Relevance of DNA preservation for future Botany and Ecology

Running title: DNA preservation for Botany and Ecology

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Abstract

The use of molecular methods in plant systematics and taxonomy has increased during the last decades, however, the accessibility of curated genetic samples and their metadata is a bottleneck for DNA-based genetic studies in botany. Plant biodiversity DNA banks and DNA-friendly collections could be critical suppliers of curated genetic material for researchers in the current context of plant biodiversity loss. We aim to understand the potential of plant DNA banks and DNA-friendly collections to enhance the growth and openness of scientific knowledge. The preservation of genetic material should become part of a natural collection's process for the generation of extended specimens enabling the preservation of both the phenotype and genotype and contributing to the generation of data networks which cross-fertilise other fields. These curated collections are advantageous in endangered species research, detecting processes related to extinction, giving a genetic dimension to IUCN assessments or completing the Leipzig Catalogue of Vascular Plants. Therefore, DNA collections are fundamental in producing FAIR data, Responsible Research and Innovation (RRI) and meeting the goals of international conservation programs. The completion of natural collections is important for current Research efforts and furthermore, vital to support future research in an era of ongoing plant biodiversity loss.

Key words:

Biobank, DNA-friendly collection, Extended Specimen, FAIR data, Responsible Research and Innovation (RRI), Biodiversity

Introduction

Botanical vouchers are well preserved whole, or part, specimens included in a permanent collection which is accessible to researchers (Funk et al., 2005, 2017; Pleijel et al., 2008). These vouchers are fundamental tools for botanical research since they provide a reference for taxonomical and morphological consultations and verifications (Funk et al., 2005; Pleijel et al., 2008; Gaudeul & Rouhan, 2013; Buckner et al., 2021). During the last decades, the decrease in the costs of molecular methods has made molecular evidence the basis of taxonomic changes (e.g. Downie et al., 2010; Banasiak et al., 2016), thus giving renewed relevance to the morphological vouchers and their associated information as key pieces for understanding the complex and unexpected phylogenetic results (Ruedas et al., 2000; Funk et al., 2005; Pleijel et al., 2008; Peña & Malm, 2012). Nonetheless, although DNA technology is popular in Botany and is quickly advancing thanks to the high-throughput genotyping technologies (HTG), the accessibility to high-quality and properly managed genomic samples and their metadata is a bottleneck for some biodiversity research projects (Blackmore, 2002; Hodkinson et al., 2007; Astrin et al., 2013; Droege et al., 2014; Gostel et al., 2016; Kumar & Kumar, 2018; McKain et al., 2018).

In this context, DNA banks or biobanks—biorepositories focused on the long-term preservation of high-quality and integrity-guaranteed genomic samples (DNA, RNA or DNA-rich tissue) which are associated with specific data about the specimen (such as the date of collection, the location or the ecosystem in which it was collected)—and other DNA-friendly centralised collections (e.g. tissue collections preserved in silica gel) are fundamental suppliers of genetic material for taxonomic and systematic studies (Jenkins, 2003; Corthals & Desalle, 2005; Rice, Shepherd, et al., 2006; Astrin et al., 2013; Gaudeul & Rouhan, 2013; Watson, 2014; Droege et al., 2016). The role of these curated collections is more critical today, as global estimations based on historical analyses of genetic diversity indicators of 91 animal species covering an average of 97 years indicate that around 6% of populations' genetic diversity has been lost (Leigh et al., 2019). No such study has yet been conducted in wild plant species, however we might predict that the same amount of biodiversity loss in plants would have a more critical effect taking into account the reduced percentage of wild plant species genomes have been sequenced (Willis, 2017). Moreover, the current plant biodiversity loss and its associated extinctions, whose consequences for other organisms are difficult to predict given the vital ecosystemic role of plants (Grime, 2002; Schleuning et al., 2016), are aggravated by the insufficient funding for non-commercial endangered plant species (Suarez & Tsutsui, 2004; Balding & Williams, 2016; Roberson & Meyer, 2020).

Unfortunately, despite the recent technical advances, the estimations of extinctions, and the pleas of Hodkinson et al. (2007) and Gaudeul and Rouhan (2013) for the integration of plant DNA biobanks and DNA-friendly collections in the already existing biodiversity preservation facilities, these have not been fully integrated. In this context, we aim to conduct bibliographic research to understand the potential of plant DNA banks and DNA-friendly collections to enhance the growth and openness of scientific knowledge.

1. *DNA preservation and the Extended Specimen*

Webster (2017) applied a holistic view of voucher material when he coined the term "Extended Specimen" (ES) to a set of different voucher material derived from the same individual which aims to preserve its phenotype, its genotype and its ecological context. Although this author created this concept for ornithological collections, plants and fungi collections are not falling behind in creating protocols aiming to transform their specimens into ESs by, for example, generating photovouchers (Gómez-Bellver et al., 2019). Nonetheless, even when historic natural collections of museums, herbaria and botanic gardens contain a large number of well-documented specimens, many of them have been treated —with chemical compounds, heat, or alcohol— to preserve their phenotype damaging their DNA in the process (Jenkins, 2003; Suarez & Tsutsui, 2004; Hanner & Gregory, 2007; Staats et al., 2011; Särkinen et al., 2012; Gaudeul & Rouhan, 2013; Bradley et al., 2014; Droege et al., 2014; Forrest et al., 2019). As a result, the DNA obtained from voucher specimens, especially from the older specimens, is highly fragmented (<50pb), making DNA amplification challenging and hindering Sanger and next generation sequencing (NGS) methods due to the lack of the necessary quality (Xu et al., 2015; Gutaker & Burbano, 2017; Marinček et al., 2022). Other drawbacks associated with the use of voucher specimens as a source of genetic material are the decay speed (6 times faster than that observed in animals), the cytosine to thymine substitution trend of ancient DNA (aDNA) or the presence of exogenous microbial DNA (Weiß et al., 2016; Bieker et al., 2020). The latter two aspects can be of relevance in phylogenetics and barcoding (Xu et al., 2015), as the necessary destructive sampling required for DNA extraction would damage the voucher specimen to obtain incomplete and/or modified DNA sequences which could lead to erroneous conclusions.

The current technology allows the long-term preservation of DNA and DNA-rich tissues in various ways: at ultracold temperatures (-80ºC) in mechanical freezers, by cryopreservation in liquid nitrogen (LN_2), at room temperature in silica gel, in stabilizing solutions or in in air-proof containers (Hodkinson et al., 2007; Wan et al., 2010; Spooner & Ruess, 2014; Zimkus & Ford, 2014). These methods require different machinery and level of investment however, the effectiveness and reliability of DNA-rich tissue preservation in silica gel (Staats et al., 2011; Wilkie et al., 2013; Forrest et al., 2019), means that morphological vouchers could be extended through DNA-friendly collections at low costs (Gaudeul & Rouhan, 2013). Thus, the genotype of a morphological voucher could be accessed via their DNA-friendly preserved tissue sample without having to destroy part of the voucher material. Hence, obtaining DNA from herbarium vouchers could be restricted to endangered species when sample collection is bound by strict limitations or prohibited, to extinct taxa when no alternative source of DNA is available and the use of the voucher material does not represent a great loss of phenotypical information or to scientific research aiming to understand the historical processes such as domestication (e.g. Roullier et al., 2013) or hybridizations events and related to invasive expansion (e. g. Martin et al., 2014).

Lendemer et al. (2020) recently proposed giving another dimension to the ESs, through the improvement of the accessibility and the interconnection of the digitalized data derived from ESs so as to enhance the scientific potential of the specimens in a process called Extended Specimen Network (ESN) (See Fig. 1). In this sense, DNA biobanks and DNA-friendly collections could play a fundamental role in enhancing the accessibility of ES's data as they would contribute to nucleotide databases and public inventories such as NCBI (https://www.ncbi.nlm.nih.gov/) or the Global Biodiversity Information Facility (https://www.gbif.org/) and facilitate international barcoding projects such as the International Barcode of Life Project (iBOL) (https://ibol.org/), Encyclopedia of Life (EOL) (https://eol.org/) or The Barcode of Life Data System (BOLD) (https://www.boldsystems.org/) to achieve their goals of gathering genetic information about all possible living forms (Hanner & Gregory, 2007; Astrin et al., 2013). Other emerging fields depending on the capacity of identifying taxa based on DNA such as environmental DNA (eDNA) or well-established fields like pharmacology would benefit from the increase in the knowledge and higher accuracy of barcoding technology (Chase & Fay, 2009; Schori & Showalter, 2011; Dick & Webb, 2012; Li et al., 2015; Bell et al., 2016; Gostel & Kress, 2022).

2. *Future applications of DNA collections*

Under the current scenario of species extinction and genetic biodiversity loss, plant biodiversity needs to be further studied and understood, and this must happen at an accelerated pace. Therefore, preserving molecular vouchers in DNA biobanks or DNA-friendly collections should be a central part of the *ex situ* conservation strategy since the future of the current plant populations depends, in part, on their genetic structure and our advances in genomic studies. *In situ* conservation efforts for endangered species may thus be complemented with genetic knowledge gained from DNA biobanks or DNA-friendly collections, and environmental impacts of invasive species could be readily monitored via HTS and barcoding (Rice, Shepherd, et al., 2006; Coissac et al., 2016; Davis & Borisenko, 2017; Breed et al., 2019). Another engaging subject in which the application of genomics would shed light is the detection of events of extinction through hybridization (e.g Arrigo et al., 2016; Rita et al., 2018), which may be relevant in mountainous areas in the future (Gómez et al., 2015) or when invasive species have been introduced (Mooney & Cleland, 2001; Geddes et al., 2021).

Institutions like DNA biobanks could also contribute to a better plant coverage of the IUCN Red List and the Botanic Gardens conservation international threatSearch database (https://tools.bgci.org/threat_search.php) by monitoring the changes in distribution ranges and evaluating the conservational status of plant species and populations based also on genetic parameters (e. g. the effective population size (Ne)) as this is one of the weaknesses of the IUCN assessments (Garner et al., 2020).

Furthermore, plant DNA banks have already proven useful for fungal biodiversity studies as plant tissue preservation methods also preserve associated fungi (i.e. Datlof et al., 2017), which means that significant advances in cataloguing fungi could also be prompted by establishing more plant DNA banks and other types of DNA curated collections.

Curated collections of DNA material could generate more complete inventories that are useful in understanding plant biodiversity patterns and evolutionary history (e.g. Molina-Venegas et al., 2017) and future spatial distributions (e.g. González-Orozco et al., 2016). Furthermore, their curated information could improve present and future conservational efforts in terms of efficiency if it is implemented with geospatial information as the NEXTGENDEM project does (Laity et al., 2015; Caujapé-Castells et al. 2021). Moreover, DNA banks could concentrate their sampling efforts both on the type localities of species and on different locations of species' distribution range. By focusing on these two sampling strategies, phylogenetic studies and conservational efforts would become more efficient and accurate in clarifying the taxonomic status of taxa, determining genetic diversity, finding and understanding adaptive genetic variations and establishing proper conservational units for both *ex situ* and *in situ* conservational strategies. In this context, both the digitalization and basic research on plant taxonomy and phylogenetics would benefit databases and initiatives like the Leipzig catalogue of vascular plants (LCVP) (Freiberg et al., 2020), as this list could be updated.

3. *DNA collections and FAIR data*

DNA collections and DNA-friendly collections are fundamental for creating Findable Accessible Interoperability and Reusable (FAIR) data within DNA sequences databases, but they are also new tools to implement legislation regarding the use of genetic patrimony as contemplated in the Nagoya Protocol (Ebert et al., 2006; Rice, Henry, et al., 2006; Wilkinson et al., 2016; Lannom et al., 2019). For instance, managing this kind of collection could accelerate and improve the implementation of the Access and Benefit-Sharing (ABS) established by the Nagoya Protocol at a national level, since the law reinforcements required by national legislation regulating the terms of the required Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) would be clarified (Global Genome Biodiversity Network (GGBN), 2015; Davis & Borisenko, 2017; Consortium of European Taxonomic Facilities (CETAF), 2020). In this sense, encouraging national implementations could have a energising effect when it comes to establishing new legal agreements between countries that share valuable natural areas (e.g. the Pyrenees, the Alps) regarding the application of ABS. Besides, the curated collection of DNA would also create more FAIR data, as many researchers would have access to the original material from which molecular data derive. Furthermore, the generation of more FAIR data and the implementation of the Nagoya Protocol would promote research ethics and open access, while contributing to achieve the targets of adequate assessment of plant species' conservation status, preservation of their genetic diversity and effective populations' management and restoration of the objectives for the documentation and conservation of plant biodiversity of the United Nations' Global Strategy for Plant Conservation (GSPC) (Sharrock, 2012; Botanic Gardens Conservation International (BGCI), 2021; United Nations, 2021) and the target European Union's Horizon Europe (European Union, 2021) for research improvement and sustainability, while addressing the current major issue for phylogenetic and conservational advances—sampling strategy, cost and availability of samples (Rice, Shepherd, et al., 2006; McKain et al., 2018). Hence, researchers' requirements for plant material would be addressed, while reinforcing research ethics through compliance with the Nagoya Protocol and the applications of some of the principles of Responsible Research and Innovation (RRI) (Owen et al., 2012; Williams et al., 2020). In this sense, the RRI principles of public participation in science, the involvement of institutions, and the establishment of the framework in research (Owen et al., 2012) would be achieved by, for instance, the generation of educational programs oriented to citizens with or without a scientific background (e.g. Funk et al., 2017), the diagnosis and coverage of the existing gaps in scientific knowledge of plant biodiversity and genetics or the generation of FAIR data (Williams et al., 2020).

Conclusions

Extending morphological vouchers through DNA curated collections would help the advances of taxonomy and phylogenetics on a global scale by addressing the voids in scientific knowledge, for instance, sampling on the type location of the taxa. In the current context of Biodiversity Loss and Climate Change, collecting and preserving DNA or DNA-rich tissues is critical to add the genetic dimension to the current conservational status assessments, thus developing more efficient conservation strategies in the long term. In this sense, generating more FAIR data is not only beneficial for present-day researchers but also aids to achieve global targets of plant conservation and preserving and managing plant genetic heritage. Moreover, as DNA technology has advanced so much that herbarium vouchers more than 100 years old can be subject to DNA studies, the material preserved today can be of use for diverse studies in the future without having to damage a voucher specimen. Thence, in an uncertain future, completing natural collections with DNA collections today means preserving genetic biodiversity for future generations.

Author Contribution

C.G.T and E. C. conceived this paper and wrote the manuscript. C.G.T. created the figures. Both authors contributed critically to the drafts and gave final approval for publication.

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Data Accessibility and Benefit-Sharing Section

Data Accessibility Statement

No data, scripts, or other artefacts have been produced for this article.

References

Arrigo, N., Bétrisey, S., Graf, L., Bilat, J., Gerber, E. & Kozlowski, G. (2016). Hybridization as a threat in climate relict *Nuphar pumila* (Nymphaeaceae). *Biodiversity and Conservation, 25(10)*, 1863–1877. https://doi.org/10.1007/s10531-016-1165-z.

Astrin, J.J., Zhou, X., & Misof, B. (2013). The importance of biobanking in molecular taxonomy, with proposed definitions for vouchers in a molecular context. *ZooKeys, 365,* 67–70. https://doi.org/10.3897/zookeys.365.5875.

Balding, M., & Williams, K.J.H. (2016). Plant blindness and the implications for plant conservation. *Conservation Biology, 30(6)*, 1192–1199. https://doi.org/10.1111/cobi.12738.

Banasiak, Ł., Wojewódzka, A., Baczyński, J., Reduron, J-P., Piwczyński, M., Kurzyna-Młynik, R., Gutaker, R., Czarnocka-Cieciura, A., Kosmala-Grzechnik, S., & Spalik, K. (2016). Phylogeny of Apiaceae subtribe Daucinae and the taxonomic delineation of its genera. *Taxon, 65(3),* 563–585. https://doi.org/http://dx.doi.org/10.12705/653.8.

Bell, K.L., De Vere, N., Keller, A., Richardson, R.T., Gous, A., Burgess, K.S., & Brosi, B.J. (2016). Pollen DNA barcoding: Current applications and future prospects. *Genome, 59(9),* 629–640. https://doi.org/10.1139/gen-2015-0200.

Bieker, V.C., Sánchez Barreiro, F., Rasmussen, J.A., Brunier, M., Wales, N., & Martin, M.D. (2020). Metagenomic analysis of historical herbarium specimens reveals a postmortem microbial community. *Molecular ecology resources*, *20(5)*, 1206-1219. https://doi.org/10.1111/1755-0998.13174

Blackmore, S. (2002). Biodiversity Update-Progress in Taxonomy Downloaded from. *Science, 298(5592),* 365. https://doi.org/10.1126/science.1075026.

Botanic Gardens Conservation International (BGCI) (2021). *The Global Strategy for Plant Conservation (GSPC).* https://www.bgci.org/wp/wp-content/uploads/2019/04/GSPC_flyer.pdf#:~:text=Target 1%3A An online Flora,the Strategy developed and shared. Accessed: October 19, 2021.

Bradley, R.D., Bradley, L.C., Garner, H.J. & Baker, R.J. (2014). Assessing the Value of Natural History Collections and Addressing Issues Regarding Long-Term Growth and Care, *BioScience,64(12),* 1150-1158. https://doi.org/10.1093/biosci/biu166

Breed, M.F., Harrison, P.A., Blyth, C., Byrne, M., Gaget, V., Gellie, N.J.C., Groom, S.V.C., Hodgson, R., Mills, J.G., Prowse, T.A.A., Steane, D.A. & Mohr, J.J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20(10),* 615–628. https://doi.org/10.1038/s41576-019-0152-0

Buckner, J.C., Sanders, R.C., Faircloth, B.C., & Chakrabarty, P. (2021). The critical importance of vouchers in genomics. *eLife,* 10. https://doi.org/10.7554/ELIFE.68264

Caujapé-Castells, J., Nebot, R., Naranjo-Suárez, J., Molina, R.J., González Pérez, M.A., Guillermes Vázquez, I., Díaz Pérez, A., Viera Ruiz, G., Cabrera, N., Gómes, I., Barreto, A., Rodríuez, J.F., Fuertes-Aguilar, J., García-Verdugo, C., Sanmartín, I., Zurita Pérez, N., Moreno, P., Reyes, D., Curbelo, A., Romeiro, M., Correia, M.J., & Caraballo, C. (2021). NEXTGENDEM: información genética, geoespacial y supercomputación para mejorar la gestión de especies y espacios en Macaronesia. *Conservación Vegetal, (25),* 29-33. https://doi.org/10.15366/cv2021.25.005

Chase, M.W., & Fay, M.F. (2009). Barcoding of plants and fungi. *Science, 325(5941),* 682–683. https://doi.org/10.1126/science.1176906

Coissac, E., Hollingsworth, P.M., Lavergne, S., & Taberlet, P (2016). From barcodes to genomes: extending the concept of DNA barcoding. *Molecular Ecology, 25(7),* 1423–1428. https://doi.org/10.1111/mec.13549

Consortium of Europena Taxonomic Facilities (CETAF). (2020). *Code of conduct & best practices for Access and Beneficr-Sharing. Exploring and Documenting Diversity in Nature.* Retrieved june 16, 2020, from Consortium of Europena Taxonomic Facilities website: https://www.cetaf.org/wp-content/uploads/CETAF-Code-of-conduct-Bestpractice-for-ABS-A4-leaflet.pdf

Corthals, A., & Desalle, R. (2005). An application of tissue and DNA banking for genomics and conservation: the Ambrose Monell Cryo-Collection (AMCC). *Systematic biology, 54(5),* 819–823. https://doi.org/10.1080/10635150590950353

Datlof, E.M., Amend, A.S., Earl, K., Hayward, J., Morden, C.W., Wade, R., Zahn, G. & Hynson, N.A. (2017). Uncovering unseen fungal diversity from plant DNA banks. *PeerJ, 5(8),* e3730. https://doi.org/10.7717/PEERJ.3730

Davis, K., & Borisenko, A. (2017). *Introduction to Access and Benefit-Sharing and the Nagoya Protocol: What DNA Barcoding Researchers Need to Know*. Advanced Books, 1, e2257. https://doi.org/10.3897/ab.e22579

Dick, C.W., & Webb, C.O. (2012). Plant DNA Barcodes, Taxonomic Management, and Species Discovery in Tropical Forests, in Kress, W., Erickson, D. (eds.) *DNA Barcodes. Methods in Molecular Biology (Methods and Protocols)* (pp. 379–393). Totowa: Humana Press. https://doi.org/10.1007/978-1-61779-591-6_18

Downie, S.R., Spalik, K., Katz-Downie, D.S., & Reduron, J-P. (2010). Major clades within Apiaceae subfamily Apioideae as inferred by phylogenetic analysis of nrDNA ITS sequences. *Plant Diversity and Evolution, 128(2),* 111–136. https://doi.org/10.1127/1869-6155/2010/0128-0005

Dröge, G., Barker, K., Astrin, J.J., Bartels, P., Butler, C., Cantrill, D., Coddington, J., Forest, F., Gemeinholzer, B., Hobern, D., Mackenzie-Dodds, J., Ó Tuama, É., Petersen, G., Sanjur, O., Schindel, D., & Seberg, O. (2014). The Global Genome Biodiversity Network (GGBN) Data Portal. *Nucleic Acids Research, 42(D1),* D607–D612. https://doi.org/10.1093/nar/gkt928

Dröge, G., Barker, K., Seberg, O., Coddington, J., Benson, E., Berendsohn, W.G., Bunk, B., Butler, C., Cawsey, E.M., Deck, J., Döring, M., Flemons, P., Gemeinholzer, B., Güntsch, A., Hollowell, T., Kelbert, P., Kostadinov, I.,Kottmann, I.R., Lawlor, R.T., Lyal, C., Mackenzie-Dodds, J., Meyer, C., Mulcahy, D., Nussbeck, S. Y., O'Tuama, E., Orrell, T, Petersen, G., Robertson, T., So¨ hngen, C., Whitacre, J., Wieczorek, J., Yilmaz, P., Zetzsche, H., Zhang, Y., & Zhou, X. (2016). The Global Genome Biodiversity Network (GGBN) Data Standard specification. *Database, 201*, baw125. https://doi.org/10.1093/database/baw125

Ebert, A.W., Karihaloo, J.L., and M.E. Ferreira. "Opportunities, limitations and needs for DNA banks". *DNA Banks: Providing Novel Options for Genebanks?* Edited by M.C. de Vicente and M.S. Andersson, International Plant Genetic Resources Institute (IPGRI)., 2006, pp. 61–68.

European Union (EU) (2021). *Horizon Europe. The next EU research and innovation invest programme (2021-2027).* https://ec.europa.eu/info/sites/default/files/research_and_innovation/ec_rtd_he-presentation_062019_en.pdf Accessed: October 19, 2021.

Freiberg, M., Winter, M., Gentile, A., Zizka, A., Muellner-Riehl, N.A., Weigelt, A. &

Wirth, C. (2020). LCVP, the Leipzig catalogue of vascular plants, a new taxonomic reference list for all known vascular plants. *Scientific data, 7(1),* 1–7. https://doi.org/10.1038/s41597-020-00702-z

Forrest, L.L., Hart, M.L., Hughes, M., Wilson, H.P., Chung, K.F., Tseng, Y.H., & Kidner, C.A. (2019). The Limits of Hyb-Seq for Herbarium Specimens: Impact of Preservation Techniques. *Frontiers in Ecology and Evolution, 7,* 439. https://doi.org/10.3389/fevo.2019.00439

Funk, V.A., Hoch, P. C., Prather, L. A. & Wagner, W. L. (2005). The importance of vouchers. *Taxon, 54(1),*127–129. https://doi.org/10.2307/25065309.

Funk, V.A., Gostel, M., Devine, A., Kelloff, C. L., Wurdack, K., Tuccinardi, C., Radosavljevic, A., Peters, M. & Coddington, J. (2017). Guidelines for collecting vouchers and tissues intended for genomic work (Smithsonian Institution): Botany Best Practices. *Biodiversity Data Journal, 5(1),* e11625. https://doi.org/10.3897/BDJ.5.e11625

Garner, B.A., Hoban, S., & Luikart, G. (2020). IUCN Red List and the value of integrating genetics. *Conservation Genetics, 21(5),* 795–801. https://doi.org/10.1007/S10592-020-01301-6/FIGURES/2

Gaudeul, M., & Rouhan, G. (2013). A plea for modern botanical collections to include DNA-friendly material. *Trends in Plant Science, 18(4),* 184–185. https://doi.org/10.1016/j.tplants.2012.12.006

Geddes, P., Murphy, L., Astudillo-Scalia, Y., Blasini, D., Nugent, S., Ríos, M.J., Schirmer, A.E., & Olfelt, J. P. (2021). Microsatellite markers reveal unprecedented high frequencies of hybridization among *Typha* species in the Midwestern US. *Wetlands, 41(2)*, 1-9. https://doi.org/10.1007/s13157-021-01429-2

Global Genome Biodiversity Network (GGBN). (2015). *Global Genome Biodiversity Network (GGBN) Guidance: Code of Conduct. The Access and Benenfit-Sharing Clearing-House (ABSCH).* Retrievede june 16, 2020 from Global Genome Biodiversity Network (GGBN) website: https://absch.cbd.int/api/v2013/documents/74D3F16E-CF01-CD4B-96EC-669B45CE36AD/attachments/GGBN Guidance _Code_of_Conduct_June_2015-Final.pdf

Gómez-Bellver, C., Ibáñez, N., López-Pujol, J., Nualart, N. & Susanna, A. (2019). How photographs can be a complement of herbarium vouchers: A proposal of standardization. *Taxon, 68(6),* 1321–1326. https://doi.org/10.1002/tax.12162

Gómez, J.M., González-Megías, A., Lorite, J., Abdelaziz, M. & Perfectti, F. (2015). The silent extinction: climate change and the potential hybridization-mediated extinction of endemic high-mountain plants. *Biodiversity and Conservation, 24(8),* 1843–1857. https://doi.org/10.1007/s10531-015-0909-5

González-Orozco, C.E., Pollock, L.J., Thornhill, A.H., Mishler, B.D., Knerr, N., Laaan, S.W., Miller, J.T., Rosauer, D.F., Faith, D.P., Nipperess, D.A., Kujala, H., Linke, S., Butt, N., Külheim, C., Crisp, M.D., & Gruber, B. (2016). Phylogenetic approaches reveal biodiversity threats under climate change. *Nature Climate Change, 6(12),* 1110–1114. https://doi.org/10.1038/NCLIMATE3126

Gostel, M.R., Kelloff, C., Wallicqk, K. & Funk, V.A. (2016). A Workflow to Preserve Genome-Quality Tissue Samples From Plants in Botanical Gardens and Arboreta. *Applications in Plant Sciences, 4(9),* 1600039. https://doi.org/10.3732/apps.1600039

Gostel, M.R., & Kress, W.J. (2022). The Expanding Role of DNA Barcodes: Indispensable Tools for Ecology. *Evolution, and Conservation. Diversity, 14(3),* 213. https://doi.org/10.3390/d14030213

Grime, J.P. (2002). Declining plant diversity: empty niches or functional shifts? *Journal of Vegetation Science, 13(4),* 457–460. https://doi.org/10.1111/j.1654-1103.2002.tb02072.x

Gutaker, R.M., & Burbano, H.A. (2017). Reinforcing plant evolutionary genomics using ancient DNA. *Current opinion in plant biology*, *36*, 38–45. https://doi.org/10.1016/j.pbi.2017.01.002

Hanner, R.H., & Gregory, T.R. (2007). Genomic Diversity Research and the Role of Biorepositories. *Cell Preservation Texhnology, 5(2)*, 93–103. https://doi.org/10.1089/cpt.2007.9993

Hodkinson, T.R., Waldren, S., Parnell, J.A.N., Kelleher, C.T., Salamin, K., & Salamin, N. (2007). DNA banking for plant breeding, biotechnology and biodiversity evaluation. *Journal of Plant Research, 120(1),* 17–29. https://doi.org/10.1007/s10265-006-0059-7

Jenkins, M. (2003). Prospects for Biodiversity. *Science, 302(5648),* 1175–1177. https://doi.org/10.1126/science.1088666

Kumar, R., & Kumar, V. (2018). A review of phylogeography: biotic and abiotic factors. *Geology, Ecology, and Landscapes, 2(4),* 268–274. https://doi.org/10.1080/24749508.2018.1452486

Laity, T., Laffan, S.W., González-Orozco, C.E., Faith, D.P., Rosauer, D.F., Byrne, M., Miller, J.T., Crayn, D., Costion, C., Moritz, C.C. & Newport, K. (2015). Phylodiversity to inform conservation policy: An Australian example. *Science of the Total Environment, 534,* 131–143. https://doi.org/10.1016/j.scitotenv.2015.04.113

Lannom, L., Koureas, D., & Hardisty, A.R. (2019). FAIR data and services in biodiversity science and geoscience. *Data Intelligence, 2(1–2),* 122–130. https://doi.org/10.1162/dint_a_00034

Leigh, D.M., Hendry, A.P., Vázquez-Domínguez, E. & Friesen, V.L. (2019). Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. *Evolutionary Applications, 12(8),* 1505–1512. https://doi.org/10.1111/eva.12810

Lendemer, J., Thiers, B., Monfils, A.K., Zaspel, J., Ellwood, E.R., Bentley, A., LeVan, K., Bates, J., Jennings, D., Contreras, D., Lagomarsino, L., Mabee, P., Ford, L.S., Guralnick, R., Gropp, R.E., Revelez, M., Cobb, N., Katja Seltmann, K., & Aime, M.C.

(2020). The Extended Specimen Network: A Strategy to Enhance US Biodiversity Collections. *Promote Research and Education, BioScience, 70(1),* 23–30. https://doi.org/10.1093/BIOSCI/BIZ140

Li, X., Yang, Y., Henry, R.J., Rossetto, M., Wang, Y. & Chen, S. (2015). Plant DNA barcoding: from gene to genome. *Biological reviews, 90(1),* 157–166. https://doi.org/10.1111/brv.12104

Marinček, P., Wagner, N.D., & Tomasello, S. (2022). Ancient DNA extraction methods for herbarium specimens: When is it worth the effort? *Applications in Plant Sciences*, *10(3)*, e11477. https://doi.org/10.1002/aps3.11477

Martin, M.D., Zimmer, E.A., Olsen, M.T., Foote, A.D., Gilbert, M.T.P., & Brush, G.S. (2014). Herbarium specimens reveal a historical shift in phylogeographic structure of common ragweed during native range disturbance. *Molecular Ecology, 23(7),* 1701–1716. https://doi.org/10.1111/mec.12675

McKain, M.R., Johnson, M.G., Uribe-Convers, S., Eaton, D., & Yang, Y. (2018). Practical considerations for plant phylogenomics. *Applications in Plant Sciences, 6(3).* https://doi.org/10.1002/APS3.1038@10.1111/(ISSN)1365-313X.PLANTS_DAY_COLLECTION

Molina-Venegas, R., Aparicio, A., Lavergne, S., & Arroyo, J. (2017). Climatic and topographical correlates of plant palaeo-and neoendemism in a Mediterranean biodiversity hotspot. *Annals of botany, 119(2),* 229–238. https://doi.org/10.1093/aob/mcw093

Owen, R., Macnaghten, P. & Stilgoe, J. (2012). Responsible research and innovation: From science in society to science for society, with society. *Science and public policy, 39(6),* 751–760. https://doi.org/10.1093/scipol/scs093

Mooney, H.A., & Cleland, E.E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, *98(10),* 5446-5451. https://doi.org/10.1073/pnas.091093398

Peña, C., & Malm, T. (2012). VoSeq: A Voucher and DNA Sequence Web Application. *PLoS ONE, 7(6),* e39071. https://doi.org/10.1371/JOURNAL.PONE.0039071

Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., Sundberg, P., & Thollesson, M. (2008). Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution, 48(1),* 369–371. https://doi.org/10.1016/j.ympev.2008.03.024

Rice, N., Shepherd, M., Bundock, P.C., & Henry, R.J. (2006). DNA Banks and their role in facilitating the application of genomics to plant germplasm. *Plant Genetic Resources, 4(1),* 64–70. https://doi.org/10.1079/PGR2006113

Rice, N., Henry, R., & Rossetto, M. (2006). "DNA banks: a primary resource for conservation research". *DNA Banks: Providing Novel Options for Genebanks*, edited by M.C. De Vicente and M.S. Andersson, M.S., International Plant Genetic Resources

Institute (IPGRI), pp. 41–48.

Rita, J., Capó, M., Moragues, E., Bota, J., & Cursach, J. (2018). Hybridization processes in an introduced subpopulation of an endangered plant: Management strategies to guarantee the conservation of Helosciadium bermejoi (Apiaceae). *Journal for Nature Conservation, 41,* 26–34. https://doi.org/10.1016/j.jnc.2017.10.006

Roberson, E., & Meyer, A.H. (2020). Funding for Plant Conservation View project. *Science, 367(6475),* 258. https://doi.org/10.1126/science.aba4360

Roullier, C., Benoit, L., Doyle, B.M., & Lebot, V. (2013). Historical collections reveal patterns of diffusion of sweet potato in Oceania obscured by modern plant movements and recombination. *Proceedings of the National Academy of Sciences of the United States of America, 110(6),* 2205–2210. https://doi.org/10.1073/pnas.1211049110

Ruedas, L.A., Salazar-Bravo, J., Dragoo, J.W., & Yates, T.L. (2000). The Importance of Being Earnest: What, if Anything, Constitutes a 'Specimen Examined?'. *Molecular phylogenetics and evolution, 17(1),* 129–132. https://doi.org/10.1006/mpev.2000.0737

Särkinen, T., Staats, M., Richardson, J.E., Cowan, R.S., & Bakker, F.T. (2012). How to Open the Treasure Chest? Optimising DNA Extraction from Herbarium Specimens. *PLoS ONE, 7(8)*. https://doi.org/10.1371/journal.pone.0043808

Schleuning, M., Fründ, J., Schweiger, O., Welk, E., Albrecht, J., Albrecht, M., Beil, M., Benadi, G., Blüthgen, N., Bruelheide, H., Böhning-Gaese, K., Dehling, D.M., Dormann, C.F., Exeler, N., Farwig, N., Harpke, A., Hickler, T., Kratochwil, A., Kuhlmann, M., Kühn, I., Michez, D., Mudri-Stojnić, S., Plein, M., Rasmont, P., Schwabe, A., Settele, J., Vujić, A., Weiner, C.N., Martin Wiemers M., & Hof, C. (2016). Ecological networks are more sensitive to plant than to animal extinction under climate change. *Nature Communications, 7(1),* 1–9. https://doi.org/10.1038/ncomms13965

Schori, M., & Showalter, A., (2011). DNA barcoding a menas for identifying medical plants of Pakistan, *Pakistan Journal of Botany, 43(Special Issue),* 1–4.

Sharrock, S. (2012). GSPC. *Global strategy for Plant conservation. AGuide to the GSPC. All targets, objectives and facts*, Botanic Gardens Conservation International (BGCI), 2012 https://www.bgci.org/wp/wp-content/uploads/2019/04/GSPC_flyer.pdf#:~:text=Target 1%3A An online Flora,the Strategy developed and shared.

Spooner, D.M., & Ruess, H. "DNA banking for the 21st century: proceedings of the US Workshop on DNA Banking", *DNA banking for the 21st century: proceedings of the US Workshop on DNA Banking*, edited by in W. L. Applequist and L.M. Campbell, Missouri Botanical Garden, pp. 71–80.

Staats, M., Cuenca, A., Richardson, J.E., Vrielink-Van Ginkel, R., & Petersen, G. (2011). DNA Damage in Plant Herbarium Tissue. *PLoS ONE, 6(12),* 28448. https://doi.org/10.1371/journal.pone.0028448

Suarez, A. V., & Tsutsui, N.D. (2004). The Value of Museum Collections for Research and Society. *BioScience, 54(1),* 66. https://doi.org/10.1641/0006-3568(2004)054[0066:tvomcf]2.0.co;2

United Nations (UN) (2021). *The Global Strategy for Plant Conservation*. Retrieved 19 September, 2019, from BGCI website: https://www.bgci.org/wp/wp-content/uploads/2019/04/GSPC_flyer.pdf#:~:text=Target 1%3A An online Flora, the Strategy developed and shared. Accessed 19 September 19, 2021.

Wan, E., Akana, M., Pons, J., Chen, J., Musone, S., Kwok, P.Y., & Liao, W. (2010). Green technologies for room temperature nucleic acid storage. *Current Issues in Molecular Biology, 12(3),* 135–142. https://doi.org/10.21775/cimb.012.135

Watson, P.H. (2014). Biobank Classification: Communicating Biorepository Diversity. *Biopreservation and Biobanking, 12(3),* 163–164. https://doi.org/10.1089/bio.2014.1231

Webster, M.S. (2017).“The Extended Specimen”. *The Extended Specimen: Emerging Frontiers in Collections-Based Ornithological Research*, edited by M.S. Webster, CRC Press, pp. 1–9.

Weiß, C.L., Schuenemann, V.J., Devos, J., Shirsekar, G., Reiter, E., Gould, B.A., Stinchcombe, J.R., Krause J., & Burbano, H. A. (2016). Temporal patterns of damage and decay kinetics of DNA retrieved from plant herbarium specimens. *Royal Society open science*, *3(6),* 160239. https://doi.org/10.1098/rsos.160239

Wilkie, P., Poulsen, A.D., Harris, D., & Forrest, L.L. (2013). The collection and storage of plant material for DNA extraction: The Teabag Method, Gardens’ *Bulletin Singapore, 65(2),* 4.

Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.-W., da Silva Santos, L.B., Bourne, P.E., Bouwman, J., Brookes, A.J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C.T., Finkers, R., Gonzalez-Beltran, A., Gray, A.J.G., Groth, P., Goble, C., Grethe, J.S., Heringa, J., C ’t Hoen, P.A., Hooft, R., Kuhn, T., Kok, R., Kok, J., Lusher, S.J., Martone, M.E., Mons, A., Packer, A.L., Persson, B., Rocca-Serra, P., Roos, M., van Schaik, R., Sansone, S-A., Erik Schultes, Sengstag, T., Slater, T., Strawn, G., Swertz, M.A., Thompson, M., van der Lei, J., van Mulligen, E., Velterop, J., Waagmeester, A., Wittenburg, P., Wolstencroft, K., Zhao, J., & Mons, B. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Scientific Data, 3(1),* 1–9. https://doi.org/10.1038/sdata.2016.18

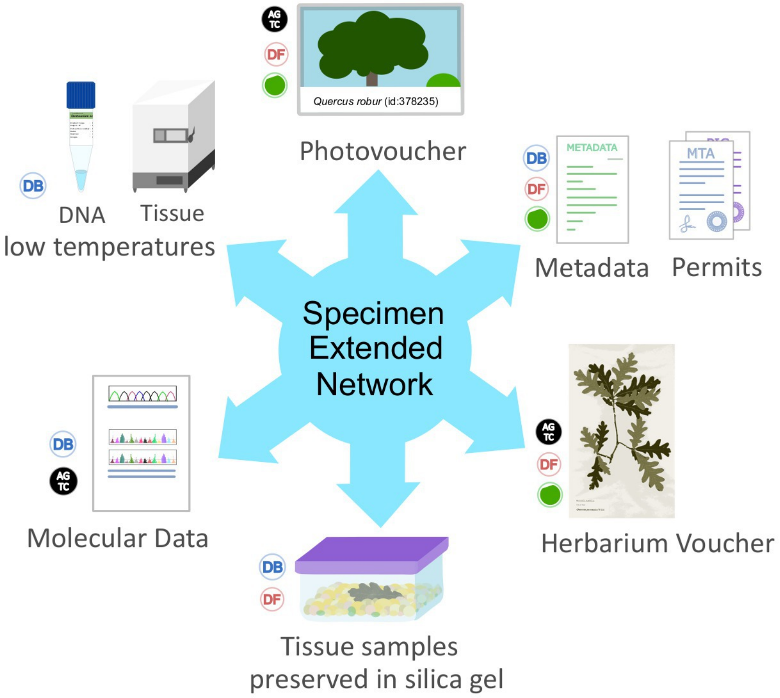
Williams, C., Walsh, A., Vaglica, V., Sirakaya, A., da Silva, M., Dalle, G., Winterton, D., Annecke, W., Smith, P., Kersey, P.J., Way, M., Antonelli, A., & Cowell, C. (2020). Conservation Policy: Helping or hindering science to unlock properties of plants and fungi. *Plants, People, Planet, 2(5),* 535–545. https://doi.org/10.1002/PPP3.10139

Willis, K.J. (2017). *State of the World’s Plants 2017. Report.* London (UK): Kew Royal Botanic Gardens (Kew), Sfumato Foundation.

Xu, C., Dong, W., Shi, S., Cheng, T., Li, C., Liu, Y., Wu, P., Wu, H., Gao, P., & Zhou,

S. (2015). Accelerating plant DNA barcode reference library construction using herbarium specimens: improved experimental techniques. *Molecular Ecology Resources*, *15(6)*, 1366–1374. https://doi.org/10.1111/1755-0998.12413

Zimkus, B.M., & Ford, L.S. (2014). Best practices for genetic resources associated with natural history collections: Recommendations for practical implementation. *Collection Forum, 28(1–2),* 77–112. https://doi.org/10.14351/0831-0005-28.1.77



● Classic Herbaria

Focused on the preservation of the phenotype

The preservation protocols may harm the genetic material

Usually stores Herbarium vouchers and specimen's metadata

Sometimes a phtovoucher is preserved

DF DNA-friendly Collections

Focused on the preservation of both genotype and phenotype

The preservation protocols focused on minimizing the damages on the genetic material

May preserve Herbarium vouchers in a DNA-friendly way or present a separate DNA-friendly tissue collection

Specimen's metadata are routinely stored and sometimes a photovoucher is preserved

DB DNA banks

Focused on the preservation of genotype

The preservation protocols focused on minimizing the damages on the genetic material

May preserve DNA aliquots and/or DNA-rich tissues at cold or ultracold temperatures. Tissue collecions at room temperature in silica gel may serve as back-up

Detailed protocols of preservation and DNA extraction. Metadata are preserved as well.May be directly involved in generating molecular data

AG TC Databases and public inventories

Focused on the preservation and accesibility of the obtained data

May preserve DNA/RNA sequences with unique IDs associated to papers. Sometimes other kind of data such as microsatellites data, phylogenetic trees are available

The could potentially store photovouchers as well

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