 44, 1, 1, 1, 1; *P. rotundifolia* Wall. ex Morris, *Cantlie* M. 2103 (MO), 1993, 18, 0, 0, 1, 2; *P. rotundifolia*, *Melendez* 929 (MO), 2005, 6, 1, 2, 1, 0; *P. rupestris* (Hassk.) Krapov. & Cristobal, *Perez* 504 (MO), 1991, 20, 1, 2, 1, 3; *P. sagittata* A. St.-Hil., *Knapenicka* 35349 (MO), 1980, 31, 0, 0, 1, 2; *P. schiedemae* Steud., *Dwyer* 7937 (MO), 1967, 44, 0, 0, 0, 1; *P. schiedemae*, *Oliviria* 287 (MO), 1981, 30, 0, 0, 1, 2; *P. schiedemae*, *Reyes-Garcia* 7436 (MO), 2004, 7, 0, 0, 0, 1; *P. schiedemae* Hinch. & Dalziel, *Frits* 8856 (MO), 1998, 13, 1, 1, 1, 2; *P. schiedemae* Spreng., *Knapenicka* 40761 (MO), 1987, 24, 0, 1, 2, 3; *P. senegalensis* Leistner, *Davide* 6379 (MO), 1974, 37, 0, 0, 1, 2; *P. senegalensis* Leistner, *Long* 429 (MO), 1987, 24, 1, 1, 2, 3; *P. senegalensis* Leistner, *Serrano* 30 (MO), 2002, 9, 1, 3, 2, 1; *P. spinipes* Fryxell & Krapov., *Harling* 22308 (MO), 1985, 26, 1, 1, 2, 3; *P. spinipes*, *Ner* 45332 (MO), 1994, 17, 1, 3, 1, 0; *P. stipitata* Kunth, *Guareco* 248 (MO), 1995, 16, 1, 3, 1, 2; *Talliaris* *hastatum* (L.) Fryxell, *Gaskin* s.n. (FLAS), 1959, 54, 1, 1, 1, 2; *T. tiliaceum* (L.) Fryxell, *Weber* s.n. (FLAS), 1949, 64, 0, 0, 1, 1.

C) Frozen at -20°C and -80°C: *Chrysanthemum* L. (Asteraceae), *Urbach* 7055 (LSU), 1995, 18, 1, 3, 1, 2; *Chrysanthemum* *depressum* Nutt. (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Chrysanthemum* Nutt. (Asteraceae), *NA* (NA), 2000, 13, 1, 3, 1, 2; *Leucanthemoides* *spatulifolium* (L.C. Anderson) Urbach, R.P. Roberts & Neubig (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Colombia* *leptophylla* G.L. Nesom (Asteraceae), *NA* (NA), 2000, 13, 1, 3, 1, 1; *Rudbeckia* *amplexicaulis* Vahl (Asteraceae), *Urbach* 6640 (LSU), 1990, 23, 1, 3, 1, 2; *Echinacea* *angustifolia* DC. (Asteraceae), *Bodick* s.n. (LSU), 1995, 18, 1, 3, 1, 1; *Echinacea* *purpurea* (L.) Moench (Asteraceae), *Urbach* 7050 (LSU), 1995, 18, 1, 3, 1, 1; *Echinacea* *purpurea* (L.) Moench (Asteraceae), *Urbach* 5879 (LSU), 1989, 24, 1, 3, 1, 1; *Echinacea* *sum-*

guinea Nutt. (Asteraceae), *Urbach* 6001 (LSU), 1989, 24, 1, 3, 1, 1; *Ericameria* *bloomeri* (A. Gray) J.F. Macbr. (Asteraceae), *NA* (NA), 2000, 15, 1, 3, 1, 1; *Ericameria* *gilmanii* (S.E. Blake) G.L. Nesom (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Ericameria* *laricifolia* (A. Gray) Shinnens (Asteraceae), *NA* (NA), 1992, 21, 1, 3, 1, 1; *Ericameria* *linearifolia* (DC.) Urbach & Wissow (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Ericameria* *nana* Nutt. (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Ericameria* *paniculata* (A. Gray) Rydb. (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Ericameria* *parviflora* (Greene) H.M. Hall (Asteraceae), *NA* (NA), 1995, 18, 0, 0, 1, 2; *Ericameria* *teretifolia* (Durand & Hilg.) Jeps. (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Ericameria* *zionis* (L.C. Anderson) G.L. Nesom (Asteraceae), *NA* (NA), 2000, 13, 1, 3, 1, 2; *Eurybia* (Cass.) Cass. (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Gaillardia* *seminivalis* (Walter) H. Rock (Asteraceae), *Urbach* 7057 (LSU), 1995, 18, 1, 3, 1, 1; *Helenium* *bigelovii* A. Gray (Asteraceae), *Urbach* 6798 (LSU), 1990, 23, 1, 3, 1, 1; *Ratibida* *pinnata* (Vahl.) Barnhart (Asteraceae), *Urbach* 6518 (LSU), 1990, 23, 1, 3, 1, 1; *Rudbeckia* *alpicola* Piper (Asteraceae), *Urbach* 6810 (LSU), 1990, 23, 1, 3, 1, 1; *Rudbeckia* *foliigera* Aitton (Asteraceae), *Urbach* 7117 (LSU), 1996, 17, 1, 3, 1, 2; *Rudbeckia* *foliigera* Aitton (Asteraceae), *Urbach* 7118 (LSU), 1996, 17, 1, 3, 1, 2; *Rudbeckia* *foliigera* Aitton (Asteraceae), *Urbach* 7106 (LSU), 1996, 17, 1, 3, 1, 2; *Rudbeckia* *grandiflora* (Sweet) J.F. Gmel. ex DC. (Asteraceae), *Urbach* 5866 (LSU), 1989, 24, 1, 3, 1, 1; *Rudbeckia* *hirta* L. (Asteraceae), *Urbach* 6607 (LSU), 1990, 23, 1, 3, 1, 2; *Rudbeckia* *hirta* L. (Asteraceae), *Urbach* 7115 (LSU), 1992, 21, 1, 3, 1, 2; *Rudbeckia* *klamathensis* P.B. Cox & Urbach (Asteraceae), *Urbach* 6801 (LSU), 1990, 23, 1, 3, 1, 1; *Rudbeckia* *laevigata* L. (Asteraceae), *NA* (NA), 1992, 21, 1, 3, 1, 1; *Rudbeckia* *missouriensis* Engelm. ex C.L. Boynton & Beadle (Asteraceae), *NA* (NA), 1996, 17, 1, 3, 1, 1; *Rudbeckia* *missouriensis* Engelm. ex C.L. Boynton & Beadle (Asteraceae), *Urbach* 7115 (LSU), 1992, 21, 1, 3, 1, 2; *Rudbeckia* *mobrii* A. Gray (Asteraceae), *Urbach* 6701 (LSU), 1990, 23, 1, 3, 1, 1; *Rudbeckia* *mobrii* A. Gray (Asteraceae), *Urbach* 6703 (LSU), 1990, 23, 1, 3, 1, 1; *Rudbeckia* *nitida* Nutt. (Asteraceae), *Urbach* 6659 (LSU), 1990, 23, 1, 3, 1, 1; *Rudbeckia* *triloba* L. (Asteraceae), *Urbach* 6262 (LSU), 1989, 24, 1, 3, 2, 1; *Silphium* *compositum* Michx. (Asteraceae), *Urbach* 6626 (LSU), 1990, 23, 1, 3, 1, 1; *Silphium* *compositum* Michx. (Asteraceae), *Urbach* 6678 (LSU), 1990, 23, 1, 3, 1, 1; *Solidago* *speciosa* (L.) Willd. (Asteraceae), *Urbach* 7020 (LSU), 1994, 19, 1, 3, 1, 1; *Towmania* A. Nelson (Asteraceae), *NA* (NA), 2001, 12, 1, 3, 1, 1; *Chrysanthemum* Nutt. (Asteraceae), *NA* (NA), 2000, 13, 1, 3, 1, 1; *Wyethia* *amplexicaulis* (Nutt.) Nutt. (Asteraceae), *Urbach* 6809 (LSU), 1990, 23, 1, 3, 1, 1; *Wyethia* Nutt. (Asteraceae), *Urbach* 6800 (LSU), 1990, 23, 1, 3, 1, 1; *Glechoma* *trivialis* L. (Labiatae), *Urbach* 7041 (LSU), 1995, 18, 1, 3, 1, 2; *Sanicula* *albida* (Nutt.) Nees (Umbelliferae), *Urbach* 6507 (LSU), 1990, 23, 1, 3, 1, 1; *Amianthium* *multitrichum* (Walter) A. Gray (Melanthiaceae), *NA* (NA), 1995, 18, 1, 3, 1, 1; *Chamaelirium* Willd. (Melanthiaceae), *NA* (NA), 1999, 14, 0, 0, 0, 1; *Venidium* *californicum* Durand (Melanthiaceae), *NA* (NA), 1995, 18, 1, 3, 1, 2; *Zigadenus* *densus* (Dess.) Fernald (Melanthiaceae), *NA* (NA), 1995, 18, 0, 0, 0; *Zigadenus* *densus* (Dess.) Fernald (Melanthiaceae), *NA* (NA), 1995, 18, 1, 3, 1, 1; *Zigadenus* *nuttallii* (A. Gray) S. Watson (Melanthiaceae), *NA* (NA), 1995, 18, 1, 3, 1, 1; *Trident* *flavis* (L.) Hitchc. (Poaceae), *NA* (NA), 1998, 15, 1,

- 3, 1, 2; *Tridens flavus* (L.) Hitchc. (Poaceae), NA (NA), 1998, 15, 1, 3, 1, 2; *Tridens flavus* (L.) Hitchc. (Poaceae), NA (NA), 1998, 15, 1, 3, 1, 1; *Tridens strictus* (Nutt.) Nash (Poaceae), NA (NA), 1998, 15, 1, 3, 1, 1; *Zanthoxylum clava-herculis* subsp. *fruticosum* (A. Gray) Reynel (Rutaceae), NA (NA), 1998, 15, 1, 3, 1, 2. Silica-dried and in falcon tubes: *Altissmeria* L. (Asteioemerellaceae), Zomlefer 722 (FLAS), 1999, 14, 0, 1, 1, 1; *Zephyanthes* Herb. (Amaryllidaceae), Pintillo s.n. (FLAS), 1999, 14, 1, 1, 1, 1; *Zephyanthes* Herb. (Amaryllidaceae), Pintillo s.n. (FLAS), 1999, 14, 1, 1, 1, 1; *Zephyanthes* Herb. (Amaryllidaceae), Pintillo s.n. (FLAS), 1999, 14, 0, 1, 1, 1; *Buxus madagascariensis* (Lam.) Ker Gawl. (Celastraceae), Herbst s.n. (NA), 1995, 18, 0, 2, 1, 3; *Podophyllum peltatum* L. (Berberidaceae), Soltis 2522 (NA), 1995, 18, 1, 3, 1, 1; *Breva madagascariensis* (Lam.) Ker Gawl. (Celastraceae), Herbst s.n. (NA), 1995, 18, 1, 3, 1, 1; *Gloriosa superba* L. (Colchicaceae), Zomlefer 659 (FLAS), 1997, 16, 0, 1, 1, 1; *Juglans nigra* L. (Juglandaceae), Soltis 2520 (NA), 1995, 18, 0, 1, 2, 3; *Lilium catesbeianum* Walter (Liliaceae), Zomlefer 767 (FLAS), 1999, 14, 1, 3, 1, 1; *Amianthium muscitoxicum* (Walter) A. Gray (Melanthiaceae), NA (FLAS), 1999, 14, 0, 1, 1, 1; *Amianthium muscitoxicum* (Walter) A. Gray (Melanthiaceae), NA (FLAS), 1998, 15, 0, 1, 1, 1; *Amianthium muscitoxicum* (Walter) A. Gray (Melanthiaceae), Zomlefer 716 (FLAS), 1999, 14, 0, 1, 1, 1; *Amianthium muscitoxicum* (Walter) A. Gray (Melanthiaceae), Zomlefer 705 (FLAS), 1999, 14, 0, 1, 1, 1; *Chamaelirium luteum* (L.) A. Gray (Melanthiaceae), Wieboldt 10170 (FLAS), 1999, 14, 1, 3, 1, 1; *Melanthium latifolium* Desr. (Melanthiaceae), NA (FLAS), 1999, 14, 1, 3, 1, 1; *Melanthium latifolium* Desr. (Melanthiaceae), Wieboldt s.n. (FLAS), 1999, 14, 1, 3, 1, 1; *Melanthium latifolium* Desr. (Melanthiaceae), Pintillo s.n. (FLAS), 1999, 14, 1, 3, 1, 1; *Melanthium parviflorum* (Michx.) S. Watson (Melanthiaceae), Pintillo s.n. (FLAS), 1999, 14, 0, 1, 1, 1; *Melanthium parviflorum* (Michx.) S. Watson (Melanthiaceae), Pintillo s.n. (FLAS), 1999, 14, 0, 1, 1, 1; *Melanthium parviflorum* (Michx.) S. Watson (Melanthiaceae), Pintillo s.n. (FLAS), 1999, 14, 0, 1, 1, 1; *Schoenocaulon dubium* (Michx.) Small (Melanthiaceae), F-673 (FLAS), 1998, 15, 0, 1, 1, 1; *Schoenocaulon texanum* Scheele (Melanthiaceae), Sivinski 4712 (FLAS), 1999, 14, 0, 1, 1, 1; *Schoenocaulon texanum* Scheele (Melanthiaceae), Sivinski 4836 (FLAS), 1999, 14, 1, 3, 1, 1; *Schoenocaulon texanum* Scheele (Melanthiaceae), Worthington 28255 (FLAS), 1998, 15, 1, 1, 1, 1; *Schoenocaulon texanum* Scheele (Melanthiaceae), Wieboldt 7016 (FLAS), 1999, 14, 1, 3, 1, 2; *Stenanthium gramineum* (Ker Gawl.) Morong (Melanthiaceae), Ghosh 12683 (FLAS), 1999, 14, 1, 3, 2, 1; *Stenanthium gramineum* (Ker Gawl.) Morong (Melanthiaceae), Wykoff 308 (FLAS), 1999, 14, 0, 1, 1, 2; *Stenanthium gramineum* (Ker Gawl.) Morong (Melanthiaceae), Pintillo s.n. (FLAS), 1999, 14, 1, 3, 2, 1; *Trillium maculatum* Raf. (Melanthiaceae), Whitten s.n. (FLAS), 1998, 15, 1, 3, 2, 1; *Vestrum album* L. (Melanthiaceae), K. Siebach s.n. (FLAS), 1999, 14, 1, 3, 1, 1; *Vestrum californicum* Durand (Melanthiaceae), Roth 1012a (FLAS), 1999, 14, 1, 1, 1; *Vestrum californicum* Durand (Melanthiaceae), R-1012b (FLAS), 1999, 14, 1, 3, 1, 1; *Vestrum californicum* Durand (Melanthiaceae), Sivinski 4985 (FLAS), 1999, 14, 1, 3, 2; *Melanthium parviflorum* (Michx.) S. Watson (Melanthiaceae), Wykoff 307 (FLAS), 1999, 14, 1, 1, 1, 1; *Venatrum* (Melanthiaceae), Gerald Smith s.n. (FLAS), 1999, 14, 1, 3, 1; *Venatrum viride* Aitton (Melanthiaceae), Wykoff 306 (FLAS), 1999, 14, 1, 3, 2, 1; *Venatrum viride* Aitton (Melanthiaceae), Wieboldt 10172 (FLAS), 1999, 14, 1, 3, 1, 1; *Melanthium albidum* (J.W. Robbin ex Alph. Wood) Bodkin (Melanthiaceae), (FLAS), 1998, 15, 1, 3, 2, 1; *Melanthium woodii* (J.W. Robb. ex Alph. Wood) Bodkin (Melanthiaceae), Ghosh s.n. (FLAS), 1999, 14, 1, 3, 1, 1; *Zigadenus densiflorus* (Desr.) Fernald (Melanthiaceae), NA (FLAS), 1998, 15, 1, 3, 2, 1; *Zigadenus densiflorus* (Desr.) Fernald (Melanthiaceae), NA (FLAS), 1998, 15, 0, 1, 1; *Zigadenus elegans* Pursh (Melanthiaceae), Sivinski 4 (FLAS), 1999, 14, 1, 1, 1, 1; *Zigadenus elegans* Pursh (Melanthiaceae), NA (FLAS), 1999, 14, 1, 1, 1, 1; *Zigadenus glaberrimus* Michx. (Melanthiaceae), Zomlefer 696a (FLAS), 1999, 1, 1, 1, 1; *Zigadenus elegans* Pursh (Melanthiaceae), NA (FLAS), 1999, 14, 1, 3, 1, 1; *Zigadenus elegans* Pursh (Melanthiaceae), NA (FLAS), 1999, 14, 1, 3, 1, 1; *Zigadenus elegans* Pursh (Melanthiaceae), Sivinski 4 (FLAS), 1999, 14, 1, 3, 1, 1; *Zigadenus elegans* Pursh (Melanthiaceae), Wieboldt 7013 (FLAS), 15, 14, 1, 3, 1, 1; *Zigadenus microthrix* Eschscholtz (Melanthiaceae), NA (FLAS), 1999, 14, 0, 1, 1, 1; *Zigadenus macrocarpus* W.J. Hess & Sivinski (Melanthiaceae), Sivinski Tonno 5106 (FLAS), 1999, 14, 1, 3, 1, 0; *Zigadenus nuttallii* Gray S. Watson (Melanthiaceae), Wieboldt 7013 (FLAS), 15, 14, 1, 3, 1, 1; *Zigadenus virescens* (Kunth) J.F. Macbr. (Melanthiaceae), Sivinski 4793 (FLAS), 1999, 14, 1, 3, 2, 1; *Melanthium albidum* L. (Melanthiaceae), Soltis 2518 (NA), 1995, 18, 1, 2, 1, 3; *Melanthium latifolium* L. (Phytolaccaceae), Soltis 2523 (NA), 1995, 18, 1, 3, 1, 1; *Populus grandidentata* Michx. (Salicaceae), Pintillo s.n. (FLAS), 1999, 14, 0, 1, 1, 1; *Populus grandidentata* Michx. (Salicaceae), Pintillo s.n. (FLAS), 1999, 14, 0, 1, 1, 1; *Vitis* (Vitaceae), Soltis 2519 (NA), 1995, 18, 0, 1, 2, 3; Silica-dried in glass jars: *Acinetia chrysanthus* (C. Moore) Lindl. & Pax (Orchidaceae), Whitten 25031 (FLAS), 1995, 18, 1, 3, 1; *Benzingia reichenbachiana* (Schltr.) Dresler (Orchidaceae), (FLAS), 1995, 18, 0, 1, 1, 1; *Cirrhaea dependens* Loudon (Orchidaceae), Whitten 23152 (FLAS), 1995, 18, 0, 1, 1, 1; *rhizae pallidae* Lindl. (Orchidaceae), NA (FLAS), 1995, 18, 0, 1, 1; *Coelogyne hyacinthina* Rchb. E. (Orchidaceae), Whitten 93153 (FLAS), 1995, 18, 0, 1, 1, 1; *Gongora cassidea* Rchb. (Orchidaceae), Whitten 30532 (FLAS), 1995, 18, 0, 1, 1; *Gongora charvensis* Jenny (Orchidaceae), Whitten 91 (FLAS), 1995, 18, 0, 1, 1, 1; *Gongora erecta* Whitten D.E. Benn. (Orchidaceae), Bennett 4700-1 (FLAS), 1995, 18, 1, 1, 1; *Gongora hirtzii* Dodson & N.H. Williams (Orchidaceae), Whitten 93109 (FLAS), 1995, 18, 0, 1, 1, 1; *Gongora lechleriana* L. (Orchidaceae), Whitten 3241 (FLAS), 1995, 18, 1, 1, 1; *Gongora seaphermae* Rchb. F. & Warsz. (Orchidaceae), Whitten 87145 (FLAS), 1995, 18, 0, 1, 1, 1; *Kegeliella atropurpurea* L.O. Williams & A.H. Heller (Orchidaceae), Hill 87 (FLAS), 1995, 18, 0, 0, 0, 0; *Kegeliella kupperi* Mandl. (Orchidaceae), NA (FLAS), 1995, 18, 0, 0, 0, 0; *Lunddeggmannia* R. E. (Orchidaceae), NA (FLAS), 1995, 18, 0, 1, 1, 1; *Peristylus hookeri* (Orchidaceae), Whitten 90121 (FLAS), 1995, 18, 0,

- 1, 1; *Pescatoria* Rchb. f. (Orchidaceae), Whitten 2304 (FLAS), 1995, 18, 0, 1, 1, 1; *Stanhopea grandiflora* (Lodd.) Lindl. (Orchidaceae), Chase 90176 (FLAS), 1995, 18, 0, 1, 1, 1; *Stanhopea xytriophora* Rchb. f. (Orchidaceae), NA (FLAS), 1995, 18, 0, 1, 1, 1; *Xylobium colleyi* (Baseman ex Lindl.) Rolfe (Orchidaceae), Whitten 2225 (FLAS), 1995, 18, 0, 1, 1, 1; *Xylobium leucoglossum* (Rchb. f.) Rolfe (Orchidaceae), Whitten 91384 (FLAS), 1995, 18, 0, 1, 1, 1. Silica-dried in Ziploc[®] bags: *Agathis lanceolata* Wash. (Araucariaceae), Plunkett 4676 (NA), 1997, 16, 0, 0, 0; *Balanophora* J.R. Forst. & G. Forst. (Balanophoraceae), Plunkett 4702 (NA), 1997, 16, 0, 0, 0; *Berberis lemannii* Hieron. (Berberidaceae), Whitten 2314 (FLAS), 2003, 10, 1, 3, 1, 1; *Apteria aphylla* (Nutt.) Barnhart ex Small (Burmanniaceae), Whitten 2321 (FLAS), 2003, 10, 1, 3, 1, 1; *Erigone anatolica* Maxim. (Ericaceae), Tangerman s.n. (NA), 1994, 19, 0, 0, 0, 1; *Cladonia lutescens* (Michx.) K. Koch (Fabaceae), Gentou s.n. (NA), 1993, 20, 1, 3, 1, 1; *Bowlesia* Thomas (Gesneriaceae), Whitten 2320 (FLAS), 2003, 10, 0, 0, 0; *Ribes aureum* Pursh (Grossulariaceae), Brownfield s.n. (NA), 2005, 8, 1, 3, 1, 1; *Aglaia meridionalis* Pannell (Meliaceae), NA (NA), 1997, 16, 0, 1, 1, 1; *Myrsinæ* O. Berg (Myrtaceae), Whitten 2311 (FLAS), 2003, 10, 1, 3, 1, 1; *Cryptocentrum latifolium* Schltr. (Orchidaceae), Whitten 2349 (FLAS), 2003, 10, 1, 3, 1, 1; *Cryptocentrum lehmannii* (Rchb. f.) Garay (Orchidaceae), Whitten 2325 (FLAS), 2003, 10, 1, 3, 1, 1; *Cryptocentrum* Benth. (Orchidaceae), Whitten 2322 (FLAS), 2003, 10, 1, 3, 1, 1; *Cryptocentrum* Benth. (Orchidaceae), Whitten 2324 (FLAS), 2003, 10, 1, 3, 1, 1; *Cryptocentrum standleyi* Ames (Orchidaceae), Whitten 2323 (FLAS), 2003, 10, 0, 0, 0; *Cynochilum auratum* (Lindl.) Senghas (Orchidaceae), Whitten 2354 (FLAS), 2003, 10, 1, 3, 1, 1; *Cynochilum* Kuenzli (Orchidaceae), Whitten 2352 (FLAS), 2003, 10, 1, 3, 2, 1; *Dichaea panamensis* Lindl. (Orchidaceae), Whitten 2348 (FLAS), 2003, 10, 1, 3, 1, 1; *Dichaea* Lindl. (Orchidaceae), Whitten 2329 (FLAS), 2003, 10, 1, 3, 1, 1; *Ellenanthus sumatranus* (Lindl.) Rchb. f. (Orchidaceae), Whitten 2355 (FLAS), 2003, 10, 1, 2, 3, 1; *Epidendrum* L. (Orchidaceae), Whitten 2336 (FLAS), 2003, 10, 1, 3, 1, 1; *Epidendrum* L. (Orchidaceae), Whitten 2345 (FLAS), 2003, 10, 1, 3, 1, 1; *Erycinis pumilio* (Rchb. f.) N.H. Williams & M.W. Chase (Orchidaceae), Whitten 2341 (FLAS), 2003, 10, 1, 3, 1, 1; *Janusia utricularioides* (Sw.) Lindl. (Orchidaceae), Whitten 2346 (FLAS), 2003, 10, 1, 3, 2, 1; *Koellensteinia graminea* (Lindl.) Rchb. f. (Orchidaceae), Whitten 2319 (FLAS), 2003, 10, 1, 3, 1, 1; *Konanecia mitisiflora* Dodson & N.H. Williams (Orchidaceae), Whitten 2371 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria angustifolia* Ames, ET. Hubb. & C. Schweinf. (Orchidaceae), Whitten 2338 (FLAS), 2003, 10, 0, 1, 1, 1; *Maxillaria angustifolia* Ames, ET. Hubb. & C. Schweinf. (Orchidaceae), Whitten 2361 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria* Poep. & Endl. I.O. Williams (Orchidaceae), Whitten 2316 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria* ayampetensis Foldas (Orchidaceae), Whitten 2347 (FLAS), 2003, 10, 1, 3, 2, 1; *Maxillaria ayampetensis* Foldas (Orchidaceae), Whitten 2360 (FLAS), 2003, 10, 1, 3, 2, 1; *Maxillaria boliviensis* C. Schweinf. (Orchidaceae), Whitten 2365 (FLAS), 2003, 10, 0, 0, 0; *Maxillaria confusa* Ames & C. Schweinf. (Orchidaceae), Whitten 2367 (FLAS), 2003, 10, 1, 3, 2, 1; *Maxillaria cyathophylloides* Carnevali & J.T. Arwood (Orchidaceae), Whitten 2300 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria curvata* Lindl. (Orchidaceae), Whitten 2301 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria diabolus* (G. Lodd. ex Lindl.) Rchb. f. (Orchidaceae), Whitten 2350 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria diurna* Ames & C. Schweinf. (Orchidaceae), Whitten 2305 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria equitans* (Schltr.) Garay (Orchidaceae), Whitten 2309 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria evoluta* Kraenzl. C. Schweinf. (Orchidaceae), Whitten 2332 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria evoluta* (Kraenzl.) C. Schweinf. (Orchidaceae), Whitten 2333 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria gracilis* Lodd. (Orchidaceae), Whitten 2303 (FLAS), 2003, 10, 1, 3, 1, 1; *Sauvagesia laevilabris* (Lindl.) M.A. Blanco (Orchidaceae), Whitten 2358 (FLAS), 2003, 10, 1, 3, 1, 1; *Sauvagesia laevilabris* (Lindl.) M.A. Blanco (Orchidaceae), Whitten 2372 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria nardina* Kraenzl. (Orchidaceae), Whitten 2359 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria nigrescens* Lindl. (Orchidaceae), Whitten 2302 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria phoenixanthus* Benth. Rodr. (Orchidaceae), Whitten 2304 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria posseggii* Rchb. f. (Orchidaceae), Whitten 2363 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria pseudoreichenheimiana* Dodson (Orchidaceae), Whitten 2330 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria pseudoreichenheimiana* Dodson (Orchidaceae), Whitten 2335 (FLAS), 2003, 10, 1, 3, 1, 1; *Mormolyce richii* (Dodson) M.A. Blanco (Orchidaceae), Whitten 2362 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), Whitten 2317 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), Whitten 2318 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), Whitten 2337 (FLAS), 2003, 10, 0, 1, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), Whitten 2368 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), Whitten 2373 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria triplaris* E. Morren (Orchidaceae), Whitten 2366 (FLAS), 2003, 10, 1, 3, 1, 1; *Maxillaria whittemeri* Dodson (Orchidaceae), Whitten 2364 (FLAS), 2003, 10, 1, 3, 2, 1; *Oncidium adamsii* (Dodson) M.W. Chase & N.H. Williams (Orchidaceae), Whitten 2339 (FLAS), 2003, 10, 1, 3, 1, 1; *Oncidium obryantoides* Kraenzl. (Orchidaceae), Whitten 2343 (FLAS), 2003, 10, 1, 3, 1, 1; *Oncidium Sw.* (Orchidaceae), Whitten 2331 (FLAS), 2003, 10, 1, 3, 1, 1; *Oncidium Sw.* (Orchidaceae), Whitten 2340 (FLAS), 2003, 10, 1, 3, 1, 1; *Oncidium weinmannianum* (Königer) M.W. Chase & N.H. Williams (Orchidaceae), Whitten 2328 (FLAS), 2003, 10, 1, 3, 1, 1; *Ornithocephalus bicolor* Lindl. ex Benth. (Orchidaceae), Whitten 2375 (FLAS), 2003, 10, 1, 1, 1, 1; *Ornithocephalus kruegeri* Rchb. f. (Orchidaceae), Whitten 2370 (FLAS), 2003, 10, 1, 3, 1, 1; *Ornithocephalus Hook.* (Orchidaceae), Whitten 2369 (FLAS), 2003, 10, 1, 1, 1, 1; *Ornithocephalus murecii* Dodson (Orchidaceae), Whitten 2374 (FLAS), 2003, 10, 1, 1, 1, 1; *Pachyphyllum* Kunth (Orchidaceae), Whitten 2313 (FLAS), 2003, 10, 1, 3, 1, 1; *Rodriguezia Lehmannii* Rchb. f. (Orchidaceae), Whitten 2342 (FLAS), 2003, 10, 1, 3, 1, 1; *Trichoceras suemijifer* (Bonpl.) Kunth (Orchidaceae), Whitten 2353 (FLAS), 2003, 10, 1, 3, 2, 1; *Metadactylum antecardoides* (Broen. & Gris.) Baum-Bod. ex Melikyan & A.V. Bobrov (Podocarpaceae), Plunkett 4675 (NA), 1997, 16, 1, 2, 1, 3; *Na* (Podocarpaceae), Plunkett 4683 (NA), 1997, 16, 0, 1, 1, 1; *Monnieria crassifolia* (Bonpl.) Kunth (Polygalaceae), Whitten 2357 (FLAS), 2003, 10, 1, 3, 1, 2; *Monnieria subspicata* Chodat (Polygalaceae), Whitten 2356 (FLAS), 2003, 10, 0, 0, 0, 0; *Anthonoxys splans* (R. Br.) Coompton (Taxaceae), Plunkett 4724

(NA), 1997, 16, 0, 1, 1, 2.

D) Samples for comparison of freshly collected, new extraction of old silica, and original extractions: *Braea* R. Br. (Orchidaceae), *Williams* N519 (FLAS), 2001, 12, 1, 1, 1; *Braea* R. Br. (Orchidaceae), *Williams* N519 (FLAS), 2013, 0, 1, 3, 1, 1; *Cochleanthes flabelliformis* (Sw.) R.E. Schult. & Garay (Orchidaceae), *Whitten* 3025 (FLAS), 2013, 0, 1, 3, 2, 1; *Cochleanthes flabelliformis* (Sw.) R.E. Schult. & Garay (Orchidaceae), *Whitten* 3025 (FLAS), 2005, 8, 1, 3, 1; *Maxillaria crassifolia* (Lindl.) Rehb. f. (Orchidaceae), *Whitten* 2544 (FLAS), 2013, 0, 1, 3, 1, 1; *Maxillaria crassifolia* (Lindl.) Rehb. f. (Orchidaceae), *Whitten* 2544 (FLAS), 2003, 10, 1, 3, 2, 1; *Inni* M.A. Blanco (Orchidaceae), *Whitten* 2752 (FLAS), 2004, 9, 1, 3, 1, 1; *Inni* M.A. Blanco (Orchidaceae), *Whitten* 2752 (FLAS), 2013, 0, 1, 3, 1, 1; *Maxillaria luteosulca* Lindl. (Orchidaceae), *Whitten* 2998 (FLAS), 2005, 8, 1, 3, 2, 1; *Maxillaria luteosulca* Lindl. (Orchidaceae), *Whitten* 2998 (FLAS), 2013, 0, 1, 3, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), *Whitten* 3230 (FLAS), 2006, 7, 1, 3, 1, 1; *Maxillaria Ruiz & Pav.* (Orchidaceae), *Whitten* 3230 (FLAS), 2013, 0, 1, 3, 1, 1; *Mormolyce ringens* (Lindl.) Schltr. (Orchidaceae), *Whitten* 2858 (FLAS), 2004, 9, 0, 1, 1, 1; *Mormolyce ringens* (Lindl.) Schltr. (Orchidaceae), *Whitten* 2858 (FLAS), 2013, 0, 1, 3, 1, 1; *Oncidium leucochilum* Bateman ex Lindl. (Orchidaceae), *Whitten* 2961 (FLAS), 2005, 8, 1, 3, 1, 1; *Oncidium leucochilum* Bateman ex Lindl. (Orchidaceae), *Whitten* 2961 (FLAS), 2013, 0, 1, 3, 1, 1; *Stanhopea anfracta* Rolfe (Orchidaceae), *Whitten* 3244 (FLAS), 2006, 7, 1, 3, 1, 1; *Stanhopea anfracta* Rolfe (Orchidaceae).

Whitten 3244 (FLAS), 2013, 0, 1, 3, 2, 1; *Stanhopea grandiflora* (Lodd.) Lindl. (Orchidaceae), *Whitten* 3012 (FLAS), 2013, 0, 1, 3, 1, 1; *Stanhopea oreata* (G. Lodd.) Lindl. (Orchidaceae), *Whitten* 3306 (FLAS), 2006, 7, 1, 3, 2, 1; *Stanhopea oreata* (G. Lodd.) Lindl. (Orchidaceae), *Whitten* 3306 (FLAS), 2013, 0, 1, 3, 1, 1; *Stanhopea Fruittii* Hook. (Orchidaceae), *Whitten* 2984 (FLAS), 2005, 8, 1, 3, 1, 1; *Stanhopea tigrina* Bateman ex Lindl. (Orchidaceae), *Whitten* 3007 (FLAS), 2005, 8, 1, 3, 1, 1; *Stanhopea tigrina* Bateman ex Lindl. (Orchidaceae), *Whitten* 3007 (FLAS), 2013, 0, 1, 3, 1, 1; *Stanhopea tricornis* Lindl. (Orchidaceae), *Whitten* 2985 (FLAS), 2005, 8, 0, 1, 1, 1; *Stanhopea tricornis* Lindl. (Orchidaceae), *Whitten* 2985 (FLAS), 2013, 0, 1, 3, 1, 1; *Trichopilia setigera* Rehb. f. (Orchidaceae), *Whitten* 2947 (FLAS), 2006, 7, 1, 3, 1, 1; *Trichopilia setigera* Rehb. f. (Orchidaceae), *Whitten* 2947 (FLAS), 2013, 0, 1, 3, 1, 1.

E) Silica-dried, photobleached samples: *Cattleya* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 0, 1, 1, 1; *Cattleya* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 3, 1, 1; *Canavalia* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 1, 1, 1; *Canavalia* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 3, 1, 2; *Catopsis* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 3, 1, 2; *Catopsis* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 3, 1, 1; *Catopsis* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 1, 1, 2; *Catopsis* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 3, 1, 2; *Catopsis* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 1, 1, 2; *Catopsis* Rich. ex Kunth (Orchidaceae), *NA* (NA), 1998, 15, 1, 3, 1, 2.