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EDITORIAL

WHY VOUCHERS MATTER IN BOTANICAL RESEARCH[1]

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What is a voucher and why is it important in research? As a preserved specimen of an identified taxon deposited in a permanent and accessible storage facility, the voucher serves as the supporting material for published studies of the taxon and ensures that the science is repeatable. Vouchers are crucial in authenticating the taxonomy of an organism, as a tool for identifying localities of the taxon, and for additional taxonomic, genetic, ecological, and/or environmental research.

Key words: climate change; herbarium specimen; taxa; taxon; taxonomy; voucher.

Many journals focused on the plant sciences, including the Botanical Society of America's journals, *Applications in Plant Sciences* (*APPS*) and the *American Journal of Botany*, require submission of vouchers as a condition for publication of articles. This is not new, as there have been repeated calls for requiring voucher citations within publications as a means to document scientific data (e.g., Goldblatt et al., 1992; Funk et al., 2005). What is a voucher, and more importantly, why do vouchers even matter? This is a question that we have heard in recent months, both from authors of *APPS* submissions and from new authors entering the publishing arena.

A voucher can be broadly defined as a representative sample of an expertly identified organism that is deposited and stored at a facility from which researchers may later obtain the specimen for examination and further study. As such, a voucher is a specimen that has been specifically collected and accessioned to support a research project (e.g., genetic analysis of a taxon) or activity (e.g., a floristic survey of a park). In some cases, a voucher specimen may be collected in the field and then identified later. For plants, a voucher typically consists of a herbarium specimen, a pressed and dried sample of an individual containing aboveground structures (leaves, stems, flowers, and/or fruits) and belowground structures when possible. The sample is usually mounted onto acid-free paper, although depending on the type of specimen, it can also be stored in a box, packet, or jar under controlled environmental conditions (see Leisner, 2013). The voucher must include an identification label that ideally lists the recognized scientific name of the plant, its accepted taxonomic authority, the name of the person who identified the sampled plant, the collector's name, date of collection, habitat of the collection site, locality of the site (preferably consisting of GPS coordinates, with the corresponding datum and degree of accuracy), and perhaps a collection number assigned by the collector; a unique accession number is sometimes added later by the herbarium. The specimen might also include annotations indicating taxonomic changes made by an expert familiar with the taxon. The voucher specimen should be deposited in an official herbarium that is located at a recognized institution and committed to the long-term maintenance of its collection, such as those herbaria registered with Index Herbariorum (http://sweetgum.nybg.org/ih/). Because of the need for accessibility, especially by future researchers, vouchers stored in personal herbaria are not considered acceptable.

Researchers can access vouchers through loan requests that physically transfer specimens to a local herbarium for study or, at some institutions, through direct, online access to highly detailed images of vouchers generated through digitization initiatives. For example, nearly two million high-resolution images of type specimens are now available through the Global Plants Initiative website (http://plants.jstor.org). Although such images offer a distinct advantage due to their almost instantaneous accessibility, examining the actual voucher specimen still remains the preferred method for many researchers because some critical morphological features may remain hidden, be too small, or are difficult to discern from two-dimensional images.

As samples deposited by a researcher, vouchers are essential because they provide credibility and are the only concrete and verifiable evidence about the taxon that is the focus of the published research. Furthermore, vouchers can have importance well beyond simply documenting the organism used in a study. Some of these uses that may not be immediately apparent are detailed below:

1. Vouchers are critical for authenticating the identification of a taxon

According to the *International Code of Nomenclature for algae, fungi, and plants* (McNeill et al., 2012), the published name of a species must be associated with a "type specimen," a representative sample of the entity being described. As a permanent record, a type specimen can be used for comparison to determine whether a newly discovered plant is novel or has already been described. Because species identification can change over time as our knowledge about plants grows and

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evolves, the type specimen and other vouchers under the same name or synonyms also serve as cross-references linking changes in taxonomy (e.g., Curtis, 2013). Previously identified vouchers can also be valuable tools to assist researchers in confirming the identity of an unknown plant after dichotomous keys have been used to narrow down the possibilities to several plausible options. Furthermore, vouchers also provide a means for other researchers to verify published taxonomic determinations and ensure that a taxonomist's work is reliable and reproducible—fundamental requirements of scientific investigation. In cases where the taxonomic determination in a published paper is questioned, the voucher can be examined to confirm the identification (see Eisenman et al., 2012 for examples). If there is no voucher and the determination cannot be checked, the value of the study diminishes considerably. In practice, the identity of each voucher deposited in a herbarium should always be confirmed directly by an expert or by comparing it to a specimen authenticated by a specialist in that particular family or genus. This can prevent mistakes in identification that even well-trained biologists can make with problematic species, especially if key diagnostic traits such as fruits or flowers are missing from a specimen. Certain federal agencies, such as the United States Department of Agriculture (USDA) Forest Service in northwestern states, now require that researchers conducting floristic surveys collect a voucher from each vascular plant species at each sampling site and that the specimen be placed in a public repository for verification of identification (USDA Forest Service, 2011). Unknown plant species may also be collected in the field and identified later before being deposited in a herbarium.

There have been several cases where the existence of voucher specimens enabled researchers to make new taxonomic discoveries that otherwise would have been impossible. For example, Jolles (in review, personal communication) observed inconsistencies in a molecular phylogeny of *Pyrola* L. and then carefully examined the voucher specimens corresponding to the outlier DNA samples. The vouchers were found to exhibit distinct differences in floral morphology and phenology that had previously gone undetected, revealing a new species of *Pyrola*. Without the vouchers, such a discovery would never have been possible, reinforcing the importance of backing up any empirical work with voucher specimens. Unfortunately, not all researchers collect vouchers during their work. For example, an allozyme analysis of a geographically distant *Viola pubescens* Aiton population indicated the unexpected presence of two distinct groups of genotypes (Culley and Wolfe, 2001); unfortunately, vouchers had not been collected during the initial collecting trip, so the population had to be revisited before the different genotypes were confirmed as two subspecies growing intermixed at the site.

The use of vouchers for taxon identity is crucial in ethnobotanical research in which botanical information collected from native inhabitants of an area is frequently limited to common plant names, often in local dialects. Vouchers provide a verifiable means to identify and distinguish these plants from one another, especially if they share the same common name. For example, vouchers were used to help identify a plant species known locally in China as 'MuTong' after patients experienced kidney failure following ingestion of Chinese herbal remedies containing this plant (Lord et al., 1999). The species was later identified as *Aristolochia manshuriensis* Kom., which is nephrotoxic and may have been inadvertently used in the herbal remedy as it shares the same common name as other nonlethal plant species growing in the area. This reinforces the necessity of proper identification of plant species and accompanying physical specimens, especially in distinguishing taxa with similar morphology or common names.

In the pharmaceutical community, herbarium specimens are essential for documenting the source material used for drug discovery (Eisenman et al., 2012). Because the efficacy of compounds in phytochemical and biological assays is often limited to a particular plant species, subspecies, or even an individual within a locality, the misidentification of a sample can dramatically hamper drug development. For example, anti-HIV michellamine compounds were obtained from plant material originally identified as *Ancistrocladus abbreviatus* Airy Shaw, but when more material was needed for testing, additional samples of the species from a different locality were found to lack bioactivity (see Eisenman et al., 2012). Because a voucher had been made of the original sample, researchers were able to revisit the initial collection site, and upon closer examination, they identified the population as a new plant species, *A. korupensis* D. Thomas & Gereau, which did contain the desired michellamines. Similarly, vouchers have been instrumental in clarifying the correct identity of *Tithonia* Desf. ex Juss. species from which the medicinally important compound, sesquiterpene lactone, had previously been extracted (La Duke and Difeo, 1981). Correct identification of study species is absolutely critical, as illustrated in these examples, and had vouchers not been collected during these investigations, therapeutic compounds may have been lost forever or their source remained unknown.

2. Vouchers serve as a tool for identifying locations of a taxon

Vouchers are often used to locate extant populations of a taxon for study because they represent known localities of the plant. Although locality information on older specimens may only contain the county or even country of collection, more recent specimens often include a detailed description of the collection site (e.g., location near a highway or cross streets) or highly accurate GPS coordinates. Any disagreement among researchers as to whether a taxon occurs at a given site can often be quickly resolved using a well-documented voucher as indisputable evidence. As verification of a species within a given area, vouchers are indispensable for authors writing floras of specific regions or taxonomic monographs. Herbarium specimens underlie much of the species locality information in the USDA PLANTS database (USDA NCRS, 2013), and are the basis for data on rare species curated by state natural heritage programs.

Although vouchers are valuable tools when used in this manner, caution is required in using older voucher data alone to confirm the occurrence of a species in a locality because a taxon may or may not currently occupy the site (Applequist et al., 2007), depending on the age of the voucher and the physical condition of the site (i.e., human alterations of the habitat). In addition to the possibility of erroneous determinations of taxonomy, herbarium collections often suffer from collector bias such that collections tend to represent certain genera or localities (i.e., along highways or near urban centers); this bias must be adequately addressed if the data will be used in distributional studies (Pyke and Ehrlich, 2010; Feeley, 2012). In the special case of vouchers collected from gardens and arboreta, locality information may only refer to the site at which the plant is cul-

tivated and not to the locality of the original source material. To assist future ecological and environmental research, researchers are encouraged to include as much information as possible on voucher labels or in accompanying computer databases (Pyke and Ehrlich, 2010); this includes detailed descriptions of nearby habitats or co-occurring plant species, notations of which specimens were collected in the same area or at the same time, and links to published data generated from that particular specimen (see also Ferren et al., 1995).

3. Vouchers deposited over time form a valuable database that can be used for additional research

Although herbarium collections were originally created as taxonomical repositories, researchers now recognize their applicability for genetic, ecological, and environmental studies (e.g., Pyke and Ehrlich, 2010). Because of their historical relevance, vouchers represent a unique opportunity to obtain data on individuals from past time periods (Primack and Miller-Rushing, 2009; Vellend et al., 2013). For example, studies of impacts of climate change on plants have used herbarium specimens to show how temperature variation causes shifts in geographic distributions (Feeley, 2012) and flowering times (Primack et al., 2004; Lavoie and Lachance, 2006; Miller-Rushing et al., 2006; Robbirt et al., 2011; Panchen et al., 2012; Calinger et al., 2013). In addition, herbarium data have been used to pinpoint locations in alpine areas for monitoring of especially sensitive native plants (Gallagher et al., 2009), and to assess diversity gradients in different areas of the world in relation to climate change (Droissart et al., 2012). Historical specimens have also been used to examine how plants have been affected by changing air quality and alterations in nutrient cycling. For example, historical specimens collected in 1804–1806 by the U.S. explorers Lewis and Clark were analyzed using carbon isotope ratios to generate baseline data on the amount of carbon present in the atmosphere over 200 yr ago (Teece et al., 2002). In addition, there is now evidence that reduced stomatal density in leaves is related to rising CO_2 levels over the past 200 yr (Woodward, 1987; Beerling and Chaloner, 1993), and increasing levels of nitrogen and other nutrients in bryophytes is associated with human activities within the past 100 yr (Peñuelas and Filella, 2001).

Uses of herbarium collections for investigations other than climate change include documentation of shifts in plant biodiversity with increasing urbanization (Dolan et al., 2011), population extinction of environmentally sensitive plants (Lienert et al., 2002), conservation priorities for taxa in endangered habitats (MacDougall et al., 1998), and human-induced reductions in individual plant size (dwarfism; Law and Salick, 2005). Herbarium specimens have also been used to track the movement of invasive species across a region, as in the cases of the ornamental Callery pear tree (*Pyrus calleryana* Decne.) following its commercial introduction in the United States in 1962 (Vincent, 2005), and *Typha* species, which have been spreading across North America for well over 100 yr (Shih and Finkelstein, 2008).

Despite the potential for degradation due to age and storage conditions, DNA can sometimes be obtained from historical and modern herbarium specimens (e.g., Lister et al., 2010; Zuntini et al., 2013), thus allowing genetic comparisons across time to examine population bottlenecks and other processes (e.g., Wandeler et al., 2007). For example, Cozzolino et al. (2007) used DNA from herbarium specimens to show that levels of genetic variation in the endangered orchid *Anacamptis palustris* (Jacq.) R. M. Bateman, Pridgeon & M. W. Chase had declined over time from the nineteenth and early twentieth centuries, with certain alleles having gone extinct. Historical DNA extracted from pre-1910 herbarium specimens of common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) was compared to that of modern samples, revealing that a nonnative, aggressive strain of the species has displaced native genotypes over the past 150 yr (Saltonstall, 2002). DNA from herbarium specimens has also been useful in phylogeographic studies (Lister et al., 2010), for example, by providing direct evidence of the origin of the European potato in relation to outbreaks of potato blight disease (Ames and Spooner, 2008). In many phylogenetic analyses, historical DNA has been indispensable because it provides samples of taxa that are difficult or impossible for a researcher to obtain otherwise, such as historical samples of extinct species or species occurring in diverse areas of the world (e.g., Tepe et al., 2011).

Information gleaned from herbarium collections is not restricted to plants alone. For example, the controversial origin of the invasive horse-chestnut leaf-mining moth in Europe was resolved by examining historical vouchers of host plant species (Lees et al., 2011). Similarly, the source of a bacterial pathogen outbreak responsible for citrus canker in the United States was identified using bacterial DNA extracted from infected leaves present in historical collections of the host plant (Li et al., 2007). Finally, the discovery that microinvertebrate extremophiles known as tardigrades could emerge from rehydrated samples of a historical moss specimen in a herbarium collection led to a much larger study of these microorganisms (Meininger et al., 1985; G. Uetz, personal communication).

CONCLUSIONS

This editorial outlines only a few of the most critical reasons for why vouchers matter. Because of the importance of vouchers for backing up botanical research, our editorial policy is that manuscripts submitted to *Applications in Plant Sciences* are required to cite one voucher per species; if appropriate, a voucher for each population is strongly recommended. The editors may waive the voucher requirement only in certain circumstances, as when the rarity of a taxon may preclude its collection in the field; in such cases, either accurate GPS coordinates are required for collection localities (if allowed for publication) or citations can consist of previously collected vouchers deposited by other collectors from the same location or from a nearby site. In only very rare cases will photographic vouchers be deemed acceptable; these must be of high resolution and clearly show the key diagnostic traits underlying the taxonomic identification. Our editorial review form specifically asks editors to confirm whether appropriate voucher information has been included in the manuscript (Funk et al., 2005). Vouchers are considered especially critical for primer note studies in which markers are developed for individual taxa, the correct identification of which is critical to the successful application of these markers by other researchers. Information regarding where to access these vouchers must be reported within the main text of the paper, in a table, or as an appendix (see the Instructions for Authors [http://www.botany.org/apps/APPS_Author_Instructions.html]). DNA samples are not accepted in lieu of standard herbarium vouchers because morphological traits remain the backbone of

traditional species identification criteria and historical comparisons. However, DNA voucher specimens may be considered in combination with herbarium vouchers if an appropriate and accessible repository can be identified, within either the current herbaria network or in a future, national repository (similar to GenBank). At *Applications in Plant Sciences*, we strongly believe not only that the inclusion of well-documented and accurate voucher information is good science, but also that it paves the way for future research into new areas that remain to be discovered.

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