Propagation of Endangered Aquatic Plants: An Experience that Promotes *ex situ* Conservation and Environmental Education

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ABSTRACT

Wetlands are among Mexico's most threatened habitats as they have not received the degree of protection and conservation as they have in North America. To promote their conservation, the Botanical Garden at the Institute of Biology at the National Autonomous University of Mexico (UNAM), located south of Mexico City, has established a collection of aquatic plants with the intent to have a representative sample of the country's aquatic plants, with emphasis on the "Cuenca of Mexico". The collection is being used to propagate aquatic species, conduct research, promote conservation of aquatic plants, and to serve as the foundation for environmental education programs to increase public awareness of these species and the challenges they face.

WHY CONSERVE AQUATIC PLANTS IN THE BOTANICAL GARDEN OF THE INSTITUTE OF BIOLOGY – UNAM?

In Mexico, aquatic ecosystems are among the country's most threatened natural habitats. They have received little attention for their conservation and there are few studies related to Mexican aquatic species (Mora-Olivo et al. 2013). Aquatic plants have been linked to man since very ancient times - the element water has been a very important factor for the establishment and development of great civilizations including the great Tenochtitlan in the "Cuenca de México" (the ancient capital of the Aztec empire). Since pre-Hispanic times, aquatic plants have been used for various purposes (Miranda 1980). One of the most important uses was to build the "chinampas" - the agricultural production systems (e.g., "floating gardens") that helped to shape the city itself and served to "gain" space in the water and form new sections of "land" for the very expansion of the great city (for an example, see https://aztecexplorers.com/2018/07/26/travelling-in-time-exploring-the-chinampas-of-tlahuac-mexico-city/). Species such as *Lilaeopsis schaffneriana*, *Schoenoplectus tabernaemontani*, *Hydrocotyle ranunculoides*, *Polygonum amphibium*, *Lemna gibba* and *Bidens aurea* were used specifically for the construction of "chinampas" (Lot and Novelo 2004).

Despite their historic significance, there are currently very few studies for these types of aquatic plants in both natural populations and *ex situ* conditions. The Aquatic Plant Collection (APC) is a botanical collection of the few of its kind that exist in Mexico and its goal is to have a representative sample of the aquatic vascular plants of the flora of our country, with emphasis on the "Cuenca of Mexico."

WHO ARE WE?

The Botanical Garden of the Institute of Biology - UNAM (JB IB-UNAM) is located south of Mexico City and occupies an area of 2.75 hectares (Caballero Nieto 2012). The Aquatic Plant Collection (APC) is distributed in 17 ponds lo-

FIGURE 1. Panoramic view of one of the ponds in the Aquatic Plant Collection containing plants with medicinal and/or food use (e.g., *Equisetum hyemale* and *Nymphaea mexicana*). (Photo by Surya Ivonne González Jaramillo.)

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cated in different sections of the botanical garden and covers an area of approximately 473 m^2 (Figure 1). It also contains a greenhouse of 95 m^2 for the propagation, growth and development of different species of aquatic plants (Figure 2).

Originally, the founders and collaborators of the JB IB-UNAM made various collections for the production of species that show the plant diversity of Mexico. Historical records indicate that in some of the botanical expeditions, aquatic plants were collected (*Equisetum hyemale* var. *affine, Equisetum giganteum, Hydrocotyle* sp., *Sagittaria sagittifolia* and *Typha* sp. among others) however at that time no specific collection was consolidated strictly for aquatic plants. From 2006, on the initiative of Dr. Javier Caballero Nieto (then Head of the Botanical Garden) all the Garden's collections were inventoried, strengthened and redefined into two different types of collections according to their composition and function: taxonomic collections and thematic collections. It was under this concept, that the Aquatic Plant Collection (APC) was formalized as a thematic collection. At the invitation of Dr. Javier Caballero, Dr. Antonio Lot Helgueras (specialist researcher in aquatic plants of the Institute of Biology UNAM) and landscape architect Pedro Camarena developed a project for the establishment of this collection. For this project, a theme was defined for each of the existing ponds that included a plant palette of aquatic species and an educational proposal for the use of the collection; this was accomplished by biologist Teodolinda Balcázar Sol (Coordinator of the Educational Area). From then on biologist José Luis López was in charge of the maintenance of the ponds and the asexual propagation counting for it, with the support of the gardener Jesús Rebollo. Some of the collection sites of the plant species were the states of Michoacán, Morelos, Hidalgo and Jalisco.

NEW FLIGHT PLAN: ACTIONS TO BOOST APC

Starting in 2015, I was appointed curator responsible for the APC to continue developing the master plan designed by Dr. Antonio Lot Helgueras. Each pond shows the different life forms, endemism, introduced species (naturalized species are shown, i.e. those that have dispersed to new environments and have succeeded in these sites and also those species considered weeds, due to the excessive growth of their populations that harms other species), as well as the species used as medicine, food, fibers for the elaboration of handicrafts and building materials. Species used for ornamental (various types and colors of flowers) and ceremonial purposes are also included.

After analyzing the conditions of the APC and the greenhouse, it was determined that the main actions to be taken would be: 1) boost the sexual spread of species, 2) conduct different studies to learn more about the development of species

FIGURE 2. Aquatic plant propagation tank in the greenhouse. (Photo by Surya Ivonne González Jaramillo.)

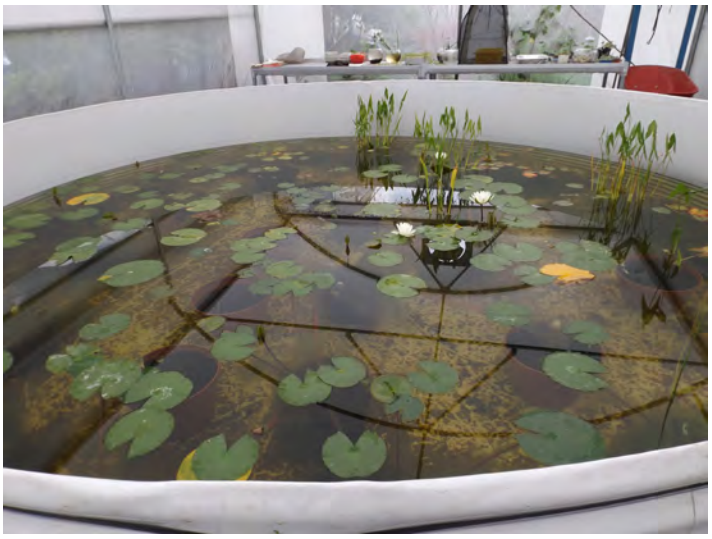
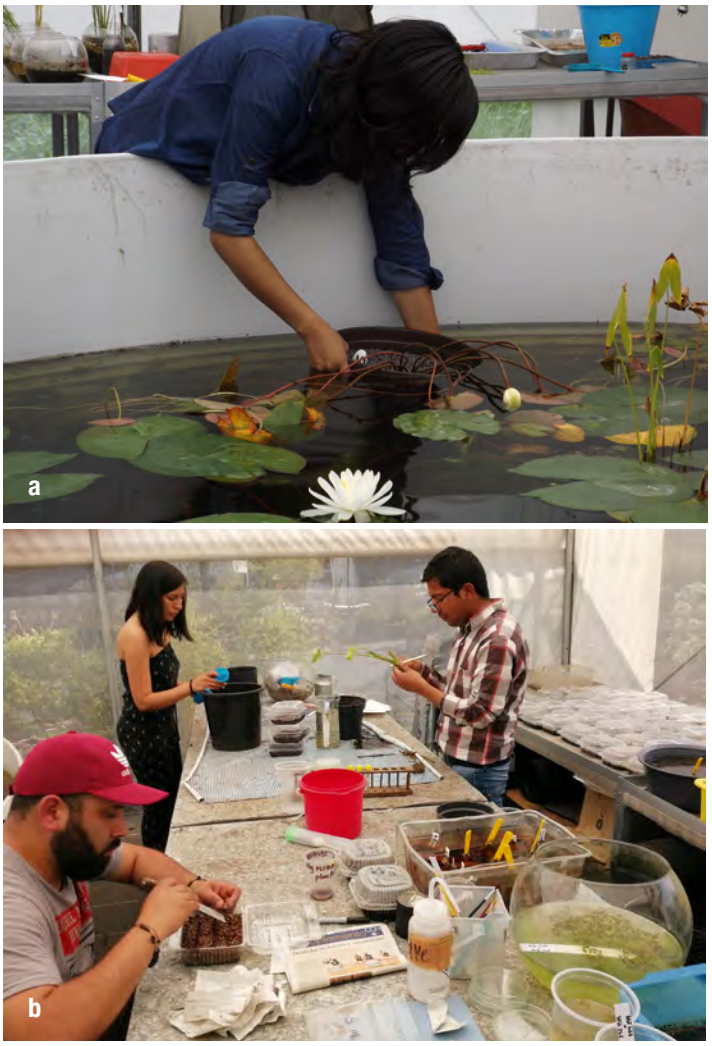


FIGURE 3. Students working on various projects: Surya González (a) working with *Nymphaea odorata* and university students - Sara Díaz, Isaac Avalos, and Francisco Mendoz (b) working in the greenhouse. (Photos by Diana Ferrusca Domínguez – a, and Nayeli González – b.)



under *ex situ* conditions, and 3) promote environmental education activities for the revaluation of Mexican aquatic plants. These actions have been made possible through multidisciplinary projects that integrate high school students and college undergraduates in research topics related to aquatic plants and even fauna associated with the ponds in the collection.

Currently the APC includes 35 species from 19 botanical families with the ultimate goal to increase the number of species and individuals per species. Since 2016, 34 students of high school or university levels have been performing volunteer activities, summer stays, professional internships, social service, or thesis research. Basic horticulture activities are carried out in addition to the design and execution of research that promotes the study of aquatic plant species. This work expands our knowledge about the biology of species, while encouraging students to develop technical skills for hydrophytic plant management and the revaluation of wetlands (Figure 3).

SEXUAL REPRODUCTION RESEARCH

Studies are carried out with some of the most attractive species of the Family Nymphaceae that are subject to more environmental pressures. The APC includes five species from this family, of which *Nymphaea gracilis, N. odorata* and *N. mexicana* (Figure 4) are those for which germination and growth tests have been initiated. These three species were abundant in the "Cuenca of Mexico" and have been elements of the landscape with a great cultural significance (López Martínez 2018). Since their populations have declined or disappeared completely they are now considered as threatened species of extinction, according to current environmental regulations. The JB IB-UNAM contributes to their *ex situ* conservation as studies are carried out to understand and improve their propagation.

N. gracilis or "*atzazamolli*" is a water lily with white flowers that stand out from the water level and possesses large green leaves (Figure 4a). Its cultural value is very important as it is represented in the "Florentino Codex" in the section that mentions edible wild plants. This species is endemic to Mexico and, unfortunately, is highly vulnerable to the loss and contamination of water bodies.

Nymphaea mexicana called "*atlacuetzon*" or "*paskurinda*" inhabited the Mexico Basin and unfortunately its natural populations have declined drastically. This species with bright yellow flowers (Figure 4b) was a very important element in the consolidation of the "*chinampas*." It is even represented in pre-Hispanic murals that demonstrate its cultural value. In addition, it had an ornamental use for the Day of the Dead offerings and its leaves were used in the preparation of a special food that was made with the mixture of other elements of the "*chinampas*" (López Martínez 2018)

N. odorata or "*apapatla*" is a very elegant and showy species whose white flowers float on the surface of the water and offer a very pleasant sweet aroma (Figure 4c). This species was a very important element for the formation of "chinampas" however it has not been seen in the Basin of Mexico since the 1940s (*Note*: It is the most common water lily in North America). Its leaves are large, which favors the colonization by aquatic fauna, both below water (i.e., for the deposit of snail eggs) and above their leaves (e.g., some small birds perch on them in order to feed on various insects).

Since the summer of 2016, individuals of *Nymphaea odorata, N. gracilis* and *N. mexicana* have been monitored in the different exhibition ponds and/or in the propagation

FIGURE 4. Three water lilies being studied: *Nymphaea gracilis* (a), *N. mexicana* (b), and *N. odorata* (c). (Photos by APC – a, and Nayeli González – b and c.)

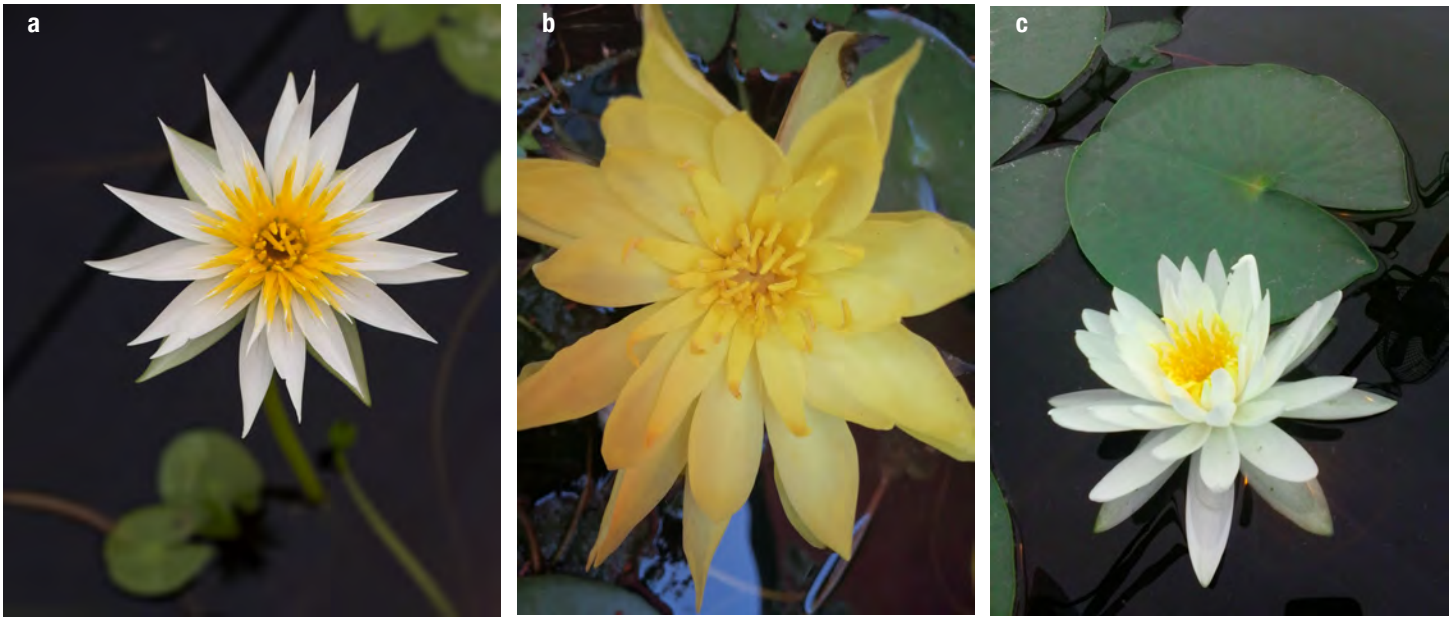


TABLE 1. Summary of studies on propagation of aquatic plants.

	N. mexicana	*N. odorata*	*N. gracilis*
Vegetative propagation	Successful	Successful	Successful
Fruits	Not enough have been obtained.	Bee pollination and cross pollination. Abundant fruits in older specimens.	Successful cross pollination.
Seeds	Scarce	Abundant	Abundant
Germination	Viable seeds have not been obtained.	Low germination. Stimulation with constant temperature and dark conditions.	Successful germination. Stimulation with constant temperature conditions.
Next actions	Fertilization tests for flower and fruit production.	Pre-germination treatments in seeds.	Germination in different light and temperature conditions.

FIGURE 5. From left to right: the first two images (with the yellow margin) shows the formation of stoloniferous rhizomes in N. mexicana for vegetative propagation; the central pair of images (with the orange margin) shows the horizontal rhizome of N. odorata that can be cut for production of new individuals and N. odorata observed in fruit that is collected to obtain seeds; the images on the right (with the blue margin) show N. gracilis - its rhizome with small leaves initiating growth and a student performing artificial pollination. (Photos by APC.)

FIGURE 6. Diana Ferrusca separating rhizomes from *N. mexicana*. (Photo by Surya Ivonne González Jaramillo.)

FIGURE 7. One of the new species added to the APC collection: *Anemopsis californica*. (Photo by Nayeli González.)

greenhouse. Sites were periodically reviewed to monitor the formation of flowers and fruits. Fruits were collected and seeds obtained. Species leaflets or rhizomes were also collected to determine favorable characteristics for their spread. In addition, the greenhouse pollination of *Nymphaea odorata* and *N. gracilis* produced fruit, viable seeds, and seedlings of both species. Although some seeds of *N. mexicana* were produced, no seedlings have been established. However *N. mexicana* did achieve favorable vegetative reproduction by means of rhizomes. There are different factors (e.g., substrate, light, and water level) that influence both germination and the establishment of seedlings so obtaining good-sized individuals from seeds involves a slower process but with greater advantages promoting genetic diversity (Bornette and Puijalon 2011). Table 1 highlights the main results and the next actions with each of the species, while Figure 5 shows the propagation process.

PROPAGATING SPECIES UNDER *EX SITU* CONDITIONS

When plants are grown in greenhouses and gardens, situations arise that must be dealt with and often quickly to maintain a healthy and productive environment. Plant cultivation at APC requires an investment to time to ensure the well-being of aquatic species in the collection.

Phenological monitoring of species and their horticultural management. Fortnightly tours are carried out to identify the stages of development of the different species in the collection. The formation of flowers, fruits and/or seeds is mainly identified and recorded. These observations also help determine whether the specimens require pruning or transplantation.

Comprehensive pest and disease management. Fortnightly observations also detect the presence of an insect or any other organism whose presence may cause some damage to aquatic species. Upon detection, Dr. Bonifacio Don Juan (responsible for the phytosanitary management of the collections) is notified and the recommended treatment is applied. Some problems caused by aphids, molluscs, and fungi have been detected. Their treatment has been timely and health problems have been kept under control (Figure 6).

Wildlife program: native versus exotic species. The ponds provide habitat for wildlife. In some of the ponds and according to the season, you can observe various birds such as the Mexican duck (*Anas platyrhynchos* ssp. *diazi*), blue heron (*Nycticorax nycticorax*), frogs (e.g., *Lithobathes montezumae*), and dragonflies (e.g., *Rhionaeschna multicolor*). Other organisms flock to the ponds to get their prey (e.g., some insects become part of the diet of the collar lizard - *Sceloporus torquatus*) or to drink water and cool down like "tlacuache" (*Didelphis virginiana*).

Unfortunately, however, there are also exotic wildlife such as the Japanese turtle (*Traechemys scripta elegans*) abandoned by visitors. Turtles are abandoned because their owners believe that ponds are a shelter option for them, but they are not. The ponds do not have a warm and constant temperature for them and in many of them there is no food suitable for them. Also many of the turtles arrive sick and fight each other as there are many (more than 25 individuals) for a small pond (60 m^2 approx.). Their presence poses a threat to the development of water lilies and the proper functioning of the system. The turtles consume leaves and buttons preferably of water lilies and climb the leaves and damage them. Since 2017, monitoring of ponds has been carried out for the detection of exotic aquatic organisms. In 2019, a study was conducted to assess the effect of the overpopulation of the Japanese turtle on one of the APC ponds. The turtle population was assessed, individual health was determined, then the turtles were removed from the pond, treated, and when they were in good condition they were put up for adoption so that they could be removed from the pond to evaluate the response of the plants by removing an element from the system. Four workshops have been held to inform visitors about the problem of abandonment of these turtles in the ponds.

Incorporation of new species into exhibition ponds. Through links with other botanical gardens of the Mexican Association of Botanical Gardens A.C. specimens or seeds of aquatic plants have been received in donation for the strengthening of APC's collection. The main donors have been the Botanical Garden of Fundación Xochitla, A.C. in the State of Mexico and the Botanical Garden of Culiacán, Sinaloa. Also some academics of the JB IB-UNAM, like biologist Ivonne Olalde, have collected aquatic plants that are currently propagated in the greenhouse for later incorporation into the ponds. In the last three years seven new species have been added to the collection: *Anemopsis californica* (Figure 7), *Ludwigia* sp., *Thalia geniculata, Hydrocotyle ranunculoides, Limnobium laevigata, Marsilea mollis,* and *Lilaeopsis schaffneriana*.

Environmental education activities: the revaluation of Mexican aquatic plants. The general purpose of educational activities is to promote the public's interest in aquatic plants and to recognize the importance of aquatic environments and their plant diversity. For four years (2015-2018) we participated in environmental education events: National Day of the Botanical Gardens and summer courses with the development of different activities related to the APC. More than 300 people have participated in the programs. Some activities were guided tours, children's workshops or didactic games (Figure 8). The activities were based on the "Environmental Education Action Plan for the Botanical Gardens of Mexico" which promotes meaningful learning by involving different audiences and diverse strategies considering that it is not only important to know the biology of plants but to have a comprehensive vision of plant resources (Martínez-González et al. 2012). We have observed after the implementation of the activities that 90% of

the participants were unaware that there was so much diversity of aquatic plants as they only knew the common water hyacinth (*Eichornnia crassipes*). They were most surprised to learn that some aquatic plants are so small (*Lemna* and *Wolffia*) while others are insectivorous (*Utricularia*). Participants also learned about the uses of aquatic plants since pre-Hispanic times and their relationship with aquatic fauna. Some suggestions for future activities include: 1) perform new activities considering different educational levels and age ranges, 2) implement workshops involving aquatic plant-animal relationships and 3) develop horticultural workshops for propagation of particular species such as horsetail (*Equisetum hyemale*).

CHALLENGES: THE IMMEDIATE FUTURE

APC will continue to develop the three basic lines of action: 1) sexual propagation of species, particularly for species of other botanical families or others with a different conservation status, 2) optimal development of species under *ex situ* conditions (e.g., assessing their growth and reducing the incidence of health problems), and 3) conducting environmental education activities and planning new strategies for educational intervention in locations (field sites) that still have natural populations of native aquatic vegetation.

The Aquatic Plant Collection provides an experience that allows not only the study of Mexican aquatic plants, but is also like a blue and green island in an urban environment that allows the development of an artificial wetland with the presence of wildlife. In addition, it contributes to the development of new professionals (Figure 9) and offers Botanical Garden visitors an introduction to aquatic plants - learning more about them (e.g. biology, ecology or ethnobotany) and the need for their conservation. ■

FIGURE 8. Examples of environmental education activities to revalue aquatic resources: guided tours (a) and children's workshops (b). (Photos by APC – a, and Surya Ivonne González Jaramillo – b.)

FIGURE 9. Students in the greenhouse (Surya González, Diana Ferrusca, Nayeli González, Luis Silva, and Rogelio Yañez). (Photo by Surya Ivonne González Jaramillo.)

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