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**From genes
to genomes:
botanic gardens
embracing new
tools for conservation
and research**



**BOTANIC
GARDENS**
CONSERVATION
INTERNATIONAL

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Cover Photo: Young and aspiring scientists assist career scientists in sampling plants at the U.S. Botanic Garden for the Global Genome Initiative (U.S. Botanic Garden).

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EDITORIAL: FROM GENES TO GENOMES: BOTANIC GARDENS EMBRACING NEW TOOLS FOR CONSERVATION AND RESEARCH



Young and aspiring scientists assist career scientists in sampling plants at the U.S. Botanic Garden for the Global Genome Initiative (U.S. Botanic Garden)



Welcome to the latest edition of BGJournal, which is produced in partnership with the the Global Genome Initiative for Gardens

Programme, part of the Global Genome Biodiversity Network (GGBN). Our cover photograph is of the United States Botanic Garden in Washington DC, which has provided both financial and technical support to the Smithsonian's Global Genome Initiative – particularly GGI-Gardens that has engaged productively with our community of botanic gardens. BGCI is very grateful to USBG for the support they have provided to this initiative, which is just the latest in a long standing and important partnership between our two organisations.

The GGBN was founded in 2011 in order to promote access to information about, and legal exchange of, the biodiversity genomic samples maintained by its members. It provides a platform to unite biobanks from across the world in order to:

1. Enable data-mining and analysis via one consistent database of global genomic resources,
2. Collaborate to ensure consistent quality standards for DNA and tissue collections,
3. Improve best practices for the preservation and use of such collections,
4. Harmonize exchange and use of genetic materials in accordance with national and international legislation and conventions, in particular the Nagoya Protocol, and

5. Enable targeted, strategic collection to fill crucial biodiversity gaps.

To date, 97 organizations have joined GGBN, mainly natural history collections and botanical gardens, but also seed banks, culture collections as well zoos, aquariums, veterinary and agricultural collections.

As Morgan Gostel and Jean Linsky explain in their article on pages 21-24, in 2015, recognizing the vast plant diversity that is housed in the world's botanic gardens, the Smithsonian's Global Genome Initiative (GGI) founded GGI-Gardens, an international partnership dedicated to sampling and preserving plant biodiversity from the world's extraordinary living collections.

GGI-Gardens coordinates with the GGBN to ensure the samples are preserved in well-managed biorepositories and that genomic samples are made available for the international research community. Their article indicates how you can contribute to this initiative and, aptly, our interview this month is with Jonathan Coddington, the Director of the Global Genome Initiative (pages 16-17).

One collaborating institution is the Rio de Janeiro Botanical Garden (JBRJ) (pages 24-27) which, gap analysis showed, has 541 unique species, that is species not being found in any other botanical garden in the world. As a contributing partner to GGI-Gardens, JBRJ has made more than 6,500 DNA and tissue samples of the Brazilian flora discoverable through the GGBN data portal, adding approximately 1,200 species to GGBN.

GGI-Gardens and the GGBN adhere scrupulously to the spirit, laws and regulations associated with the Nagoya Protocol on Access and Benefit Sharing, and on pages 28-31 Rachel Meyer at the University of California Santa Cruz, describes the journeys her students take in navigating the Nagoya Protocol, local permits, and finding synergies with fellow researchers.

On pages 32-33 the Dr. Cecilia Koo Botanic Conservation Center (KBCC) describe their impressive programme that aims to preserve and safeguard high-quality tropical plant materials for future genomic and transcriptomic studies. To this end they have established a cryopreservation facility at KBCC comprising 40 liquid nitrogen tanks, each with a capacity to hold 6,000 samples. To date, a total of 8,489 accessions, relating to 4,392 taxa, have been cryopreserved.

The application of DNA sampling and genomic studies to plant conservation and use is described by another GGI Gardens partner, the Balkan Botanic Garden of Kroussia (BBGK) on pages 34-39. *Origanum vulgare* L. is a typical species of Mediterranean ecosystems, growing in dry, sunny places from sea level up to 1500 masl and, due to cross pollination and plasticity, the species is extremely variable and polymorphic. However, oregano crops commonly use wild populations without the appropriate



Young and aspiring scientists assist career scientists in sampling plants at the U.S. Botanic Garden for the Global Genome Initiative (U.S. Botanic Garden)

plant material selection. For this reason, a wide variety of products with different qualities of raw material and essential oils, are produced and therefore selection and breeding represent a key role of the quality assurance system. Understanding the genetic structure and variability of different oregano species and ecotypes improves not only their botanical description and/or identification, but also the development of conservation strategies for future breeding purposes. In addition, the development of 'modern' -omics technologies and state-of-the-art analytical tools provide great opportunities for the sustainable use of, as yet 'unimproved', plant species.

Our plant collection feature in this issue is an inspiring piece by Farahnoz Khojayori, who describes her collecting trips in Texas, in the summer of 2019 in the company of Morgan Gostel and Seth Hamby (pages 13-15). Like three botanical musketeers, they travelled to 13 botanical sites, including botanical gardens, nature centres, wildlife refuges, and state parks and were inspired by the people they met and worked with. Amongst their achievements, they collected herbarium vouchers and genome quality tissues preserved in liquid nitrogen and silica gel for 250 taxa of plants. Amongst these were five new taxa at the family level and 115 new species added to the GGBN database.

Last but not least, our featured garden in this edition is the Northwestern University Ecological Park & Botanic Gardens (NUEBG), a private university garden in the Philippines established as a living laboratory of the flora of Northwestern Luzon (pages 9-12). The NUEBG is an 8-hectare haven for biodiversity in an otherwise degraded and transformed landscape, and the leadership role that the garden plays in preserving Luzon's remaining biodiversity is an inspiration to us all.

Happy reading, and please don't hesitate to get in touch with your ideas for future issues of BGjournal.

Paul Smith
Secretary General, BGCI

FEATURES

NEWS FROM BGCI

**FEATURED GARDEN:
THE NORTHWESTERN UNIVERSITY ECOLOGICAL PARK & BOTANIC GARDENS**

**PLANT HUNTING TALES:
GARDENS AND THEIR LESSONS: THE JOURNAL OF A BOTANY STUDENT**

**TALKING PLANTS: INTERVIEW WITH JONATHAN CODDINGTON,
THE DIRECTOR OF THE GLOBAL GENOME INITIATIVE**

One of the four orchid houses at the Dr Cecilia Koo Botanic Conservation Centre (KBCC)

NEWS FROM BGCI



Local villagers helping with reinforcement planting of *Magnolia omeiensis*.

Here we present a selection of the most recent news stories from BGCI. Please browse our website to keep up-to-date with the latest news and events from BGCI and the botanic garden community. www.bgci.org

Ten Golden Rules for Reforestation (or planting the right tree in the right place)

BGCI, together with researchers from RBG Kew have set out ten golden rules for reforestation to ensure that restoring forests benefits people and the planet.

The 10 golden rules, set out in a paper published in the journal *Global Change Biology*, focus on protecting existing forests first, putting local people at the heart of projects, and using natural regrowth of trees where possible. The rules highlight that planting trees is complex and there is no universal, easy solution to a successful reforestation.

A virtual conference on the topic is being held from February 24-26, organised by RBG Kew and BGCI. It will bring together a series of global perspectives to debate and challenge the myth that “all tree planting is good” and to discuss best practice for protecting and restoring the world's forests.



Quercus brandegeei seedlings planted in Mexico. (The Morton Arboretum)

Ten Golden Rules for reforestation:

1. Work with local people;
2. Maximize biodiversity recovery to meet multiple goals;
3. Select the right area for reforestation;
4. Use natural forest restoration wherever possible;
5. Select tree species that maximise biodiversity;
6. Use resilient tree species that can adapt to a changing climate;
7. Plan ahead;
8. Learn by doing;
9. Make it pay.

Find out more and read the full article here: *Ten golden rules for reforestation to optimize carbon sequestration, biodiversity recovery and livelihood benefits - Di Sacco - - Global Change Biology - Wiley Online Library*

World Biodiversity Forum

Planting the right tree in the right place was also the key message promoted by BGCI at the recent World Biodiversity Forum. BGCI joined experts from the University of British Columbia and the University of São Paulo in a virtual event that also highlighted the importance of leveraging the expertise available in botanic gardens worldwide for tree planting and restoration.

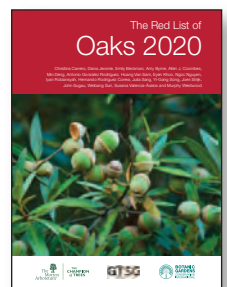
The webinar, entitled *Lipstick on a fig tree: Why planting trees avoids the real problems (and may make things worse)* can be viewed on line here: *BGCI shares expertise on tree planting at the World Biodiversity Forum | Botanic Gardens Conservation International*

One-third of the world's oaks at risk of extinction

An estimated 31% of the world's oak species are threatened with extinction according to data compiled in a new report by BGCI, The Morton Arboretum and the International Union for Conservation of Nature (IUCN) Global Tree Specialist Group. The Red List of Oaks 2020 details for the first time the distributions, population trends and threats facing the world's estimated 430 oak species, and will serve as a roadmap for conservation action.

According to the researchers, an estimated 41% of the world's 430 oak species are of conservation concern. Nearly one-third (31%) are considered threatened with extinction. This proportion of threatened species is higher than threat levels for mammals (26%) and birds (14%). The report indicates that the countries with the highest number of threatened oak species are Mexico (32 species), China (36), Vietnam (20), and the United States (16).

Find out more here; *One-third of the world's oaks at risk of extinction, according to new BGCI report | Botanic Gardens Conservation International*



(The Morton Arboretum)

Tree Conservation Fund

BGCI's newly launched Tree Conservation Fund is an initiative to enable businesses, philanthropic organisations and governments around the world to contribute to the essential effort to save the world's threatened tree species. At least 3,500 tree species are on the brink of extinction and urgent action is required. Through this fund, BGCI aims to develop partnerships with local communities and land managers to ensure that tree planting sustainably supports biodiversity and will provide the scientific and horticultural expertise to bring the right tree to the right place.

Find out more: BGCI's Tree Conservation Fund – Saving the world's threatened tree species



Desert Botanical Garden

GGI-Gardens Awards Program

The GGI-Gardens Awards Program aims to promote partnership activities to preserve and understand the Earth's genomic diversity of plants. The Awards Program aims to support collection and preservation activities at botanic gardens around the world. The Program, funded by GGI-Gardens and United States Botanic Garden, and administered through BGCI's

Global Botanic Garden Fund, is pleased to announce 14 grants to botanic gardens and arboreta in nine countries. The awardees will collect genome-quality plant tissue samples from their living collections and preserve them in a network of publicly accessible bio-repositories.

<https://www.bgci.org/news-events/14-botanic-gardens-awarded-grant-to-preserve-global-plant-biodiversity/>

Institution	Country
Atlanta Botanical Garden	United States
Core Facility Botanical Garden, University of Vienna	Austria
Desert Botanical Garden	United States
Inala Jurassic Garden	Australia
Instituto de Pesquisas Jardim Botânico do Rio de Janeiro	Brazil
Jawaharlal Nehru Tropical Botanic Garden and Research Institute	India
Kunming Botanical Garden	China
Montgomery Botanical Center	United States
Northwestern University Ecological Park & Botanic Gardens (NUEBG)	Philippines
Royal Botanic Garden	Jordan
San Diego Botanic Garden	United States
The Huntington	United States
Tooro Botanical Gardens	Uganda
Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences	China



Montgomery Botanical Center



Marsh awards

BGCI is pleased to announce Jeannie Raharimampionona from Missouri Botanical Garden's Madagascar programme as the winner of the 2020 Marsh Award for International Plant Conservation and Tara Moreau from UBC Botanical Garden as the winner of the 2020 Marsh Award for Education in Botanic Gardens.

Find out more here: [BGCI Announces 2020 Marsh Award Recipients | Botanic Gardens Conservation International](#)

Global botanic garden fund awards

BGCI's Global Botanic Garden Fund aims to support plant conservation and sustainable development, especially in smaller gardens, by disbursing small grants every year. In 2020, BGCI received 151 applications from more than 50 countries and 60 institutions. Following review, 40 grants worth more than \$82,000 have been awarded. Funding for the Global Botanic Garden Fund was made possible in 2020 by contributions from The Botanist Foundation, the Gibson Charitable Trust, crowd-sourced funding through the BigGive Christmas Challenge, and the BGCI/PlantSnap Covid-19 appeal.

A full list of grant recipients can be found here: [BGCI Announces 2020 Global Botanic Garden Fund Recipients | Botanic Gardens Conservation International](#)

Anthropology and Conservation Virtual Conference: 25-29 October, 2021

BGCI is delighted to be a co-organiser of the upcoming Anthropology and Conservation Virtual Conference, organised by the Royal Anthropological Institute. This major interdisciplinary conference aims to explore ways in which conservation and people go together, recognising that conservation can only be properly achieved with the full participation of, and in full recognition of the rights of Indigenous Peoples.

The call for panel proposals is now open, with a deadline of 12 March. BGCI members can propose panels, or join the conference as a delegate, at the Fellows rate.

Find out more: [Anthropology and Conservation 2021 \(therai.org.uk\)](#)

1st International Congress of Historical Botanical Gardens

Over recent years, there has been a renewed interest in the care and value of historic botanical gardens – those with built heritage, historical collections, or historical connections. Such gardens have made a major contribution to our understanding of the origin and development of botanical science, and to related disciplines such as herbal medicine, ethnobotany and ethnopharmacology. They have also influenced many of the crops we grow, the food we eat and the trees, shrubs and herbs that adorn our streets, parks, and gardens. However, unless prompt action is taken, many of these unique resources risk falling into neglect, decay or in some cases total loss. The 1st International Congress of Historic Botanical Gardens will be held in Lisbon, 11-12 October 2021.



Find out more: [1st International Congress of Historic Botanical Gardens \(historicalbotanicgardenscongress.org\)](#)

BGCI accreditation

BGCI's Accreditation Scheme distinguishes botanic gardens from non-botanic gardens.

<https://www.bgci.org/our-work/services-for-botanic-gardens/bgci-accreditation-scheme/>

The following botanic gardens achieved BGCI Botanic Garden Accreditation since the last issue of *BGJournal*:

- Universitetshagene, Norway
- Botanic Garden, Lund University, Sweden
- Jardin Botanique de l'Université de Fribourg, Switzerland



The following botanic gardens achieved BGCI Advanced Conservation Practitioner Accreditation since the last issue of *BGJournal*:

- Leon Levy Native Plant Preserve, Bahamas



FEATURED GARDEN

THE NORTHWESTERN UNIVERSITY ECOLOGICAL PARK & BOTANIC GARDENS



The NUEBG expedition team (Michael Agbayani Calaramo)

Racing against time to conserve the critical flora of the Northwestern Luzon, Philippines - The challenging role of a private university garden working to secure local plant diversity in a typhoon prone region of the country.

The Northwestern University Ecological Park & Botanic Gardens (NUEBG) is a private university garden in the Philippines established as a living laboratory of the flora of the Northwestern Luzon. The eight-hectare garden is ultimately designed to carry out plant conservation in response to the alarming decline of forest cover. This loss of habitat is due to a number of factors, including: rapid rural infrastructure development; changing climate that has triggered prolonged dry periods during the summer and intense monsoon rains during the wet season; and the over exploitation of natural resources that aggravates the loss of local biodiversity. Annually there are an average of twenty typhoons that pass over the country. As the prevalence of

super typhoons is increasing, the fear of losing the very delicate floristic blanket is a growing concern, especially as disturbances on critical landscapes constrict the natural provenance of narrow endemic species to a critical level. A further threat for this archipelagic country is its location in the Pacific ring of fire where earthquakes and volcanoes are active. This is the reality of what is happening in the NW Luzon in the Philippines - a megadiverse country yet among the hotspots of diversity loss.

As a result of the Northwestern University management review & forum in 2007, the concept of saving plant diversity in the NW Luzon was envisaged, and this was the reason for the establishment of NUEBG.

However, with no government support and dependent solely on the university's annual budget, this is a challenging task.

In preserving the natural heritage of the NW Luzon, the plant conservation program is seen as the institution's social obligation. Its mission and vision is to conserve plant diversity for the future of the Filipino people, as a sustainable source of food, medicine, clothing and building materials as well as the source of livelihood for many locals.

Botanical collections

The garden has an extensive plant collection for research and education purposes especially the Philippine gymnosperms, ferns and allies, aroids, orchids, Zingibers, Asclepiads, Aralias, Myrtaleans, Legumes, Malvids, Euphorbs, and Philippine endemic trees. It also houses numerous exotic species and important flora of the different continents.

As a member of the worldwide botanic community, it engages with a wide range of visitors from the scientific community who use botanical specimens for DNA and phytochemistry work. Its living collections are also arranged or grouped systematically and provide a conducive place for the study of plant systematics or field botany, while special ecosystems constructed to display specific flora are used by ecology classes. Today the botanic garden is immensely popular and is becoming the central hub in the northern Philippines for taxonomists and plant researchers, especially since the opening of the Herbarium of the Northwestern Luzon Philippines (HNUL), which houses more than twenty thousand collections to date.

Rediscovering the vegetation of the NW Luzon.

Plant expeditions were launched from 2007-2017 where the research unit of the garden had a chance to explore the diversity of NW Luzon. The decade long fieldwork enabled the team to document important plant species which were not yet included in the existing flora list. Some are so rare that very few individuals thrive in the wild. Taken as a whole, the flora of the region is hugely diverse and a wholistic program is needed to save this unique assembly.

The NUEBG continues to explore the remaining forest fragments of the region. It has been able to identify 11 major vegetation types, from arid and mangrove



The NUEBG entrance rotunda (Michael Agbayani Calaramo)

coastal ecosystems up to the limestones and montane tropical rainforests. The NUEBG Biological Diversity Research Unit traces the collection sites of Elmer Drew Merrill from the early 1900's and is trying to recollect the 400 species that are endemic to the region. While the vegetation has changed over a century, some of the species are already extirpated from their type locality. These species are *Ixora ilocana*, *Syzygium ilocanum* and *Psychotria palimlimensis*. But because of far-reaching exploration, thanks to numerous research grants that the university has received, the NUEBG team have found new extant populations of these species outside their previous distribution range.

Extent of the biological research

The NUEBG team is comprised of botanical staff, university academic personnel, researchers, volunteers, and private enthusiasts. This team has been carrying out a meticulous documentation of the flora and fauna of the entire NW Luzon from the west of Cagayan and the entire Ilocos region down to the Zambales mountain range in the south. Aside from floristic sampling the team is also engaged in wildlife documentation. The Asian Raptor Research Conservation Network has partnered with the team since 2015 and has documented numerous migratory birds which are vital indicators of the status of the vegetation where they stay. This work documented the first Philippine country record of the Black Stork in December 2018. Moreover, the NUEBG team is an active participant in the Asian Waterbird Census and numerous wildlife conservation and monitoring advocacy programs. It has also presented numerous papers in international symposia and fora.

Working with local organisations

The NUEBG biological diversity research unit also played an important role in the floristic inventory of 5 national parks in the region and the establishment of a new protected area in the province of Ilocos Norte. It works diligently with the Department of Environment & Natural Resources to conserve key species of the region and identify critical ecosystems that need protection.



Taxonomy classes (Michael Agbayani Calaramo)



View of the gardens (Michael Agbayani Calaramo)

This is instrumental to most local government units with a key role in the protection of critical vegetation. These vegetation types can be vast mafic ecosystems or coastal intertidal zones which harbor endemic species. Dense tropical rainforests are endorsed to be part of protected landscapes. This is very timely work as the Local Government Units (LGU) are updating their Comprehensive Land Use Plans (CLUP). This identification of important biodiversity spots is therefore included in their environmental protection and conservation strategy.

Plant conservation and international collaboration

The garden's living plant collections are valued and provide hope for the flora of the NW Luzon. These botanical collections contain considerable numbers of keystone species that will augment and arrest the decline of plant populations in the wild. However, the garden itself is equally affected by devastation and natural calamities, and the NUEBG cannot afford to lose even a single species. As a long-term solution, the NUEBG must develop strategies to

ensure that there are backup specimens that can be relied upon whenever there is devastation - and the most efficient strategy is through seed conservation.

In 2013 the NUEBG started to collect and save seeds in a freezer, as stocks to maintain its living collections. They are propagated every time a typhoon damages a collection, or to increase the species population at the gardens. However, this program was on a short-term basis due to lack of seed banking protocols and management expertise. Then, in 2018, the garden director attended a seed conservation training course at the Millennium Seedbank, and NUEBG became the first botanic garden in the country to uphold the MSB seed conservation techniques and protocols.

NUEBG has spread its arms to numerous international collaborators in plant conservation. In 2020, the Dr. Cecilia Koo Botanic Conservation Center (KBCC) in Taiwan, which also experiences severe tropical storms, partnered with NUEBG to share expertise and conservation techniques to save the plants in peril along the Taiwan and Luzon island arc flora. This partnership is one among many possibilities to join forces in the battle to combat plant extinction in this typhoon struck region.



Seedlings for restoration work (Michael Agbayani Calaramo)



Screen House for newly acquired collections (Michael Agbayani Calaramo)

Ecological restoration projects

NUEBG has several *in situ* field work sites. A 21 ha *Anisoptera thurifera* forest located in Piddig Ilocos Norte is one of the most unique sites due to a remarkable stand of a dipterocarp species thriving in very arid seasonal mafic vegetation. The NUEBG team, in collaboration with the Far Eastern University Herbarium researchers in Manila (FEUH), are conducting a monitoring and conservation effort to restore the natural stand of dipterocarps and its coexisting flora. These exceptional trees are becoming endangered as they are affected by climate change and habitat loss due to annual forest fires.

Another 100 ha ecological restoration site in Tadao Pasuquin, in the same province, was launched in 2013 where a list of carefully selected framework species was used to augment the declining vegetation. The site is a limestone forest with patches of mafic vegetation. This site is also a watershed and this restoration combats the decline in forest cover and degradation that could result in low water supply for the surrounding community.

Discoveries and publications

Despite the few scientific staff at the garden, the decade-long NUEBG exploration has enabled the production of scientific writings. Eight new species to science have been published from 2015-2018. These include orchids (*Cleisostoma*

ilocoense, *Robiquetia ilocosnortensis*, *Robiquetia roycimatui*, *Thrixspermum nicolasiorum*, *Dendrobium elineae*), a carnivorous plant (*Nepenthes enigma*), a begonia (*Begonia palemlemensis*), and an endemic shrub (*Psychotria triflora*). And more exciting discoveries are waiting for submission.

The future of NUEBG

While the Northwestern University operates as a private entity, its scope and extent of biodiversity work marks an indelible achievement in the documentation and conservation

of important biodiversity restricted to the northwestern edge of the island of Luzon. It will continue to uphold its:

Vision for Mother Earth:

NUEBG will lead a group of academic plant scientists in Luzon to respond to the needs of Northwest Luzon's diminishing ecosystems and respond to the environmental needs of its local communities as affected by natural calamities, human exploitation, and the changing climate.

Mission for Humanity:

A mission that would uplift the life of the local people by educating the younger populace to conserve the region's natural resources (including the ecological aspects used for ecotourism, the fertile lands for agriculture, the marine ecosystem and forest reserves which are the backbone of biodiversity) which are vital components of human survival.

The NUEBG is seeking support from the international community to conserve the island arc flora of Luzon, Taiwan and Japan as they are of utmost conservation priority.

Michael Agbayani Calaramo,
Northwestern University Ecotourism
Park and Botanic Gardens,
Laoag City,
Ilocos Norte,
2900 Philippines



Seed banking at NUEBG (Michael Agbayani Calaramo)



PLANT HUNTING TALES GARDENS AND THEIR LESSONS: THE JOURNAL OF A BOTANY STUDENT

Dr. Morgan Gestel teaching a student how to identify and collect a plant tissue sample at the Lady Bird Johnson Wildflower Center. (Farahnoz Khojayori)

“Take the question to nature”

Many centuries ago, the philosopher Francis Bacon encouraged people to “take the question to nature” and explore the morphological diversity of the world with their own eyes and hands. This was the method of the ancients such as Aristotle and Theophrastus, the father of Botany, who through meticulous study of morphological features of the plants were able to gather valuable information about their properties and share them with the public. As time went on, botanists and philosophers became entirely reliant on this previously collected information and often failed to interact with the world around them. Thus Bacon’s frustration encouraged people to seek answers to their questions within nature itself. Recently, the acceleration of genomic tools and reduction of their costs have

proliferated a vast repository of genomic data, but often times that data’s value is undermined by the lack of precise species information. Furthermore, the existing data only encompasses a small percentage of known and existing species on earth, most of which still remain undescribed. Thus, the Global Genome Initiative (GGI) was created to facilitate the identification and collection of non-human taxa across the Tree of Life, with a special focus on dark taxa that have no existing genomic information. Situated within the Smithsonian Institution, GGI attempts to collect and preserve genome-quality tissues from all non-human species, and deposit them on the Global Genome Biodiversity Network repository so that the collections may be accessible by researchers to enable new advances and discoveries. But a far more lasting impact of the initiative is to train a new

generation of field scientists, with specialized experience in identifying and understanding the diversity of this Earth.

Significance of gardens

Botanical gardens are a vast living collection of plants. Analogous to zoos or aquaria, botanical gardens often host plants from foreign and far-flung parts of the world to provide a tangible glance at diversity to the local people. Even Linnaeus himself attempted to recreate the biblical garden of Eden in the botanical gardens of Uppsala University by sending his students to collect plants from remote parts of the world. However, not many of those plants survived the harsh winters of Sweden. Similarly, Islamic cultures fascinated with heaven, attempted to recreate paradise gardens filled with glorious plants and later memorialized those gardens through weaving onto carpets.

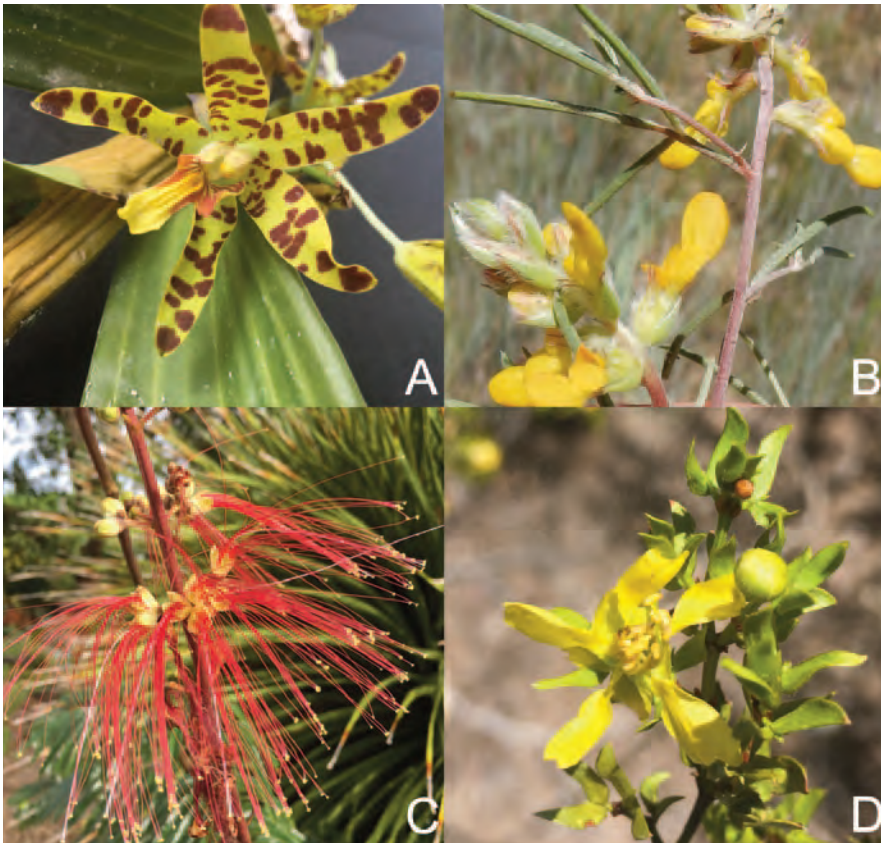


Fig. 2: Notable collections including (A) *Ansellia africana*, (B) the rare *Dalea hallii*, (C) explosive *Caesalpinia gilliesii*, and (D) common *Larrea tridentata*. (Farahnaz Khojayori)

During the renaissance, botanical gardens became a staple feature of universities, where cultivation of plants, encouraged by the medical department, fostered learning and medicinal use of plants. And as colonial pursuits vastly explored the unknown corners of the world, botanical gardens became a display of the conquests of empires. At the same time, desire to grow foreign plants rapidly increased interest in horticulture and development of glass houses to preserve and display unique tropical plants. Today, gardens embrace the history of their origins through magnificent displays of the Earth's wonders. But they also serve roles to preserve and protect valuable plants that can no longer thrive in their rapidly depleting natural habitats. Thus, gardens have become a refuge for plants, and today, some of the only living individuals of species can be found in gardens.

Once GGI began to harvest tissues across the world for the repository, Professor Vicki Funk dared the program director to start collection efforts in gardens, knowing full well the vast treasures stored in each one. Thus, the GGI for Gardens partner was born, and quickly expedited the vast collection of plants across the world. At its core GGI-Gardens fosters collection of genome quality plants, preservation of botanical

tissues, and provides access to vast genomic resources for plants to researchers across the world.

A summer of botanical discovery

During the summer of 2019, as part of the GGI for Gardens, Dr. Morgan Gostel, Seth Hamby, and I travelled across seven of the ten ecoregions of Texas (Fig. 1). Our mission was to accurately determine species and collect genome quality tissues of the local flora of Texas. In the short span of three months, we travelled to 13 botanical sites, including botanical gardens, nature centers, wildlife refuges, and state parks. As part of our efforts, we collected herbarium vouchers and genome quality tissues preserved in liquid nitrogen and silica gel for 250 taxa of plants. Amongst these were five new taxa at the family level and 115 new species added to the GGBN database. Some notable gems of the collection include *Dalea hallii*, *Aquilegia longissima*, *Ophioglossum engelmannii*, and *Larrea tridentata* (Fig. 2).

Anthropogenic angels

As we honed our collecting skills into a well-oiled three-person force, we traveled to the infamous city of Houston. Positioned uniquely on the confluence of four disparate ecoregions, Houston

offered an unprecedented explosion of botanical diversity. Over the course of three days we made 97 collections from the John Fairey Garden, nature areas around the coast, and the Mercer Botanic Gardens. When we started our trip in Peckerwood, working with Adam Black, we were not prepared for the diversity we encountered. In a short afternoon of surveying, we had managed to collect more samples than an entire day in our recent ventures in Fort Worth. Each plant was different than the next, from tall trees, to succulents and palms, to hidden ferns, and boisterous flowers with their showy personalities. The diversity before us was simply awe-striking. At one point, as we stood in the corner of the greenhouse and Adam presented specimen after specimen, we synchronized to the point of not even needing to talk. I would go and cut a specimen to press for a herbarium voucher, remove a couple leaves and pass them to Seth, who neatly catalogued and separated them into the tissue collecting vesicles. In the meantime, Morgan recorded every possible characteristic of the plant onto our field journal in iNaturalist. And then, we would restart the procedure with the next specimen. By the time, we raised our heads to look up, it was well into late afternoon, and the sun was setting. All we could do was marvel at over 70 specimens, wedged tightly between two pieces of wood.

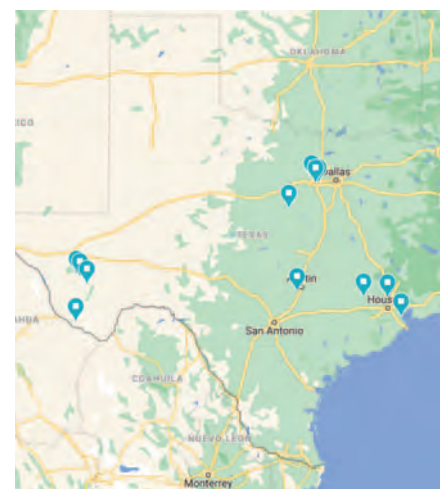


Fig. 1: Map of the collecting sites for GGI Gardens during the summer of 2019. (Farahnaz Khojayori)

It was at this point that I discovered the anthropogenic angels hidden in each garden. Botanists and naturalists like Bob O'Kennon and Adam Black, spend their entire lives dedicated to the understanding and preservations of the world's quickly disappearing biodiversity. At Mercer Botanical Gardens, I witnessed the stories of the surviving city. Overwhelmed and flooded by the disaster of Hurricane Harvey, most of the garden was once submerged underwater. Small potted plants like *Acanthostachys strobilacea*, were dislodged by the water and displaced miles away by its turbulent waves. But as the storm raged, rescuers joined the staff from across the state and country to come and recover the plants. Lost individuals were found, returned, and continued to thrive, showing the resilience of plants, people, and gardens.

At Chihuahuan Desert Research Institute, I witnessed the innovation of the human spirit. Volunteers from the local area would come together to cut down the dying inflorescence of Yucca trees, trim the leaves, and polish the wood to make walking sticks for the hickers to enjoy the desert terrain. Nearby, in the herbarium and botanical gardens of Sul Ross University, Dr. Michael and Mrs. Shirley Powell, dedicated decades to the classification, description, and illustration of the vast flora of the Trans-Pecos, in a magnificent tome the *Flora of the Trans-Pecos*.

At our home base in the Botanical Research Institute of Texas (BRIT) and its associated botanic gardens, I understood the true scope of gardens. Since their inception in 1934, the Fort Worth Botanic Gardens house 23 unique gardens, and are home to the largest collections of Begonias on the western hemisphere. On the other hand, the recently adjoined research institute houses nearly three million herbarium vouchers and fosters research on the frontiers of science. But more importantly, they provide education, conservation, and protection of the local fauna. Their education ranges from typical classroom lessons and internships for botanical students to advice and clarification in criminal botanical mysteries. More importantly, the BRIT press publishes articles, books, and reviews of botanical literature, cultivating botanical literacy across the world. And thus, each member of BRIT pushes the boundaries for the scope of gardens and the possibilities of botanical discovery.



Dr. Morgan Gostel preparing a herbarium voucher (left), Seth Hamby entering data into the field journal (right), and Farahnoz Khojayori collecting genome quality tissues (back-left). (Minette Marr)

Impact and future directions:

Despite the exhaustion, heat, and even danger posed by the elements and creatures before us, it was the single best experience in my life. The lessons of gardens transcend the boundaries of botanical research. Each garden seemed to have its own mission whether it was conservation, research, education, tourism, or a combination of those elements. Furthermore, unique programs at gardens provided a solace for the larger community, bringing people together through volunteering, or education. But largely the gardens were a refuge for plants, housing species that could no longer be found in the wild and preserving diversity through careful study and nurture of each plant into their adulthood. At Big Bend Ranch State Park and the Davis Mountains Preserve, I witnessed the complexity of nature. They taught me how plants and animals survived and responded to the convergence of drastically different climates and geographies, and the burst of diversity that was the ultimate result. In Houston, I witnessed the lessons in

resilience in the face of devastating circumstances. How a plant, a garden, a community could survive, support each other, and thrive despite all odds. In Austin, I saw how one person could plant a seed that set its roots throughout the land to inspire a culture of conservation and education. And in Fort Worth is where I learned how to live. I learned that life is far more complex and richer than one could imagine; that each venture posed an opportunity for an answer but far more doors for newer questions. I witnessed the vast human compassion of botanists, the kindness of strangers, and the wisdom of those who have lived in service of others, whether its plants or people. And so I walk a little wiser, but far more curious than ever, ready for the next adventure. Today, I am pursuing my PhD at the University of Cambridge. Seth Hamby is the head gardener of the Chihuahuan Desert Research Institute. And Dr. Morgan Gostel continues to be a lifelong friend and mentor, inspiring new students with his endless kindness and passion for botany, leaving a profound impact on our understanding of the world and the scope of GGI Gardens.

INTERVIEW

TALKING PLANTS



For this issue of BGjournal, we were delighted to have the opportunity to talk to **Jonathan Coddington**, the Director of the Global Genome Initiative.

You have been director of the Global Genome Initiative (GGI) since 2015. Can you tell us a bit about how your research on the systematics and behaviour of spiders led you to become interested in global biodiversity genomics.



An open liquid nitrogen tank at the Smithsonian's Biorepository. (Donald E. Hurlbert)

In my scientific career I have worked as a field botanist (e.g. publishing the candidate list of rare and endangered plants of Massachusetts in 1978 for the USA Endangered Species Act), was trained as an invertebrate zoologist, and then hired as a Smithsonian entomologist (spiders are not insects). I began to publish on biodiversity in general in the 1990's, saw the necessity of genomics for research and conservation in the early 2000's, and co-authored the vision for the Global Genome Initiative, "to preserve and understand the genomic diversity of Life," which I now direct.

The past year has been very challenging in many ways. Has the COVID-19 pandemic had a major impact on the work of the GGI and how have these impacts been overcome?

GGI simply switched to digital work. Genomic samples require a lot of metadata, and can be difficult to catalogue. It all happens on spreadsheets. GGI has also been supporting the inclusion of legacy genomic collections in the centralized NMNH biorepository; cleaning up and importing those data is also time-consuming. However, we usually fund a lot of field and laboratory work, and COVID-19 has prevented that.

The GGI hosts the secretariat of the Global Genome Biodiversity Network. How important is collaboration and networking to the success of the GGI and how are such relationships supported?

Networking and collaboration are fundamental to GGI. No single institution can address or ameliorate the effects of environmental change, and the Global South, is, after all, where most of megadiversity occurs. Countries own their own patrimony—partnership and collaboration is the only way forward. Even before GGI had significant funding, we hosted in 2011 a workshop on "virtual

biorepositories" involving 16 institutions from nine countries. It turned out that Germany had already solved many of the database/informatics issues in their DNA Bank Network. GGI built on that and helped to fund the development of the GGBN data standard, which is now compatible with all major natural history collection database systems. This contribution meant that any institution had a data model for their genetic resources, and those data could be shared with the world via the GGBN data portal. Membership in GGBN stands at 97 members from 35 countries, and is growing rapidly. A number of these institutions came in through GGI-Gardens. Botanical Gardens and arboreta, among all collections-based institutions, have a uniquely important opportunity and role in conserving their discipline, plant diversity. GGBN is a dues-paying organization, with its own rules and by-laws. I think biological collections-based institutions are coming to realize that genomics is such an effective research and conservation tool, that institutional survival and continued relevance depends on incorporating some version of genomics collections. Through GGBN and GGI-Gardens, GGI funds awards programs that are strongly weighted towards institutions in underserved and biodiverse countries.

Apart from the pandemic, what do you see as the major challenges facing the GGI in the coming years?

GGI was never intended to be, and is not, a permanent program. We were successful in fund raising for about two-thirds of our goal, but GGI will sunset at the end of 2021, having expensed many millions of dollars. It was always our intent to spend big, and as fast as prudence permitted. Genomic science widely acknowledges that access to high quality tissues is rate limiting, just as the still spotty "library" of DNA signatures, which permit industrial, scalable taxonomic identifications, is crucial.

Insofar as before GGI, no coherent international framework or mechanism to share computable biodiversity genomics data on samples existed, it seems possible that GGI has changed the world.

As well as leading the GGI, you also continue to have research and curatorial responsibilities. Which aspects of your job do you find most interesting and rewarding?

In spiders I still work on taxonomy, phylogeny, and evolution—the last major effort was a review of the astonishing diversity of spider sexual biology. However, it's fair to say that I am thrilled to get as many lineages as possible through the current extinction bottleneck. GGI is one of

the very few organizations that thinks about it ALL. For example, it is to me startling that only about 10,000 taxonomic families exist, and generally only about a dozen new ones are discovered per year. (That doesn't include lumping and splitting, or other rearrangements of known diversity.) I think we are pretty much done with the inventory of major lineages! GGBN has preserved, with its partners, about 5,700. We call the ones that are still out there, "dark" taxa. GGI provides guidance to many projects via gap analyses that tells you what is preserved and what is not. I enjoy focusing our efforts on the darkest taxa we can find. Just at the moment, I am mesmerized by an isolated parasite lineage found only in octopus kidneys (Dicyemida).

This journal is produced by and for the botanic garden community. Do you have a favourite botanic garden and if so, why is it your favourite?

Botany was the first natural history discipline I could really access, because of excellent local field guides. I was about eleven. At about 15 I started working as a cross-country ditch digger (don't ask), and I gobbled my lunch so I could spend the rest of the time botanizing. Only about 25 miles away from my hometown (on rotted schist) was the Housatonic River Valley—limestone! The difference astonished me. My first and sentimental favourite was Bartholomew's Cobble in Sheffield Massachusetts that specialized in native limestone ferns. Maybe it is not formally a garden, but it had a lot of labels, and I'm pretty sure the staff kept close track of their *Lygodium palmatum* (Bernh.) Swartz. Living in the DC area, I can easily get to the sumptuous gardens in the Brandywine River Valley, but I do like ones that showcase their local flora—or have Welwitschia.



(Barney Wilczak)

ARTICLES

BANKING BOTANICAL DIVERSITY WITH THE GLOBAL GENOME BIODIVERSITY NETWORK (GGBN)

**THE GLOBAL GENOME INITIATIVE FOR GARDENS:
CONSERVATION PRIORITIES AT THE INTERFACE OF BOTANIC GARDENS
AND BIODIVERSITY GENOMICS**

RIO DE JANEIRO BOTANICAL GARDEN AND THE GLOBAL GENOME INITIATIVE FOR GARDENS

LEARNING HOW TO SEQUENCE BIODIVERSITY BEFORE ITS GONE

CRYOPRESERVATION FOR THE FUTURE

**GENETIC AND GENOMIC STUDIES OF NATIVE MEDICINAL AND AROMATIC PLANTS MAINTAINED
IN BOTANICAL GARDEN AND LIVING COLLECTIONS IN GREECE: THE EXAMPLE OF *ORIGANUM* SPP.**

*Fern house at the Dr Cecilia Koo Botanical
Conservation Centre (KBCC)*



BANKING BOTANICAL BIODIVERSITY WITH THE GLOBAL GENOME BIODIVERSITY NETWORK (GGBN)

Liquid nitrogen storage tanks at the National Museum of Natural History's biorepository in Washington DC (Donald E. Hurlbert)

Excluding algae, mosses, liverworts and hornworts it is estimated that there are nearly 400,000 known plant species in the world, of which 370,000 are flowering plants. Presently around 2,000 new species are described each year – though this number is decreasing (Christenhusz and Byng, 2016). Surprisingly approximately one third of these known species may be found in botanic gardens, which also hold 41% of the known threatened species. Perhaps not surprisingly most of the species composition in botanic gardens is biased toward the temperate zone as that is where a substantial part of the world's botanic gardens are situated and it is estimated that 76% of the tropical species are absent from the collection (Mounce *et al.*, 2017). This goes to show the enormous role botanic gardens play in achieving global conservation goals, such as *ex situ* conservation of living plants, seed banks, and increasingly conservation genomics.

Traditionally botanic garden collections were used for taxonomy and general plant biology but the molecular revolution has changed this dramatically, and the need for quickly preserved, properly vouchered and correctly identified plant material has increased significantly. The use of traditionally preserved materials

from, e.g., herbaria, frequently becomes a challenge: 1) the DNA in specimens is often fragmented; 2) historical preservation techniques usually fail to inhibit endo- and exonuclease activity; or 3) the DNA can become almost inaccessible due to preservatives and fixatives that cause widespread post-mortem damage, interfering with sequencing (e.g. by cross-linking DNA and proteins in formalin-preserved tissues; see, Friedman and DeSalle, 2008; Zimmermann *et al.*, 2008). Even though advances in sequencing technology have significantly broadened the quality range of material that may potentially be sequenced, it remains easier and much more efficient to use either fresh material or material stored under optimal conditions, e.g., in biodiversity biobanks.

During the last decade, a series of genomics projects with huge taxonomic scope have been launched, e.g., Earth BioGenome Project: Sequencing life for the future of life (Lewin, 2018). Common to all larger genome projects is their heavy reliance on easy access to appropriate samples and botanic gardens are one excellent source of such relevant material. This fact has been very successfully explored by GGI-gardens and its members, who have added

substantially to the Global Genome Biodiversity Network's (GGBN: www.ggbn.org) diversity of plant samples. Together they have helped to close important taxonomic sampling gaps in conservation. However, there is no free lunch and any project, regardless of its size needs to comply with the ABS (Access and Benefit) requirements on the use of genetic resources as a consequence of the Nagoya-Protocol.

Building capacity to preserve the world's biodiversity

GGBN was founded in 2011 in order to join the efforts of promoting access to information about, and legal exchange of, the biodiversity genomic samples maintained by its members. It provides a platform to unite biodiversity biobanks from across the world in order to: 1) Enable data-mining and strategic analysis across one consistent database of global genomic resources, 2) Collaborate to ensure consistent quality standards for DNA and tissue collections, 3) Improve best practices for the preservation and use of such collections, 4) Harmonize exchange and use of genetic materials in accordance with national and international legislation and conventions, in particular the Nagoya Protocol, and 5) Enable targeted, strategic collection to fill crucial biodiversity gaps.



-80C freezers at the National Museum of Natural History's biorepository in Washington DC (Donald E. Hurlbert)

To achieve its mission, one of GGBN's principal activities is the management of a globally distributed database of genomic samples linked to voucher specimens – the GGBN Data Portal.

Connecting researchers to collections

Historically, scientists seeking genomic samples for research had no central access point to simplify their search. Thus, countless opportunities for research, development and conservation have been lost due to a lack of access to available biological resources. The GGBN Data Portal addresses this by providing researchers with a one-stop entrance to high-quality, well-documented, legally-obtained DNA and tissue samples that are compliant with access- and benefit-sharing agreements (not least the Nagoya Protocol). GGBN members contribute to the Data Portal, which is globally searchable, while samples remain the property of, and properly attributed to, the contributing member. Today 97 organizations have joined GGBN, mainly natural history collections and botanical gardens, but also seed banks, culture collections as well as Zoos and Aquaria, veterinary and agricultural collections. GGBN is working together with other stakeholders such as GBIF (Global Biodiversity Information Facility), INSDC (International Nucleotide Sequence Database Collaboration), and ISBER (International Society for Biological and Environmental Repositories) in order to provide tools and infrastructures for traceable and trackable molecular research. In this issue the importance and role of botanical collections is highlighted and we hope more collections will be

able to share their collections through infrastructures such as GGBN in the near future, for the benefit of everyone and major step towards implementing the Nagoya Protocol.

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Ole Seberg,
Natural History Museum of Denmark

Gabi Dröge,
Botanischer Garten
und Botanisches Museum,
Berlin-Dahlem, Germany

Glossary:

Biodiversity repository

A publicly accessible curated collection of biological material. Examples include museum, herbaria, botanical gardens, seed banks and zoos.

Biodiversity biobank

A subset of biodiversity repositories and collections that store DNA, RNA or tissue samples of biodiversity, (in general excluding human material).

Natural history collection

Traditional (i.e. morphological and other) museum and herbarium collections.

Genome sequencing

Genome, metagenome, transcriptome, and marker sequencing.

Genome quality

High-molecular weight DNA or RNA.

Biodiversity genomics

DNA sequencing of biodiversity.

Genomic sample

Any biological material preserved to keep its molecular properties (in general excluding human material). Examples include DNA, RNA, and tissue.

Exonuclease

Degradation of DNA or RNA in living organisms requires enzymes called nucleases, viz, deoxyribonucleases (DNAases) and ribonucleases (RNAses). These both fall in two categories exonucleases and endonucleases. Endonucleases cut the DNA or RNA molecules inside the molecule, exonucleases from either end.

DNA degradation

Chemical breakdown of the DNA molecule. The half-life of DNA is 521 years, hence fossils older than 5 or 6 million years don't contain any DNA anymore. To stop degradation a stable cold and dry environment is needed, today usually achieved by storing DNA samples in liquid nitrogen or -80C freezers.

Tissue preservation

In botany, tissues are normally stored in silica gel in a stable and dry environment. For RNA analyses tissue must be stored in liquid nitrogen or -80C immediately after sampling.



GGI-Gardens founder, Vicki Funk, collecting with interns at the U.S. Botanic Garden. L to R: Sara Gabler, Vicki Funk, Asia Hill, and Kristen van Neste. (U.S. Botanic Garden)

THE GLOBAL GENOME INITIATIVE FOR GARDENS: CONSERVATION PRIORITIES AT THE INTERFACE OF BOTANIC GARDENS AND BIODIVERSITY GENOMICS

An evolving role of botanic gardens in the genomic era

The Global Genome Initiative (GGI) is a program established with the mission “to preserve and understand the genomic diversity of life.” Accomplishing this mission means building a global infrastructure that is ready to meet the needs of the genomic community. The genomic revolution has transformed biology in the 21st century and is playing a central role in unlocking the mysteries of life. High-throughput sequencing technology is more and more dependent upon access to high-quality, preserved tissue collections as technological costs are reduced.

The set of instructions responsible for carrying out the processes of life are encoded in an organism’s genome and unfortunately that set of instructions quickly degrades and becomes fragmented after separation from a living organism. Genomic material can be preserved without degradation if it is collected from living specimens and then treated in various ways, including chemical preservatives or by storing in cold, dry conditions. Fortunately, botanic gardens play a central role in the preservation and study of genomic diversity in plants and are therefore, uniquely positioned to accomplish diverse sampling across the plant tree of life for large-scale genomic research.



In 2015, recognizing the vast plant diversity that is housed in the world’s botanic gardens, the Global Genome Initiative (GGI) founded GGI-Gardens, an international partnership dedicated to sampling and preserving plant biodiversity from the world’s extraordinary living collections. Genomic tissue sampling goals proposed by GGI-Gardens follow three phases:

- 1) all families,
- 2) 50% of genera, and
- 3) all species of vascular plants on Earth.



A GGI-Gardens collecting team at the U.S. Botanic Garden. L to R: Morgan Gostel, Asia Hill, and Vicki Funk. (U.S. Botanic Garden)



*GGI-Gardens interns collecting vouchers from *Musa textilis* at the U.S. Botanic Garden. L to R: Kathryn Faulconer, Maryam Sedaghatpour, and Samantha Vo. (Morgan Gostel)*

Since 2015, GGI-Gardens has grown to encompass nearly 40 partner gardens and has collected nearly 20,000 herbarium vouchers and genomic DNA tissues to date. GGI-Gardens coordinates with the Global Genome Biodiversity Network (GGBN) to ensure the samples are preserved in well-managed biodiversity repositories (biorepositories) and that genomic samples are made available for the international research community. In this way GGI-Gardens is working to increase the visibility of botanic gardens for highly-impactful genomics research, connect gardens with state-of-the-art research resources, and facilitate a global effort to sequence the genomes of all organisms on Earth, before their genomes are lost in the midst of a global biodiversity crisis.

Assessing goals and expanding GGI-Gardens

Two recent events have helped researchers delve deeper into the potential of botanic gardens for genomics. First is the expansion of the GGI-Gardens partnership and second is an assessment of plant diversity held in *ex situ* conservation collections by Mounce *et al.* (2017). This latter study utilized BGCI databases and has allowed GGI to assess progress toward our collecting goals and prioritize new collections and partnerships. At GGI-Gardens, we have been especially curious about two questions:

- 1) "Which plant genera have not yet been collected, preserved, and made available for genomic research?" and
- 2) "How many of these priority plant genera are contained in living plant collections at botanic gardens and how do we engage them through GGI-Gardens?"

To answer these questions a partnership was developed between BGCI and GGI-Gardens to assess genera-level sampling gaps from GGI-Gardens and determine priorities for collection (and which gardens might have the highest number of priority collections). Key public databases managed by GGBN and BGCI hold data which allows us to answer these questions and more. The GGBN Portal is a database that documents all tissue samples that have been collected and preserved in GGBN-partnered biorepositories. As of 17 January 2021 there are more than 1.7 million samples in this database. Two other key resources are the PlantSearch and ThreatSearch databases, hosted by BGCI, which document plant taxa reported in botanic gardens and other similar collections and a comprehensive database of assessments of the extinction risk of plants, respectively.

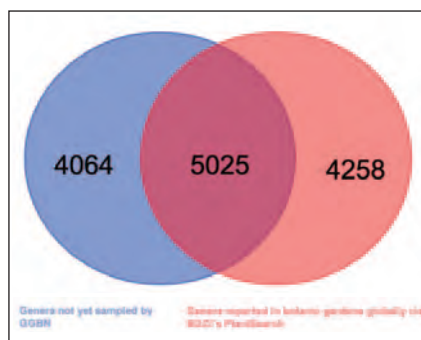


Figure 1: Results from a gap analysis of global collections databases displayed as a venn diagram. The Blue circle (L) includes all plant genera not found in the GGBN web portal and the Red Circle (R) includes all plant genera known to be found in living collections from botanic gardens worldwide (according to BGCI's PlantSearch collection database). The intersection shows that botanic gardens contain just over half of all genera of vascular plants that have not been collected, preserved, and made available for genomic research in a GGBN biorepository.

Using these databases, GGI-Gardens has been able to determine sampling progress and identify genera not yet sampled within the GGBN, but that can be found in living collections (see Figure 1). In addition, we identified unsampled genera present in the world's botanic gardens via PlantSearch and highlighted key countries and institutions to engage in the GGI-Gardens Partnership. Results of the analysis show that a total of 4,868 (or approximately 35%) vascular plant genera have been sampled and are available within the GGBN. That leaves approximately 9,000 genera which have not yet been sampled. Comparison with BGCI's PlantSearch database reveals that, of the approximately 9,000 genera not yet sampled, 5,025 are reported within the world's botanic garden living collections (Fig. 1). Data from the PlantSearch database suggest that gardens in biodiverse countries of the world including South Africa, Australia and Indonesia host unique unsampled genera not found in other gardens.

How can this data inform sampling programs?

GGI-Gardens partners have been utilizing gap analyses to coordinate their collections since the program's inception, but the addition of BGCI tools has led to significant advances. These tools have helped us reach out to botanic gardens with particularly high levels of unsampled diversity and they also indicate which taxa are not reported in living collections and therefore may need to be collected from the wild.



Reviewing living collections lists while searching for new, uncollected taxa at Lady Bird Johnson Wildflower Center. L to R: Morgan Gostel, Minnette Marr, and Seth Hamby. (Farahnoz Khojayori)



A collecting team at Mercer Botanic Gardens in Humble, Texas. L to R: Anita Tiller, Farahnoz Khojayori, and Seth Hamby. (Morgan Gastel)

In September 2020, GGI-Gardens, with support from BGCI and the United States Botanic Garden, announced an open call for applications to an awards program. These awards will support collection and preservation activities at botanic gardens around the world. Awards were announced to fourteen botanic gardens in January 2021 and will enable the collection of previously unsampled genera around the world (See News from BGCI).

GGI-Gardens has also begun collaborating with ambitious genome sequencing programs to identify and prioritize taxa in a collaborative and efficient way. Tissues collected and preserved through GGI-Gardens partnerships can be leveraged for whole genome sequencing through the 10,000 Plant Genome Project and Earth Biogenome Project. It is important to note some areas of significant gaps, particularly nonvascular plants

To find out whether your garden holds an unsampled genus, simply enter a genus level inventory list into the GGI Gap Analysis Tool (accessible via <https://www.globalgeno.me>). The output of this analysis shows the status of the genus (and associated synonyms) in the GGBN and GenBank databases. Those genera listed as 'No' in GGBN are a priority for sampling to increase the diversity of material available for preservation and research.

How your garden can contribute

Botanic gardens are already on the front lines of global plant conservation efforts and their missions have been closely aligned and informed by international policies such as the Global Strategy for Plant Conservation. As gardens lead the charge for myriad conservation programs such as seed banking, opportunity abounds to render added value from these activities and collections through voucher collection. GGI-Gardens was established to address the critical need for high quality tissues in the genomic era; but we also hope to motivate a paradigm shift in botanic garden collections that encourages the collection and documentation of voucher specimens for research and conservation. In this way, gardens have unparalleled potential to impact critical research priorities and an opportunity to render enormous scientific value from their already precious living collections.

The timing for coordinated collection of genomic tissues could not be better, as large-scale efforts such as the Darwin Tree of Life project (<https://www.darwintreeoflife.org/>) and the Earth Biogenome Project (Lewin *et al.*, 2018), are underway to sequence the genome of all species in Britain and Ireland and all species on Earth, respectively. Tissues collected by the GGI-Gardens program will help facilitate these and other sequencing projects through coordinated and targeted sampling approaches.

GGI-Gardens provides support for collection activities through awards, training, and informatics assistance. Joining GGI-Gardens is as simple as sending an email and signing a Memorandum of Cooperation on behalf of your institution. If your institution would like more information or to join GGI-Gardens, please send an email to GGI@BRIT.ORG.

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A researcher removing a rack of genomic tissues stored in a liquid nitrogen tank at the Smithsonian Institution's Biorepository. (Donald E. Hurlbert, Smithsonian Institution)

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The GGI-Gardens team at Lady Bird Johnson Wildflower Center in 2019. L to R: Seth Hamby, Minnette Marr, Morgan Gastel, and Farahnoz Khojayori. (Ashley Hackett)

RIO DE JANEIRO BOTANICAL GARDEN AND THE GLOBAL GENOME INITIATIVE FOR GARDENS



Bombax ceiba ("paineira-vermelha-da-india"). Leaf tissues of this native South Asia and Australia tree, new to GGBN, were sampled and made discoverable through the data portal. (Marcus Nadruz Coelho)

In a world of declining biodiversity, much has been said about the fundamental role botanical gardens are playing in conservation, both as storehouses of rare plants and as centers of research. This huge research agenda includes studying the amazing diversity of plant species, their relations to each other and their evolutionary origins. Like most older tropical botanical gardens, Rio de Janeiro Botanical Garden was established during the colonial era as a consequence of trade and commerce. Founded in 1808, with the transfer of the Royal family and the Portuguese Court to Brazil fleeing Napoleon's troops, it began as an acclimation garden for economically beneficial plants brought from other tropical regions of the world. The first challenge was to acclimatize the so-called spices of the East: vanilla, cinnamon, pepper and others. Yet, over the years, the mission has changed and expanded.

In 1824 the institution's director, botanical Friar Leandro do Sacramento, took the first steps towards transforming the garden, which was called "Real Horto Botânico" at the time, into a research center by identifying and scientifically organizing the existing specimens. Directors that followed him, like João Barbosa Rodrigues and Paulo Campos Porto, began organizing the living collection by dividing the Arboretum into sections and grouping plants by affinities, families, ecological requirements and regional groups (Amazon regions, Northeast and Cerrado). With a considerable and worldwide-recognized biological diversity, the Arboretum still maintains historical collections that date from its creation.

Therefore, although initially created as a place to experiment with plants sent from other Portuguese provinces, nowadays, together with other praised botanical

gardens, the Jardim Botânico do Rio de Janeiro (JBRJ) plays a pivotal role in garden science and plant conservation activities.

Rio de Janeiro Botanical Garden owns one of the most beautiful tropical collections, cultivated throughout its Arboretum. The Arboretum, where 77.9% of the specimens are determined at the species level, has 190 beds, distributed in 42 sections, within 54ha of cultivated area. The five families with the highest number of taxa represented are Bromeliaceae, Leguminosae, Arecaceae, Orchidaceae and Cactaceae. The living collection, composed by the Arboretum and Thematic Collections, has 11,877 specimens, with 3,069 taxa, of which 2,950 are species. After a thorough verification process, 541 species were identified as unique to Rio de Janeiro Botanical Garden, not being found in any other botanical garden in the world.



Nelumbo nucifera (“flor-de-lótus”). This perennial aquatic herb, distributed in tropical Asia and Australia, has been sampled and made discoverable through GGBN data portal. (Marcus Nadruz Coelho)

Of these, 522 are Brazilian and 19 are exotic species; among the Brazilian species, 317 are endemic. Amongst the 541 species unique to JBRJ, 46 are within one of the IUCN's endangered categories: Extinct in Nature (EX) - 1, Critically Endangered (CR) - 8, Endangered (EN) - 20 and Vulnerable (VU) - 17.

An article published in *Nature Plants* (Mounce *et al.*, 2017) evaluated the diversity of living collections from one-third of the existing botanical gardens. This survey revealed that these 1,116 botanical collections alone contained more than 100,000 species of plants, holding representatives from about 30% of species, 59% of genera and 75% of the families of all land plants globally and indicating the unique position of botanical gardens to help preserve and understand Earth's biodiversity.

In 2018, JBRJ received a GGI-Gardens Partnership Award and, with it, began a new genome-quality tissue sample collection, the “RBtecido” (RBtissue). Prioritizing taxa absent from other biorepositories worldwide, we vouchered and preserved genome-quality leaf tissues from 290 genera new to GGBN, 27 genera and seven families new to GGBN, new to GenBank and unique to Rio de Janeiro Botanical Garden. Together with the results of the 2018 GGBN-GGI Awards Program funding, JBRJ has made more than 6,500 DNA and tissue samples of the Brazilian flora discoverable through the GGBN data

portal, adding approximately 1,200 species to GGBN. Established in June 2004, Rio de Janeiro Botanical Garden DNA Bank (RBdna) maintains genetic information of approximately 5,700 specimens, representing the high diversity of Brazilian flora, especially among the diverse ecosystems that shape the Atlantic Rainforest biome. Our aim is to store DNA from relevant species of endangered Brazilian ecosystems, from different accessions of rare and/or endangered species, from Arboretum collections and from special taxonomic groups. DNA samples come from our researcher's expeditions and from donated plant material or DNA. DNA banks provide an efficient and long-term approach to preserve genetic resources, strengthening the power of traditional strategies by preserving the richness and diversity encoded by plant genomes (Seberg *et al.*, 2016).

Since 2005, Rio de Janeiro Botanical Garden has developed a database system for botanical collections known as JABOT system (<http://jabot.jbrj.gov.br>). The RB tissue collection database, the RB DNA sample database (RBdna) and JBRJ's other collections can also be accessed through the JABOT system.

Among the RBtissue collected specimens new to GGBN are the critically endangered and endangered bromeliads *Dyckia monticola* and *Portea kermesina*, respectively. Besides being unique to our Garden, the sampled *Wunderlichia*

azulensis (Asteraceae) and *Paratecoma peroba* (Bignoniaceae) are classified as endangered species. Vulnerable specimens, such as *Melanoxylon brauna* and *Apuleia leiocarpa* (Leguminosae), *Merianthera pulchra* (Melastomataceae) and *Acanthosyris paulo-alvini* (Santalaceae) are unique to JBRJ's arboretum. Other species with IUCN's vulnerable conservation status have been tissue-sampled and made discoverable through the GGBN data portal, such as Ucuúba (*Viola surinamensis*, Myristicaceae), Mogno (*Swietenia macrophylla*, Meliaceae), Castanha do Brasil (*Bertholletia excelsa*, Lecythidaceae) and Guabiroba, (*Campomanesia reitziana*, Myrtaceae). The enormous *ex situ* conservation significance of this garden cannot be overstated.

We believe that improving this collection will be of key importance for the scientific community. In this sense, nearly 100 genera new to GGBN have been selected as conservation priorities. Recently, JBRJ's cactus collection was awarded funding from BGCI to help maintain the collection and increase the number of species threatened with extinction in the collection. Ten cactus genera, within one of the IUCN's endangered categories, are among the selected genera new to GGBN that will be included in “RBtecido”. More than 30 Orchidaceae genera, new to GGBN and GenBank, have also been selected for sampling.



Bombax ceiba. (Marcus Nadruz Coelho)

While the “RBtecido” collection was being enriched with selected specimens from the arboretum, JBRJ – in partnership with Chico Mendes Institute for Biodiversity (ICMbio) – carried out several collection expeditions in protected areas to establish permanent parcels for long-term monitoring. During these expeditions, approximately 5,000 samples were collected for the herbarium RB, in 15 protected areas distributed throughout the Brazilian Amazon. At the same time, over 1,100 genome-quality tissue samples were preserved on silica gel and are now part of the “RBtecido” collection. Another thousand samples have yet to be cured and added to the RB tissue collection.

Due to habitat destruction and climate change, new estimates suggest two-fifths of the world's plants are at risk of extinction. The assessment from the State of the World's Plants and Fungi (Antonelli *et al.*, 2020) reaffirmed that Brazil is one of the countries with the greatest biological diversity in the world. Because devastation is happening very fast despite conservation efforts, we believe that preserving genome-quality tissue samples from plants in consolidated collections is crucial if a precise knowledge of these plant genetic resources ought to be saved for present and future generations.

In both the short and long term, the “RBtecido” collection will be a source of genetic material for collaborative genomics research on different areas of science, avoiding the destructive sampling of the herbarium exsiccates. It will also allow bioprospection of genes involved in important characteristics such as drug biosynthesis, food quality and plant resistance to pathogens and stress conditions.

Conservation activities at botanical gardens, together with development and research projects often involve collaboration and exchange of plant material between in-country institutions or botanical gardens and research laboratories in other countries. In the last thirty years, concerns involving the use of biological resources without the permission of countries of origin or sharing of benefits to the communities from where they originated, has led to the recognition of sovereign rights to control and exploit a nation's natural resources.



Dahlstedtia pinnata, also known as “timbozinho”, is absent from any other biorepository and is being preserved in RBtecido. (Marcus Nadruz Coelho)

Questions concerning access and use of plant genetic resources and associated traditional knowledge, as well as fair and equitable sharing of benefits that arise from this utilization are essentially guided by the Convention on Biological Diversity and its Nagoya Protocol.

Benefit Sharing consists of the division of benefits from the economic exploitation of finished product or reproductive material developed from access to genetic resources or associated traditional knowledge. The sharing of

benefits may occur in monetary and non-monetary modalities. The Brazilian Law establishes and delimits the negotiation, collection and application of resources, according to the type of access that gave rise to the product for which the distribution is due (access to genetic resources without associated traditional knowledge; access to associated traditional knowledge of identifiable origin; access to associated traditional knowledge of non-identifiable origin) (Law No. 13,123 / 2015 and regulated by Decree No. 8,772 / 2016).



Metternichia principis is an endemic Brazilian species, unique to Rio de Janeiro Botanical Garden. Leaf samples are being preserved in our tissue collection. (Marcus Nadruz Coelho)

Brazil's National Fund for the Sharing of Benefits (FNRB) was created by the same Law and is linked to the Ministry of the Environment (MMA). The Fund's main objective is to promote the appreciation of genetic resources and associated traditional knowledge and their use in a sustainable manner. Funds from Benefit Sharing in the monetary sense, due to economic exploitation of a product derived from access to genetic resources, are allocated to FNRB. The allocation amount may be 1% (one percent) of the annual net income obtained from the economic exploitation of the finished product or reproductive material. Monetary resources deposited in the fund, resulting from access to the genetic resources of *ex-situ* collections, can be in part (60% to 80%) allocated in benefit of these collections.

Non-monetary Benefit Sharing includes, among other initiatives, supporting projects and training of human resources on topics related to conservation or sustainable use of biodiversity. It also includes exchange of information, materials or technology between a

national scientific or technological research institution, public or private, and a research institution based abroad.

Companies can make this non-monetary Benefit Sharing by supporting conservation projects directly with research institutions, as long as the projects are approved by the Ministry of the Environment. JBRJ has three projects that involve its collections submitted in partnership with companies to MMA awaiting evaluation.

We hope that this new form of distribution will be a promising source of resources for maintaining and expanding JBRJ collections, so that they increasingly represent the high diversity of the Brazilian flora.

Biological collections play an important role in the flow of information on biodiversity, being basic components to support scientific development and technological innovation. We believe that strengthening science for the benefit of society depends on promoting wide access to data and information on biodiversity.



Pleroma marianum, popularly known as "quaresmeira", is endemic to Espírito Santo State. Leaf tissues of this bush, unique to JBRJ arboretum, were sampled and are being preserved. (Marcus Nadruz Coelho)



Metternichia principis. (Marcus Nadruz Coelho)

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LEARNING HOW TO SEQUENCE BIODIVERSITY BEFORE ITS GONE

Students join various beach goers in tide pooling for nudibranchs in Davenport Landing, California. They harvested four species for DNA extraction. (Rachel Meyer)

Facing mass extinctions of species, cultivars, and knowledge, our age of the Anthropocene sure has its wicked problems. As a planet, we struggle to meet the Aichi Biodiversity Targets to address the underlying causes of biodiversity loss (<https://www.cbd.int/aichi-targets/>), whilst still searching for the Essential Biodiversity Variables to discover cause and effect (e.g. Essential Biodiversity Variables; Pereira *et al.*, 2013; Lin *et al.*, 2020). We need to train the next leaders to be better than we are at slowing biodiversity loss and repairing our ecosystems.

At the University of California Santa Cruz, I teach a Molecular Ecology course where I tie together science policy, bioethics, and molecular initiatives that future molecular ecology must rely on. In particular, I introduce upper-division undergraduates to the standards for how biodiversity should be managed locally and across

international borders (permits; the Nagoya Protocol), and simultaneously introduce the molecular moonshot called the Earth BioGenome Project (Lewin *et al.*, 2018). Both policy and moonshot strive to understand and save global biodiversity, but there are few road maps to show a class on how to integrate them.

Why do we need students making genomes?

We don't have time to waste. If comparing species' genomes can tell us how to rank species that risk experiencing the extinction vortex (Gilpin & Soulé, 1986), we can smartly allocate resources to save them. Genome lab. work and sequencing is getting cheaper, easier, and increasingly being brought into the classroom (Jung *et al.*, 2020; Salazar *et al.*, 2020). I can imagine armies of undergraduates around the world

collecting locally available species, sequencing them, and publishing them, at a pace that competes with large science institutes. To accomplish this, they would need to become more fluent in biodiversity policy than most of the professors currently teaching them, and they would need to become extremely resourceful to access the biodiversity in private gardens, nature reserves, and collections such as those from botanical gardens, zoos and fungal culture banks.

Background on the Nagoya Protocol

In an effort to promote the conservation and celebration of biodiversity worldwide, the Convention on Biological Diversity (CBD) has put forth policies that fundamentally change the way we handle access to species and associated information for research and innovation.

These policies are bundled under the “Nagoya Protocol”, in full titled “The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity”. In brief, the Nagoya Protocol is a framework for how to obtain and utilize an organism and all the information associated with it, which includes everything from its physiology, behavior, adaptations and chemistry, to the traditional knowledge about how to use it, and potentially even, its digital genetic code.

Key practices outlined in the protocol include 1) identifying the appropriate groups who are the ‘providers’ of the organism (or knowledge), 2) articulating the aims of the ‘user’ (in most cases, the researcher) in the form of a document to be shared and negotiated until it can be signed to give Prior Informed Consent [PIC], 3) continuing communication between users and providers to write out *Mutually Agreed Terms* [MAT], and 4) registering and certifying these documents with the provider country through an *Access and Benefit Sharing Clearing House* (<https://absch.cbd.int/>) to enable countries to track and hold users accountable for adhering to and delivering on the terms.

The Nagoya Protocol meets biodiversity genomics

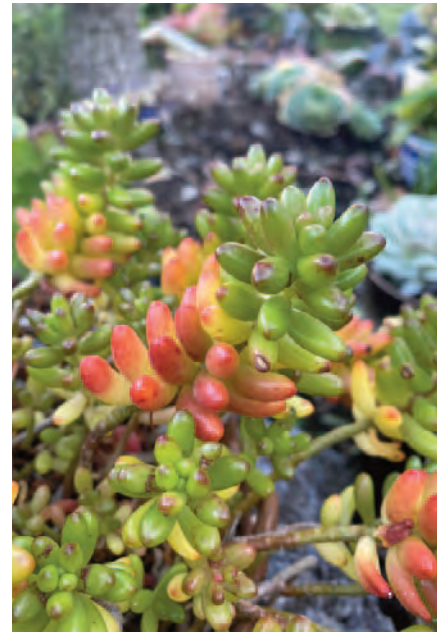
The spirit of the policy side, being the Nagoya Protocol, advocates for avoiding haste and investing the necessary time to find and bring together appropriate people to determine how certain biodiversity shall be used in a fair and equitable way. This may seem to conflict with the Earth BioGenome Project that is rapidly churning away to sequence every family — and then every species — of Eukaryote on the planet all within this chaotic decade. The take home message for my students is they do not conflict; rather, they enhance each other, if people involved are open-minded, resourceful, respectful, and willing to work hard.

Nonetheless, for most people trained in the ways of the past who want to help save biodiversity through science and particularly, through genomics, the tremendous tension between policy and moonshot is palpable. Delicate bridges could snap if we don’t build a constituency

able to work responsibly with both of them. One of the ways it could even snap this year is if digital sequence information (DNA, RNA, amino acids) becomes regulated such that capacity to compare genomes across the tree of life, is curtailed by national jurisdiction of those species’ origins (see Aubry 2019, and the CBD website <https://www.cbd.int/dsi-gr/> for more information).

The species sequencing assignment

I explained to my Fall 2020 class of 30 Molecular Ecology students that our research mission was to find species that would make great Earth BioGenome species, meaning sequencing should be a first for their evolutionary clade such as being a ‘family first’. In the pandemic, students would have to find a species that occurred in a botanical garden, a nature preserve, a state park, a private garden, or somewhere where they could obtain official permission to collect it. Once they had permission, they would be mailed a DNA extraction kit, and attempt DNA extraction on their own. They also prepared a collection identifier and a voucher for a freezer collection. Upon receiving the extracted DNA back, I would clean it to remove small degraded molecules and made TN5 shotgun sequencing libraries, sequence it on an Illumina NextSeq we have in house, and share the data back with the students. Together with the teaching assistant Joshua Kapp, we would help each



Sedum pachyphyllum (varietal ‘jelly beans’) collected from a garden by student Selena Lin. This plant is in the Crassulaceae family, which does not yet have a reference quality genome. Two *Sedum* L. organellar genomes are available, but as a large genus of ~600 species, it is important to have multiple species representing the genetic diversity of this genus. (Selena Lin)

student assemble de novo genomes from mitochondria and chloroplasts. Even though our effort was far from officially being an Earth BioGenome Project species with a nuclear genome of high standard, we would raise the visibility of certain species from certain places as accessible for genomics with permission.



Student Beny Castro meets expert Max Klepikob who has offered to share Lepidopteran collections from UCSC Fort Ord Natural Reserve. Beny selected the Tiger Moth, *Apatensis ornata* (Packard, 1864). (Rachel Meyer)



Field collections being inventoried and associated with terms of use for people who want to use them as a research resource. (Artwork by Maya Edelman)

Here are some of the student experiences:

- One student initially wanted to study iconic protected species, like monarch butterflies, and learned quickly how many hurdles it takes to develop the relationships wherewithal to navigate the regulatory system. She eventually found the UCSC Fort Ord Natural Reserve, that students can easily register a project and obtain official permission to study its species, then found graduate students studying invertebrates, and received some of their most exciting collections for DNA extraction.
- Two students wanted to study the many-colored nudibranchs in the Santa Cruz intertidal, and asked a professor who studies nudibranchs whom they had never met if he was willing to share permits as a collaboration. The professor happened to welcome this collaboration, but it was conditional on species choice. To help get the correct species, the students also had to find a local researcher with the skill to identify them, and actually get lucky enough to collect them.
- Several students wanted to sequence plants from the living collections at arboreta, botanical gardens and nurseries. For non-native local species, they had to contact staff and trace the origin of the germplasm that was being replicated. If the plant stock

was obtained before October 12, 2014, the day the Nagoya Protocol entered into force, then it did not fall under the Nagoya Protocol, and could be sequenced with permission from the garden or nursery (which often had thorough terms of use). If it was obtained after that date, they needed to follow the Nagoya Protocol and begin the process of identifying a host country and a provider community to develop the *PIC* and *MAT*, and to eventually register the project. This would be impossible to do in the short time of an academic quarter, so I recommended they switch to another species.

- Plants from a family garden were the focus of five students. As private land, if the plants were there prior to October 12, 2014, only the property owner had to give written permission to allow the students to sequence the plant. Usually this was a one sentence email. The students had to contemplate if that was fair because it was easy, and whether seeking an alternative source for the species, such as from a botanical garden, or as a permitted collection from the country of origin, would add more ethical protection for the species and the people who have a multi-generational relationship with the plant. A few students chose to change their species from an exotic one to a native one when they considered the trade-offs of collection types and origins.

- One student wanted to sequence a plant from a state park. She filled out the permit request forms, with excellent help from the Parks department to evaluate drafts applications. Then she waited, and sadly never received a review of her application to this day. She switched species and collection locations.

Learning Outcomes

The students worked hard for this assignment, and part of what drove them was to have data to contribute to the world's inventory of biodiversity through DNA, in this case, NCBI Genbank (NCBI 2021). Every week, we had in-class discussions to share our logistical struggles with obtaining official permission and our internal struggles over the most ethical source for a collection that would be sequenced. Students finished the quarter with 30 examples of how to navigate the Nagoya Protocol, local permits, and to expect many endeavors to fail. They thought about the ability of one person with experience to help others (such as the case of the nudibranchs). They thought about our responsibility to set the terms of use wisely, such as the Nagoya Protocol PIC and MAT, and that this matters locally as well as internationally, even if working with private land owners. Perhaps most important, however, is that students taught each other that failures and dead ends happen a lot in biodiversity sequencing, that there is no way to push through permissions, but if you are willing to pivot, there is plenty to focus on.



Students Beny Castro and Marisa Dobkins join UCSC graduate student Jon Detka outside of the Norris Center to select Lepidopteran collections for sequencing. Rachel (standing) crashed the party to drop off supplies. (Max Klepikov)

Based on a mid-quarter survey of 24 students, 63% of students had improved their confidence in their ability to do *in situ* collection fieldwork. Before the activity, 10 students did not rank themselves as knowing how to do fieldwork, and after the activity, all but one student felt at least somewhat skilled and able to teach others what the process of getting permission for field sampling involves.

Next Year: Focus more on botanical gardens, as they reflect ethical and policy shifts

As I reflect on the class activity and plan for next year, I think I'll add more background on how many botanical collections originated from voyages, aiming to collect the most interesting, culturally treasured and useful species so they could be studied and exploited by a completely new group of users -- and how collections increase now in the 21st century. The Hortus Botanicus in the Netherlands was filled in the 1600's with Asian species grown to help the local population remedy the bubonic plague. Now, the Hortus Botanicus has forged a model partnership with the China Academy of Chinese Medical Sciences to DNA sequence and chemically analyze the collection, returning benefits derived from

the collection back to the origins of the collections. Botanical gardens are a reflection of societies' progression through cultural imperialism and inequity to collaboration and agreements that intend to benefit the planet. Many gardens and curated nature reserves and parks play key roles in brokering fair and equitable use of plant specimens, and traditional botanical knowledge associated with them, for research and innovation, with one example being that they must guardian records of permission and conditions of use surrounding each plant collection, living or preserved. Teaching the history of some botanical collections explains the impetus for the Nagoya Protocol, and show examples of how to follow it, while possessing rich biodiversity to help species sequencing moonshots.

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Cryopreservation at KBCC. (KBCC)



Palm house (above) and cold room with constant 24°C at KBCC. (KBCC)

CRYOPRESERVATION FOR THE FUTURE

Human-driven destruction of habitats is leading us to the brink of a global biological disaster that could rival anything in evolutionary history. Tropical plants are especially under enormous threat and disappearing at an alarming rate. Conservation strategies designed to protect plants have taken place at local and international levels in recent decades. However, tropical plants with their richest diversity are often neglected, since it is difficult and costly to meet their heat and humidity growing requirements in developed temperate countries. Rescuing endangered tropical plant species is critically important to allow damaged ecosystems to be restored in the future.

The Dr. Cecilia Koo Botanic Conservation Center

The Dr. Cecilia Koo Botanic Conservation Center (KBCC), located in southern Taiwan, was established in 2007 with the mission: *to conserve the tropical and subtropical plants, in order to sustain the richest biodiversity on Earth.*

KBCC is mainly supported by Dr. Cecilia Koo Botanic Conservation and Environmental Protection Foundation and

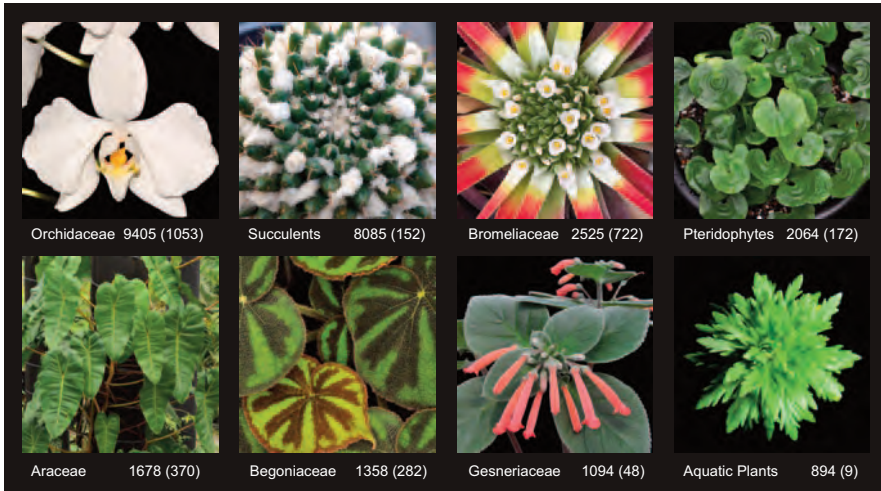
partially by the Ministry of Science and Technology, Taiwan (ROC). It focuses on *ex situ* preservation of living plants, and now houses 33,963 species and cultivars of plants, representing one of the richest living plant collections in the world.

The majority of plants growing in KBCC are kept in 17 greenhouses with 35,240 m² floor space. Every pot of plants is barcoded for easy record tracking. KBCC is not open to the general public, and thus its 30 fulltime staff can concentrate their efforts on caring for the plants.

However, scholars are welcome to collect materials for basic research and pharmaceutical and horticultural applications.

Conservation activities

The major threat posed by global warming and climate change to the cloud forest is reduced moisture. Starting from 2012, two greenhouses were built in central Taiwan at 1,100m elevation for hosting more than 1,000 species of plants coming from global cloud forests.



Every pot of plants is barcoded. (KBCC)

accessions, relating to 4,392 taxa, have been cryopreserved. Importantly, for each species, at least three organ types have been separately frozen, each having two replicate vials. The plant material is put into a cryovial which has a barcode attached, a scanner is used to link that tube to the associated data in the database. This liquid nitrogen sample is also associated with a herbarium specimen deposited in the National Museum of Natural Science, Taiwan. In total, 59,937 cryovials have been made and catalogued at KBCC since 2016. A full-time manager is currently dedicated to overseeing and growing our cryopreservation portfolio. The cryopreserved materials will greatly facilitate the ongoing efforts to broadly decode the genomic information from across the plant tree-of-life. KBCC is eager to share experiences and join forces with other botanical gardens to protect and learn from our valuable biodiversity resources before it is too late. KBCC has therefore joined the Global Genome Biodiversity Network which links institutions from around the world that share the interest in long-term preservation of genomic samples representing all non-human biodiversity on Earth.

It is important to stress, however, that this is just a beginning. In five years, we plan to have all the taxa, expecting to be around 40,000 in our living plant collection, cryopreserved.

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Above: the number of taxa in each plant group hosted in KBCC, the number in brackets representing taxa that have been deep frozen. Below: KBCC at night. (KBCC)

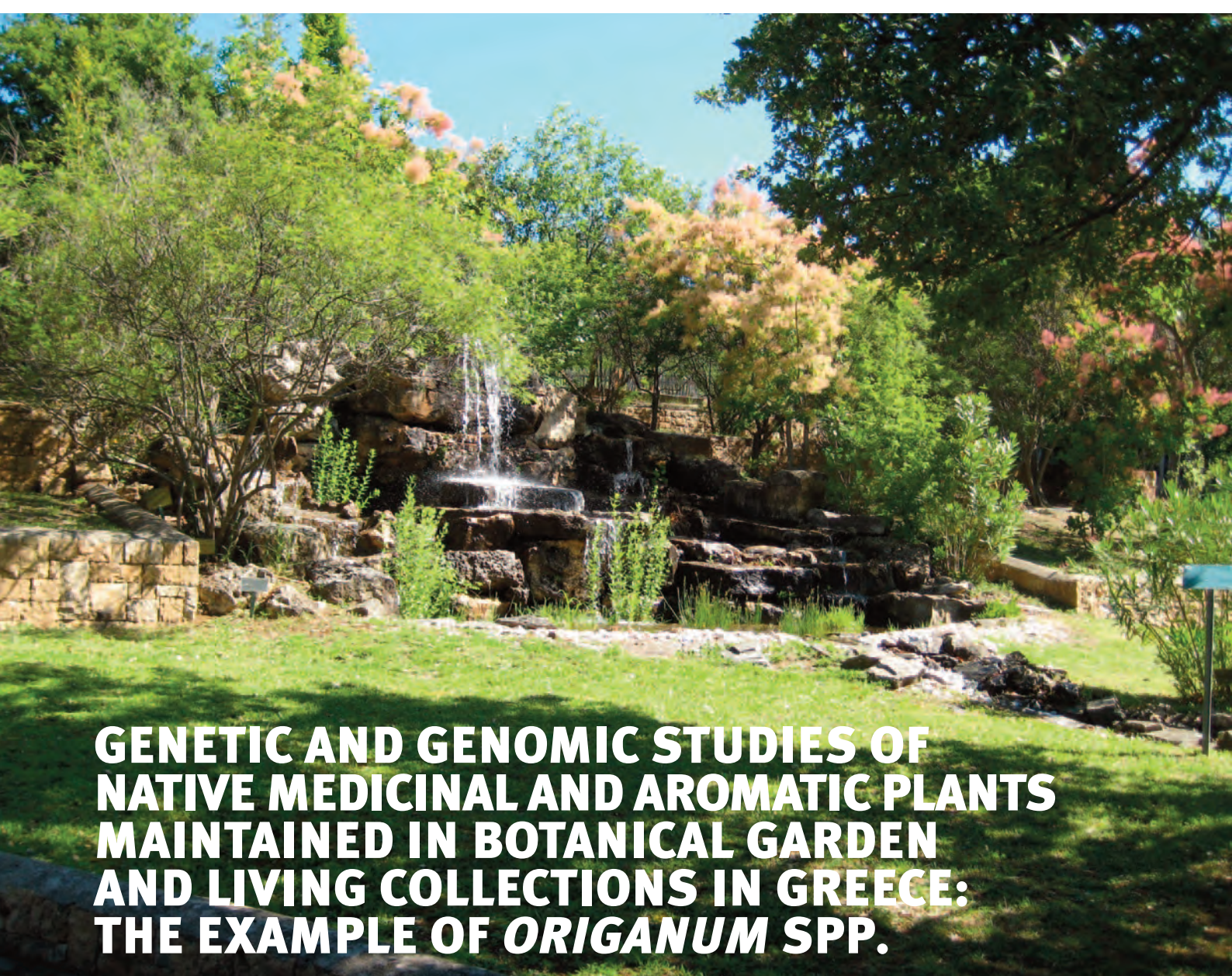
With the recent advancement of DNA sequencing technologies, it is becoming increasingly affordable and easy to obtain the entire genomic sequences from organisms of interest. For example, the genome of *Arabidopsis thaliana*—the first plant to have its genome sequenced cost over 100 million dollars and took 10 years to complete—and this can now be done with just \$500 over 2 days. Having high-quality genomes from across the plant tree-of-life will not only revolutionize our understanding of plant adaptation, but also unlock pharmaceutical and agricultural potential.

However, even with such accessible sequencing technologies, generating genomes from a “phylodiverse” collection of plants is still an extremely challenging endeavor. This is largely due to the difficulty of obtaining sufficient and high-quality materials for DNA and RNA extraction. Typically, to get enough DNA for long-read sequencing (PacBio or Oxford nanopore), one would need at least 20g of fresh or flash-frozen leaf samples. In addition, to better capture the

gene space and aid genome annotation, RNA samples from different parts of the plant would also be needed from the corresponding individual or genotype. While plant collectors often fix samples in the field for subsequent DNA work (e.g. with silica gels), they rarely yield high molecular weight DNA necessary for long-read sequencing. Furthermore, many studies have shown that the existing RNA preservation methods (e.g. RNAlater) cannot adequately prevent RNA from degradation, making it difficult to quantify gene expression level. In other words, the major hurdle now is the availability of the plant samples, and this is exactly the rationale behind our “deep frozen” initiative.

Cryopreservation

In order to preserve and safeguard high-quality tropical plant materials for future genomic and transcriptomic studies, a cryopreservation facility at KBCC was established with 40 liquid nitrogen tanks, each with a capacity to hold 6,000 samples. To date, a total of 8,489



GENETIC AND GENOMIC STUDIES OF NATIVE MEDICINAL AND AROMATIC PLANTS MAINTAINED IN BOTANICAL GARDEN AND LIVING COLLECTIONS IN GREECE: THE EXAMPLE OF *ORIGANUM* SPP.

Water loving plants at BBGK.

The **Balkan Botanic Garden of Kroussia** (BBGK) belongs to the Institute of Plant Breeding and Genetic Resources. The BBGK has been developed within a deciduous oak forest (*Quercus pubescens* and *Q. frainetto*) that has been traditionally managed for a long time, at an altitude of 600m (Mount Mavrovouni, Kroussia Mountain Range). It covers an area of 31ha and is dedicated to the combined *ex situ* and *in situ* conservation of native plants of Greece and the Balkans (15ha and 16ha, respectively), as well as to raising the environmental awareness of, and educating, the public. Since the beginning, the BBGK has been continuously supported by a well-equipped nursery and laboratory facilities.

All mother plant material is associated with explicit information regarding its collection in the wild i.e., geographical coordinates and site description (specific location, region, prefecture and country). Habitat information is also documented *in situ* for each individual accession collected (substrate, soil type, forest zone, habitat type, slope, altitude). Additionally, in many cases GIS is used to unveil the natural species-specific ecological preferences of conservation priority plants (Krigas *et al.*, 2010). This procedure represents the BBGK's 'explicit plant documentation' policy.



Greek tulips under *ex situ* conservation in BBGK.

2,400 accessions that correspond to 1.265 taxa are maintained in the Seed Bank
900 accessions that correspond to 330 taxa are maintained in the living collection (11,000 individuals)
480 accessions that correspond to 544 taxa are maintained both in the living collection and the Seed Bank
1,900 accessions that correspond to 1,103 taxa are maintained exclusively in the Seed Bank
420 accessions that correspond to 273 taxa are maintained exclusively in the living collection
18 accessions that correspond to 16 taxa are maintained in tissue culture
280 accessions that correspond to 266 taxa are under <i>ex situ</i> conservation in the BBGK
300 taxa are under <i>in situ</i> conservation in the BBGK
340 accessions that correspond to 303 taxa are under <i>ex situ</i> conservation in the Botanic Garden of Environmental Education in the premises of IPBGR

Table 1. Accessions and corresponding taxa maintained in connection to the Balkan Botanic Garden of Kroussia (BBGK).

To document the genetic identity of different accession numbers and to facilitate identification of the uniqueness of specimens, possible sustainable exploitation of selected accessions, accurate plant documentation, traceability, access and benefit sharing policies, the ‘DNA barcoding policy’ is applied with selected molecular markers (Tsoktouridis *et al.*, 2009; 2019).

The BBGK prioritizes the propagation and *ex situ* conservation of the important plant species (‘IPS first’ policy) and several species-specific propagation protocols have been produced (e.g., Gkika *et al.*, 2013; Grigoriadou *et al.*, 2011; 2014; Krigas *et al.*, 2010; Sarropoulou *et al.*, 2018). During the last 15 years, about 150 Greek endemics have been successfully propagated (Krigas *et al.*, 2016) and progressively over recent years, efforts have been intensified with >300 taxa (20% of all Greek endemics) now being conserved under *ex situ* conservation.

Any plant material shared with other institutions for research purposes or stakeholders is provided with a Material Transfer Agreement and passport data with International Plant Exchange Network (IPEN) numbering and in compliance with the EU Regulation 511/2014 implementing the Nagoya Protocol and the Greek National Law 4617, Issue A’ 88/10.6.2019 (‘Access and benefit sharing’ policy).

To date, >150 plant collecting expeditions have been organized in all phytogeographical regions of Greece and >3,700 accessions have been collected from the wild and are under conservation in the premises of the Institute of Plant Breeding and Genetic Resources (IPBGR) and the Balkan Botanic Garden of Kroussia (BBGK) (Seed Bank, living collection, tissue culture, botanic gardens). Among 3,000 maintained accessions that are systematically identified to date, there are 1,480 Greek native taxa (species or subspecies) under *ex-situ* conservation (> 22% of the Greek flora) (Table 1) (Maloupa *et al.*, 2008).

Genetic and genomic studies on *Origanum* spp.

Origanum vulgare L. is a typical species of Mediterranean ecosystems, growing in dry, sunny places from sea level up to 1500 m (Vokou *et al.*, 1993). Due to cross pollination and plasticity, the species is extremely variable and polymorphic (Azizi *et al.*, 2016). Greek oregano chemotypes have been classified according to their essential oil composition (Fleisher and Sneer, 1982). However, oregano crops commonly use wild populations without the appropriate plant material selection. For this reason, a wide variety of products with different qualities of raw material and essential oils, are produced and therefore selection and breeding represent a key role of the quality assurance system.



Amelanchier ovalis under *ex situ* conservation in the BBGK.

Especially in oregano, the extent of variability of populations offers an excellent source for selection work.

Origanum spp. genetics

Molecular breeding of medicinal and aromatic plants (MAPs) requires advanced technology such as genetic markers linked to agronomic and biochemical traits, high throughput, automated diagnostic techniques, and modification in breeding practice taking full advantage of the information provided by genome diagnostics. Lately, the continuous advances in molecular biology have offered a set of new techniques useful for systematic, phylogenetic and identification studies of MAP species. Studies reporting the use of molecular markers as significant tools for assessing the genetic variability between oregano species and populations and are summarized in Table 2. Understanding the genetic structure and variability of different oregano species and ecotypes, could improve not only their botanical description and/or identification, but also the development of conservation strategies for future breeding purposes.

The work of Gounaris *et al.* (2002) led to the discrimination of a putative hybrid *Origanum × intercedens* from Crete (Greece) and its parental taxa *O. onites* and *O. vulgare* using DNA fingerprinting with RAPD primers. Furthermore, Klocke *et al.* (2002) used RAPD fingerprints of



Species of genus *Salvia* (sages) with different pharmaceutical value.

single plants on a breeding program of *O. majorana* to illustrate the heterogeneity inside the accessions in the first cultivation year, indicating also the enhancement of the homogeneity in the next progenies caused by selfing. The same markers were also used from Katsiotis *et al.* (2009) who studied the phylogenetic relationships between *Origanum* and genera of the Menthae tribe reporting a clear distinction. In addition, the germplasm variability of the Greek oregano (*O. vulgare* subsp. *hirtum*) was investigated using RAPD markers, identifying significant genetic dissimilarities among Greek *O. vulgare* subsp. *hirtum* populations and *O. onites/O. virens* species. Such genetic variability was distributed mainly within populations and attributed mostly to



Collection of range-restricted species that are endemic (exist only) in Greece or the Balkans.

different geographical localities. Similarly, Tonk *et al.* (2010) used RAPDs for the determination of the genetic variability from Turkish oregano (*O. onites*), but the co-workers didn't identify any correlation between the genetic structure of the selected clones and the respective essential oil composition.

In order to estimate genetic diversity within *O. onites* (L.) germplasm, Ayanoglou *et al.* (2006) used Amplified Fragment Length Polymorphism (AFLP) analysis. Likewise, Azizi *et al.* (2009) reported the relative efficiencies of two PCR-based marker approaches, AFLP and selectively amplified microsatellite polymorphic loci (SAMPL) for comparable genetic diversity surveys and subspecies discrimination among 42 oregano accessions. In their study the molecular analysis confirmed the morphological classification, while concluded that SAMPL markers were slightly more efficient in differentiating accessions and subspecies in their experiments.

Species	Markers	Reference
<i>Origanum × intercedens</i> , <i>O. onites</i> , <i>O. vulgare</i>	RAPD	Gounaris <i>et al.</i> (2002)
<i>O. vulgare</i> subsp. <i>hirtum</i> , <i>O. onites</i> , <i>O. virens</i>	RAPD	Katsiotis <i>et al.</i> (2009)
<i>O. majorana</i>	RAPD	Klocke <i>et al.</i> (2002)
<i>O. onites</i>	AFLP	Ayanoglou <i>et al.</i> (2006)
<i>O. vulgare</i> , <i>O. majorana</i>	SSR	Novak <i>et al.</i> (2008)
<i>O. vulgare</i>	SSR	Helsen <i>et al.</i> (2013)
<i>O. vulgare</i> L.	SSR	Lukas and Novak (2013)
<i>O. saccatum</i> , <i>O. solymicum</i> , <i>O. husnucanbaser</i> , <i>O. bilgeri</i> , <i>O. minutiformum</i> , <i>O. majorana</i> , <i>O. onites</i> , <i>O. vulgare</i> subsp. <i>Hirtum</i>	CAPS-microsatellite	Ince <i>et al.</i> (2014)
<i>O. vulgare</i> , <i>O. viride</i> , <i>O. virens</i> , <i>O. vulgare</i> ssp. <i>hirtum</i> , <i>O. gracile</i>	AFLP, SAMPL	Azizi <i>et al.</i> (2009)
<i>O. vulgare</i> ssp. <i>glandulosum</i>	SSR	Mechergui <i>et al.</i> (2016)

Table 2. Molecular markers reported for the determination of phylogenetic relationships and genetic diversity between oregano species and populations.



Plants threatened with extinction.

Simple-sequence repeats (SSR) markers were also developed from expressed sequence tags (ESTs) of essential oil glands of *O. vulgare*, while all loci developed from *O. vulgare* successfully cross-amplified in *O. majorana* (Novak *et al.* 2008). Lukas and Novak (2013) reported for first time the complete sequenced chloroplast genome of *O. vulgare* L. which exhibited no significant rearrangements but organization, gene order and content of typical angiosperm chloroplast genome. In addition, they concluded that within the Lamiales the *O. vulgare* chloroplast genome is close related to that of *Salvia miltiorrhiza*, while from the 16 chloroplast regions that were analyzed, only one (psbA-trnH intergeneric spacer region) is suggested for studying phylogenetic relationships within *Origanum* using SSRs as the most suitable tool. One year later, expressed sequence tags (ESTs) of *Thymus* and *Salvia* were used to develop 30 LB primer pairs, for the establishment of microsatellite and CAPS-microsatellite markers in 8 different *Origanum* species (Ince *et al.* 2014).

Apart from determining the genetic diversity; microsatellites have also been used to investigate the genetic effects of *O. vulgare* colonization in restored semi-natural grassland patches. Though Helsen *et al.* (2013) reported a significantly higher inbreeding coefficient in recent populations by comparing them with the putative source populations, though without decreasing their genetic diversity.

Nowadays the efficiency of sequencing together with a decrease in cost has increased dramatically single nucleotide polymorphisms (SNPs) genotyping technologies. Identification of SNPs requires a high level of genome sequence information. Since a draft oregano

genome was published only recently (Bornowski *et al.*, 2020), it is not surprising that no SNP analyses have been reported yet on oregano.

Transcriptomic approaches

Metabolic pathway analysis of MAPs by plant transcriptome analysis (RNA-seq) opens the way to functional plant breeding. Transcriptomics study has become one of the most active areas of genome research for MAP species. Significant efforts have been made to understand the molecular basis of secondary metabolites biosynthesis, while also developing EST-SSRs in some economically important species of Iridaceae, Poaceae and Lamiaceae families, although the literature is still limited concerning oregano studies. An interesting approach that could find potential application also in oregano molecular breeding, was reported from

Meena *et al.* (2016). In this study the authors indicated the involvement of identified candidate genes in the formation of aromatic compounds through comparative essential oil profiling and mRNA expression analysis in three *Cymbopogon* species. In addition, the identified SSRs in the transcriptome linked to terpene pathway genes including the genes potentially involved in aroma biosynthesis. Concerning the Lamiaceae family, the most extended studies on transcriptome sequencing that have been reported so far, focus on *Salvia miltiorrhiza* (Chen *et al.*, 2014; Xu *et al.*, 2015), *Ocimum* species (Torre *et al.*, 2016) and *Scutellaria baicalensis* (Liu *et al.*, 2015), detecting putative genes related to numerous metabolic pathways of important secondary metabolites as diterpenes (sage), anthocyanins (sweet basil) and flavonoids (*Scutellaria*), respectively.

Considering the wide range of bioactivities and the potential uses of the phenolic monoterpenes such as carvacrol and thymol, and other phenolic compounds mentioned previously, such metabolites represent one of the most important target traits also for molecular breeding of oregano. The first deep study investigated the biosynthetic pathway of oregano terpenes and their regulation through the use of transcriptomics was reported by Crocoll *et al.* (2010). In this work, the authors were able to identify and characterize seven terpene synthases, key enzymes of terpene biosynthesis, from two cultivars of *O. vulgare*.



Garden of the Senses during springtime.

Subsequently the regulation of carvacrol biosynthesis in combination to relative gene expression was studied in *Satureja khuzistanica* and two oregano species, using RT-qPCR analysis (Ramak *et al.*, 2013; Morshedloo *et al.*, 2017).

Conclusion

In conclusion, it is clear that botanical gardens and living plant collections can contribute towards preserving the valuable diversity of MAPs populations for domestication, breeding and further scientific purposes. Meanwhile, the development of 'modern' -omics technologies and state-to-the art analytical tools provide great challenges and opportunities for the exploitation of such a great biodiversity of, as yet not improved, plant species.

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Pilot cultivation of greek native *Rhus koriaria*.

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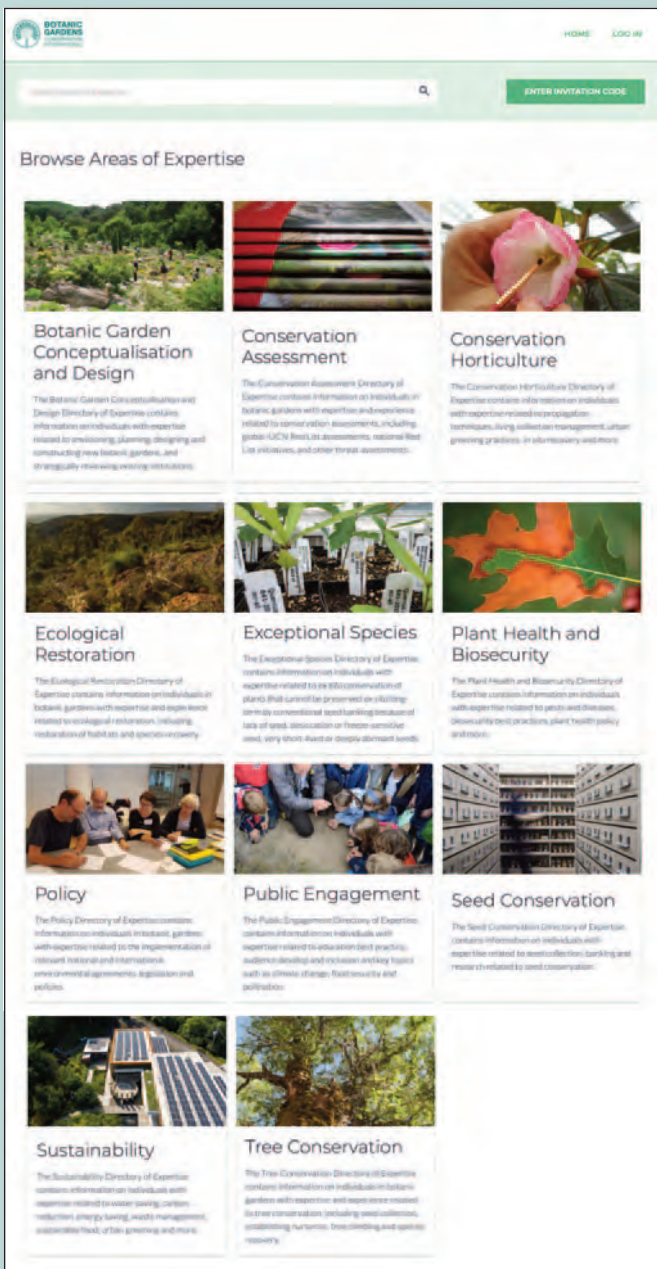
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