Peperomia Reference Collection: an *ex situ* living plant collection for scientific research

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Abstract

Research projects on several plant groups in the Ghent University Botanical Garden are largely based on living collections. Among these is the largest living *ex situ* collection of *Peperomia* (Piperaceae) in the world. The strategy of building such a major scientific collection has proved very advantageous and of unique value, creating the opportunity to follow plants throughout their life cycle and to collect fresh material for immediate morphological, anatomical and molecular studies. An overview of the setup, maintenance, workability and accessibility of a plant collection used in high-standard scientific research is given, based on our experiences with *Peperomia*.

Key words: access and benefit sharing, collection and export permit, *ex situ* collection, IPEN, *Peperomia*, PlantSearch, scientific collection, Seed List

Introduction

The Botanical Garden of Ghent University, with more than 10,000 species spread over 2.75 hectares, was founded in 1797 and has been at its present location since 1902. The director, the curator and nine gardeners are responsible for management and maintenance. The tasks of the garden lie in three main areas: 1) education, 2) research and 3) services. This paper focuses on the research side.

Research projects in several plant groups, e.g. *Peperomia* (Piperaceae), Cyperaceae, Hydrangeaceae, *Epimedium* (Berberidaceae), are largely based on our living collections. The presence of a living *ex situ Peperomia* collection is invaluable for our research in this group (e.g. Wanke *et al.*, 2006; Samain, 2008; Horner *et al.*, 2009; Samain *et al.*, 2009). Studying living plants is essential, as many characteristics are no longer visible on herbarium specimens. The succulent nature of many *Peperomia* species often causes specimens to shrivel, rather than to dry well. To date, our living *Peperomia* collection consists of approximately 2,000 specimens of 700 species (nearly half the total number) from all continents where the genus occurs (Africa, America, Asia and Australia), although with a strong emphasis on America. It is also the most representative *ex situ* collection as it contains species from all currently recognized subgenera (unpublished data).

Peperomia (Piperaceae) consists of approximately 1,600 species and is one of the most species-rich angiosperm genera (Samain *et al.*, 2009). The genus has a pantropical distribution with its highest diversity in the Neotropics. Representatives grow in virtually any habitat between sea level and 4,600 m altitude, resulting in mega-diverse morphological adaptations. Most of this variation lies within vegetative characteristics, inflorescence architecture and fruit morphology, whereas the flowers are very homogeneous throughout the genus.

Our research on *Peperomia* includes a wide range of studies, such as systematics, taxonomy, contributions to floras, checklists, phylogenies, biogeography, character and growth form evolution, anatomy, biomechanics, speciation processes, genetic diversity, etc. Sufficient material was available in the Botanical Garden for research and exploration of the

genus (Samain, 2008). Once we started to focus on particular monophyletic clades, it became necessary to collect in the field as these clades were underrepresented in living collections.

Setting up the scientific collection

Extending an existing collection or starting from scratch?
The plan for collecting and studying a genus or a family usually grows organically and/or historically. Hence, when starting a new project on a particular plant group, a living collection is often already available. However, the scientific usefulness of existing collections might be insufficient. Each plant should be labelled with a unique accession number, so its history can be traced, ideally from its collection in the wild to its current position in the garden. For most high-quality research a well-documented field locality is important, but this is often impossible to retrieve with older collections.

The requirements for high-quality research can be fulfilled more easily when a collection is started from scratch, as the researcher can personally determine what is needed for the work and will usually go into the field themselves to find and gather the necessary data. The success of setting up or expanding a collection within the research time frame has to be ensured. This is usually without problems for herbaceous plants with a relatively fast life cycle, but might be problematic for woody plants that take several years from seed or cutting to produce flowers and fruits. In the latter case, a collection should already have existed for several years in order to be useful for scientific research. It is also important that garden curators focus on setting up a limited number of groups to specialize in, rather than acquire new accessions randomly.

Obtaining plants from other gardens
A collection can be enlarged through exchange with other Botanical Gardens in several ways: 1) via Seed Lists (*Index Seminum*), allowing for specific search and selection, 2) via the PlantSearch database of Botanic Gardens Conservation International (BGCI), very useful for searching (rare) botanic garden accessions around the world, 3) during a personal visit to botanical gardens to obtain cuttings, ensuring more direct contact and more control of identification. Again, it is essential to give each accession upon its arrival a unique number which remains linked to the accession number of the original garden or the IPEN (International Plant Exchange Network) number. Membership of IPEN ensures that CBD rules are respected and that exchange of material is only aimed at conserving biodiversity and research and education. Plants can also be obtained via private collectors, but here only material that has been legally acquired can be accepted.

Collecting plants in the field
Field trips ideally are prepared based on locality data from 1) (digitalized) herbarium specimens, 2) literature, and 3) local botanists. It is challenging to keep plants alive in the field but our experience is that herbaceous plants at least can be treated as follows: 1) tape the roots together with humid mosses, forming a temporary flowerpot, 2) write the collection number on the tape and place the plants in a plastic box you can close during the day and open during the night, 3) return approximately every two weeks to a ‘base camp’ where someone can sprinkle your plants during your absence, and 4) place the temporary flower pots in pure moist TerraCottem®. At the end of the expedition, remove tape and mosses to prevent rotting during the return transport and place the plants in paper bags labelled with the collection number. Rhizomatous and tuberous plants usually do not need such treatment, but here also attention has to be paid to avoid rot and/or fungal diseases.

It is important also that local scientists, students and inhabitants can benefit from the expedition. Identification of the plant group(s) of your interest in local herbaria should be checked and updated where possible. This is not only useful for these herbaria but also for your own field trip and research. Other benefits might include 1) covering of expenses and training of local students who assist you during the trip, 2) hiring a local botanist as guide but

also allowing him/her to spend some time collecting his/her own group, 3) giving local inhabitants information and a fee when they guide you in their neighbourhood or territory, etc. With larger projects, it might be possible to invite local students and botanists to your home institute for a short or long stay, to enhance cooperation.

Setting up a legal living collection at a research institute outside the country of origin is not straightforward. The authorities in some countries do not allow export of living plants (or at least not from plants collected in the wild), and some countries do not even issue collection and/or export permits at all. Whatever the case, doing everything strictly according to the rules requires a huge administrative effort.

Although *ex situ* conservation preferably takes place in the country of origin, botanical gardens in tropical countries often suffer from a lack of financial support, poor infrastructure and/or the absence of trained staff members. Unfortunately, some permit-issuing authorities apply the Convention on Biological Diversity (CBD) protocol very strictly and do not give an export permit for living plants, even though there is an absence of the necessary infrastructure and financial means in the country of origin. An ideal intermediate solution is to provide duplicates of each plant collection for both the country of origin and the reference collection at the home institute, although this might involve collecting more individuals at each locality. Efforts have to be made to convince authorities to distinguish between collection and export of material for scientific purposes as opposed to commercial goals, and to encourage them to facilitate access to genetic resources for scientific and conservation studies.

Maintenance of the scientific collection

Databasing and digitalization

The unique accession number of every specimen is essential. The database of the collection/garden should allow for flexible transfer of uniformized data between botanical gardens. Ideally, this database includes for every accession at least country of origin, detailed locality, coordinates, altitude, collector + number, collection date, IPEN-code, and additionally should also allow for taxonomical updates. Old data should be checked, e.g. in seed lists or notes. An inventory should be made on a regular basis or continuously updated, taking note of dead plants, position, and number of specimens for each accession. In addition to the garden database, a database for specific scientific purposes might also be useful, including additional data or links to specimens with photos or high resolution scans.

Labelling and ordering

The label of every accession should be durable and the data should be written with a pencil or printed, as water-resistant pens often do not withstand permanently wet conditions. The label should at least mention: garden accession number, collection number (this reference is easier for the collector), up-to-date name and country of origin. This label should be kept together with the plant at all times. To avoid confusion during repotting, only one plant with its corresponding label should be removed from its pot at any one time. Additional coloured labels are useful to indicate ongoing work, e.g. sample has been taken for molecular work, important plant that should be checked daily, etc.

The ordering of the plants within the collection might engender a conflict of interests between researchers and gardeners – for example, whether it should be alphabetically, systematically according to their relationships, or practically according to their size or water requirements. In our *Peperomia* collection, most plants are arranged according to their relationships, with the exception of plants that need special care or are placed in other greenhouses because they favour different conditions. A walk list/inventory of all accessions with permanent greenhouse structures (paths, pillars) as reference points makes it straightforward to localize specific accessions within the collection.

Usability and workability of the scientific collection

Collecting material and herborization
Flowers, stems, leaves, etc. should be continuously collected in 70 per cent alcohol, Kew mix, etc., for ongoing or future (micro)morphological, anatomical and ontogenetic studies. For DNA isolation, 2–3 young leaves should be collected in a paper bag or tea bag placed inside a hermetically sealed bag with silica gel (preferably with colour change upon water saturation), or in liquid nitrogen for immediate processing in the lab. A herbarium specimen (ideally with all vegetative and generative parts) should be made as a voucher for scientific publications on morphology, anatomy and for deposition of sequences in databases, or simply for study by other researchers.

Accessibility for other researchers and projects
This topic has been discussed here from the viewpoint of the person setting up the collection. It is important to provide access to the collection for other researchers through online databases (e.g. uploading your data to PlantSearch of BGCI) or upon request, which might also result in new cooperations. The detailed field data of the accessions and scientific results can helpt to bring about involvement in conservation and reintroduction projects.

References

Horner, H.T., Wanke, S. & Samain, M.S., 2009. Evolution and Systematic Value of Leaf Crystal Macropatterns in the Genus *Peperomia* (Piperaceae). *International Journal of Plant Sciences*, **170**: 343–54.

Samain, M.S., 2008. *Tackling Pandora's Box. Order out of chaos in the giant genus* Peperomia *(Piperaceae).* Ghent University, Belgium: viii + 270.

Samain, M.S., Vanderschaeve, L., Chaerle, P., Goetghebeur, P., Neinhuis, C. & Wanke, S., 2009. Is Morphology Telling the Truth about the Evolution of the Giant Genus *Peperomia* (Piperaceae)? *Plant Systematics and Evolution*, **278**: 1–21.

Wanke, S., Samain, M.S., Vanderschaeve, L., Mathieu, G., Goetghebeur, P. & Neinhuis, C., 2006. Phylogeny of the Genus *Peperomia* (Piperaceae) Inferred from the *trnK/matK* Region (cpDNA). *Plant Biology*, **8**: 93–102.

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