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EFFORTS TO CONSERVE ENDANGERED TERRESTRIAL ORCHIDS *IN SITU* AND *EX SITU* AT TWO NATURAL RESERVES WITHIN CENTRAL MEXICO

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The natural vegetation in and around Mexico City once harbored an unusually high number of plant and animal (insect) species, including endemics (Vázquez 1973, Ceballos & Galindo 1984, Rzedowski 1991). The high diversity in this region has been attributed to the unusual topography resulting from a series of volcanic eruptions that ended *ca.* 1800 years ago (Siebe *et al.* 2004). In addition, two phyto-geographic regions overlap within Central Mexico that support diverse vegetation types (*e.g.*, shrubs, mature pine forests). Due to the rapid, uncontrolled growth of Mexico City's population, and surrounded Cities as Cuernavaca, many of these habitats have been destroyed, prompting the establishment of several natural reserves, especially south of the city. Two reserves are the subject of this study: El Pedregal in Mexico City, and El Corredor Biológico Ajusco-Chichinautzin limited by the southern Mexico City and Northern Morelos State. El Pedregal is a relictual area (237 ha) where some representative elements of the original fauna and flora of this Valley still prevail (Valiente-Banuet & De Luna-García 1994, Téllez 2002, Castillo-Argüero *et al*. 2004, Hágsater *et al*. 2005). A xerophytic shrub vegetation is supported by a basaltic shield where any developed soil can be found other than organic matter accumulations in depressions and fissures (Cano-Santana & Meave 1996). High plant diversity was described initially by Rzedowsky (1954) (*c.a.* 350 species) and Asteraceae, Poaceae, Leguminoseae and Orchidaceae Families are the dominant (Herrera & Almeida 1994). Terrestrial orchid diversity has been documented, especially in El Pedregal and a total of 25 orchid species have been reported, including five species on the verge of extinction (*Bletia punctata, Cyrtopodium macrobulon*, *Epidendrum anisatu*, *Habenaria strictissima*, *Liparis greenwoodiana*) (Hágsater *et al.* 2005).

In contrast, in the Chichinautzin Area, eight types of vegetation can be found. An altitudinal gradient joint with successive periods of volcanic activity are combined and pedogenetic processes through time and parental material result in a chronosequence of soils. Main vegetation type is *Pinus* forest developed in elevations from 1800 to 3500 m and in an extension of 65, 700 ha. Around 785 different plant species have been described where Orchidaceae is the more diverse Family with 125 species (six are protected and 25 are listed in the IUCN-CITES and the Red List of Threatened Plants (Espejo *et al.* 2000).

The main ecological problem in the first place is the habitat fragmentation where the degradation processes are the spread of non-controlled fires during the dry season, over collection and pollution problems, such as trash dumps replacing native for perturbed flora. Meanwhile at the second place, the main degradation processes are the conversion of forest into agricultural lands as a result of overpopulation, with subsequent irrational exploitation of wood and also uncontrolled fires. In both habitats, the Orchidaceae is one of the most endangered families because of the changes in vegetation, soil use and over collection that do not allow populations to recover (Rubluo *et al*. 1993, Mera *et al*. 2002, Téllez 2002, Koopowitz *et al*. 2003, Wotavová *et al*. 2004). This problem gets worse from the fact that no governmental effort is made to preserve biodiversity in seed collections as has been done for some forest species. In consequence, there is not any future perspective to consider soil microorgan-

isms as mycorrhizal fungi in conservation strategies like habitat restoration, for this particular group (Zettler 1997). On the other hand, it is well supported and established the use of ectomycorrhizal fungi in reforestation of gymnosperm forest with macromycetes. The main problem is that local inoculation programs do not use native fungi and when done, they do it with commercial isolates.

We have conducted an extensive project aimed at monitoring, conserving germoplasma, and isolating mycorrhizal fungi from orchids at both sites. Our aim is not to begin an uncontrolled seed collection practice without the isolation of associated mycorrhizal fungi in order to promote symbiotic germination with natural isolates. Studies at El Pedregal initiated in 2002 during the rainy season and we were able to found half of the original described orquiflora for this habitat. We started a germless storage with a total of 105 capsules and 73 different collect numbers with 38 identified isolates at the anamorphic stage (Currah *et al.* 1997). Some examples are shown in Table 1, Fig. 1.

At the second place, El Corredor Chichinautzin, because of its big extension, we started to locate well conserved forest sites with contrasting soil quality. Recently (2005) we identified a total of 25 species in sites with non-developed soils similar to the El Pedregal habitat and sites with deep and well developed Andosols. In one year of field monitoring during the rainy season, we got 250 capsules (65 collected numbers) and 18 mycorrhizal isolates, all of them nearly identified in the anamorph stage (Table 1, Figs. 1 and 2). One of this unidentified isolates belonged to an achlorphylic orchid *Corallorhiza maculata.*

A variety of morphological features were found in the different isolates as well as specificity for the plant species with their mycorrhizal fungi. There is less morphological variation between the isolates found in El Pedregal probably due to the fact that they come from fewer species of orchids in a smaller habitat. A wide morphological variation was detected in the isolates from the Chichinautzin, also because they come from more orchid species and habitats. Main morphological variations are related to the rate of development and the texture of the mycelium forming the colony in the Petri dish. *Epulorhiza* spp. isolates have consistently waxy mycelium with submerged hyphae in yellowish- pale brown colors where monilioid cells are quite similar. Molecular studies have been conducted for the isolates from El Pedregal and all teleomorphic species of *Epulorhiza* belong to Tulsnellaceae, particularly *Tullasnella calospora* (Rangel 2006). For *Ceratorhiza* spp., a less specific determination was obtained to the Family Ceratobasidae. Morphological features of *Epulorhiza* spp. isolates from Corredor Chichinautzin are more cottony-texture with aerial mycelium, white and sometimes similar to *Ceratorhiza* colonies (Fig. 2B, O and R). Instead, few *Ceratorhiza* cultures grew as *Epulorrhiza,* without concentric rings and waxy texture (Fig. 1Y). *Ceratorhiza* cultures grew faster but some moniliod cells developed more slowly in some cultures and it was difficult to appreciate them because they don not finish in clumps as *Epulorhiza* (Fig. 1E1, Fig. 2H).

Although this is a preliminary study for the mycorrhizal fungi diversity associated to terrestrial orchids in southern Mexico City, we have a very clear picture of the morphological diversity that can be found associated to plant species. It looks like some genera are highly specific for their mycobiont as the couples *Bletia - Epulorhiza, Dichromanthus – Ceratorhiza, Habenaria – Epulorhiza,* and *Malaxis – Epulorhiza*. More evidence is required to confirm whether this specificity always occurs in nature, due to the fact that some symbiotic cultures can be developed *in vitro* with different isolates (Rangel 2004). Bioassays confirm specificity at some level; we have noticed that *Bletia* species are less specific for both genus isolates than *Dichromanthus.* However, results for the *in vitro* propagation must be interpreted carefully and do not reflect the specificity in nature. *In situ* bioassays demonstrate that specificity can be developed through the life history of the plant (Rangel 2006). The *Bletia* spp. can be probably less dependent on fungi for *in vitro* germination and less specific because they photosynthesize rapidly; but in nature, populations are more endangered than *Dichromantthus* and seedlings are very difficult to observe where asexual corm propagation is common (Ortega-Larrocea and Rangel, same volume). The *Ceratorhiza* fungi grew faster *in vitro*, but we do not know whether the same behavior found in

TABLE 1. Mycorrhizal isolates from several orchids in Central Mexico.

Orchid species	Number of isolates	Source plant	Site	Anamorphic phase	Growth of colony (PDA	Ranges of growth (cm/day), and l x w of moniliod cells (µm)	Figure
Bletia campanulata Lex.	2	Adult	El Pedregal	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae; waxy colony and sometimes cottony in the center	0.43 - 0.61, 12.25 x 9.8 - 14.7 x 14.7	1B-G
Bletia urbana Dressler	8	Adult	El Pedregal	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes aerial hyphae in the margin	0.47 - 0.57	1I-M
Bletia sp. 1	1	Adult	El Pedregal	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes cottony in the center	0.57	1N-P
Bletia sp. 2	1	Adult	Corredor Chichinautzin	*Rhizoctonia* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes cottony in the center	0.27	1Q-S
Dichromanthus aurantiacus (La llave & Lexarza) Salazar & Soto Arenas	23	9 from adult, 14 from protocorm	El Pedregal	*Ceratorhiza* sp.	White to cream colored, fluffy with aerial hyphae in concentric rings and sometimes in speck. Isolates obtained from adult plants and protocorms grown in both patterns	0.71 - 1.21, 24.5 x 9.8 - 31.85 x 12.25	1T-X
D. aurantiacus	2	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Brownish to cream colored, waxy	0.56 - 0.60	1Y-Z
Dicrhomanthus cinnabarinus (Lex.) Garay	2	Adult	El Pedregal	*Ceratorhiza* sp.	Brownish to cream colored, fluffy with aerial hyphae in concentric rings	1.21	1A1-E1
D. cinnabarinus	1	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Brownish to cream colored, fluffy with aerial hyphae in concentric rings and sometimes in speck.	0.50 - 2.12	1F1-G1
D. cinnabarinus subsp. *galeotiana*	1	Adult	Gypsum mine near Tonalá, Jalisco	n. d.	Brownish, cream-colored to yellowish with submerged hyphae, waxy	n.d.	
Galeottiella sarcoglossa (A. Rich & Galeotti) Schltr.	1	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy	0.2, 12.25 x 12.25 - 9.8 x 9.8	2 A-C
G. sarcoglossa	1	Adult	Corredor Chichinautzin	*Rhizoctonia* sp.	Cream-colored to yellowish, colony develops in form of flower with waxy zones with submerged hyphae, and aerial hyphae in the petal	1.21	2D-E

TABLE 1. Mycorrhizal isolates from several orchids in Central Mexico.

Orchid species	Number of isolates	Source plant	Site	Anamorphic phase	Growth of colony (PDA (cm/day), and l x w of moniliod cells (µm)	Ranges of growth	Figure
Govenia liliacea (Lex.) Lind.	1	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Cream colored, fluffy with aerial hyphae	0.2	2F-H
Habenaria novemfida Lind.	1	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and sometimes aerial hyphae in the margin	2.12, 9.8 x 9.8 - 14.7 x 12.25	2I-K
Habenaria sp.	1	Adult	Veracruz	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy	0.33	2L-M
Malaxis sp. 1	2	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and fine aerial hyphae	0.50 - 0.53	2N-P
Malaxis sp. 3	1	Adult	Corredor Chichinautzin	*Epulorhiza* sp.	Cream-colored to yellowish with submerged hyphae, waxy and fine aerial hyphae	0.2	2Q-S
Schiedeella eriophora (B.L.Rob & Greenm.) Schltr.	1	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Cream colored, fluffy with aerial hyphae	1.21, 25.5 x 9.8 - 31.85 x 12.25	2T-X
Platanthera volcanica Lind.	2	Adult	Corredor Chichinautzin	*Ceratorhiza* sp.	Cream colored, fluffy with aerial hyphae in concentric rings	1.06, 24.5 x 12.25 - 36.75 x 12.25	2Y-A1
Spiranthes sp. 1	1	Adult	Corredor Chichinautzin	*Rhizoctonia* sp.	Cream-colored to yellowish with submerged hyphae, waxy and fine aerial hyphea	1.21	2B1-D1
Corallorhiza maculata (Raf.) Raf.	1	Adult	Corredor Chichinautzin	n.d.	n.d.	n.d.	2E1-F1

n.d. = not determined

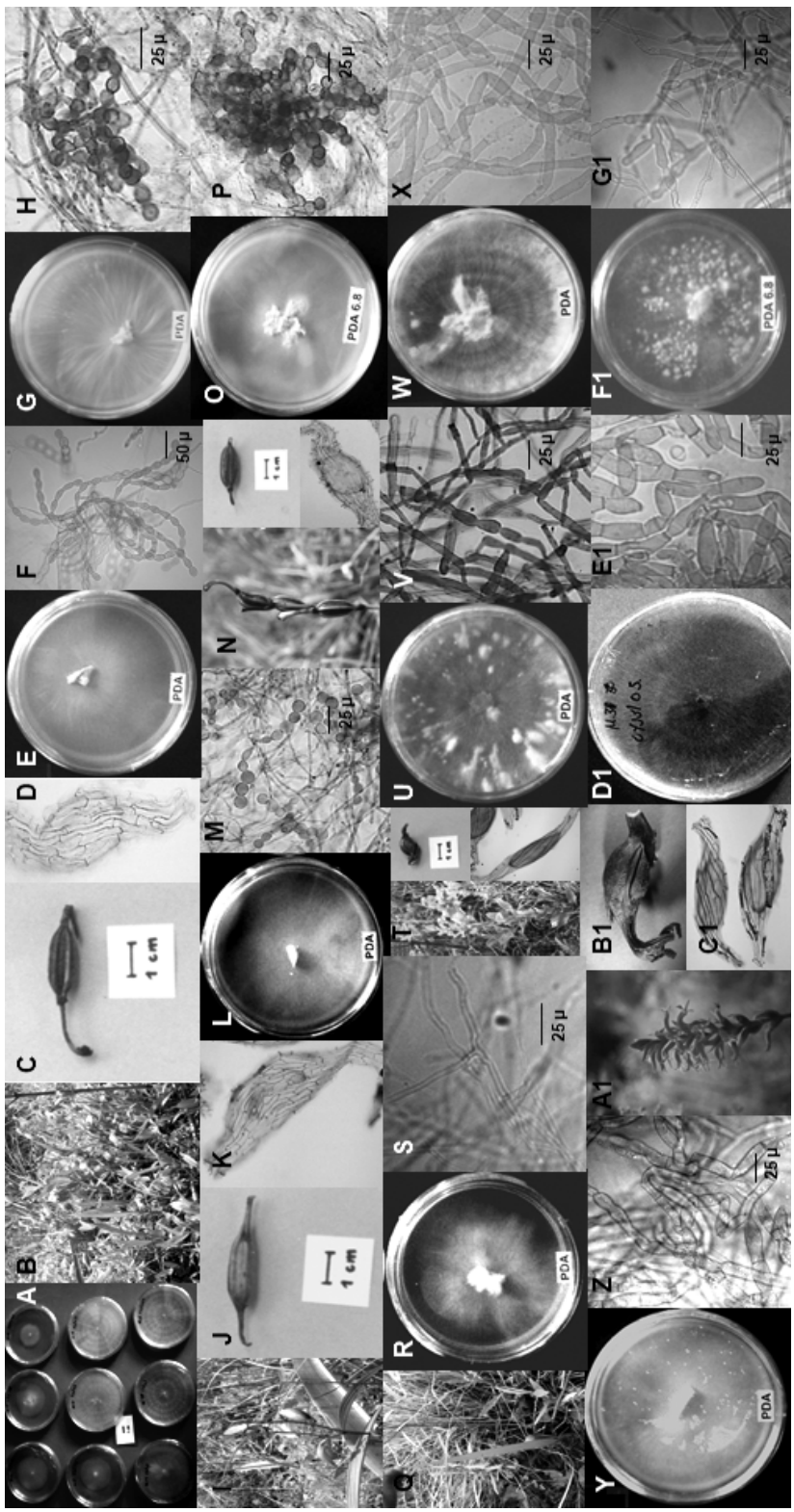

Fig. 1. **A.** General view of grow rate and colony development of mycorrhizal isolates: *Epulorhiza* spp. (first four), *Ceratorhiza* spp. (rest five). **B.** *Bletia campanulata*. **C.** Capsule. **D**. Light microscopy of seed (100 x). **E**, **G.** Colonial morphology. **F**, **H.** Microscopic morphology of monilioid cells. **I.** *Bletia urbana*. **J.** Capsule. **K.** Light microscopy of seed (100 x). **L.** Colonial morphology. **M.** Microscopic morphology of monilioid cells. **N.** Capsule of *Bletia* sp. (left), capsule detail (upper right) and light microscopy of seed (100 x) (lower right). **O.** Colonial morphology. **P.** Microscopic morphology of monilioid cells. **Q.** *Bletia* sp. **R.** Colonial morphology. **S.** Microscopic morphology of hyphae. **T.** *Dichromanthus aurantiacus* (left), capsule (upper right) and light microscopy of seed (100 x) (lower right). **U, W, Y.** Colonial morphology. **V, X, Z.** Microscopic morphology of monilioid cells. **A1.** *Dichromanthus cinnabarinus*. **B1**. Capsule. **C1.** Light microscopy of seed (100 x). **D1, F1.** Colonial morphology. **E1, G1**. Microscopic morphology of monilioid cells. Illustrations by Rangel.

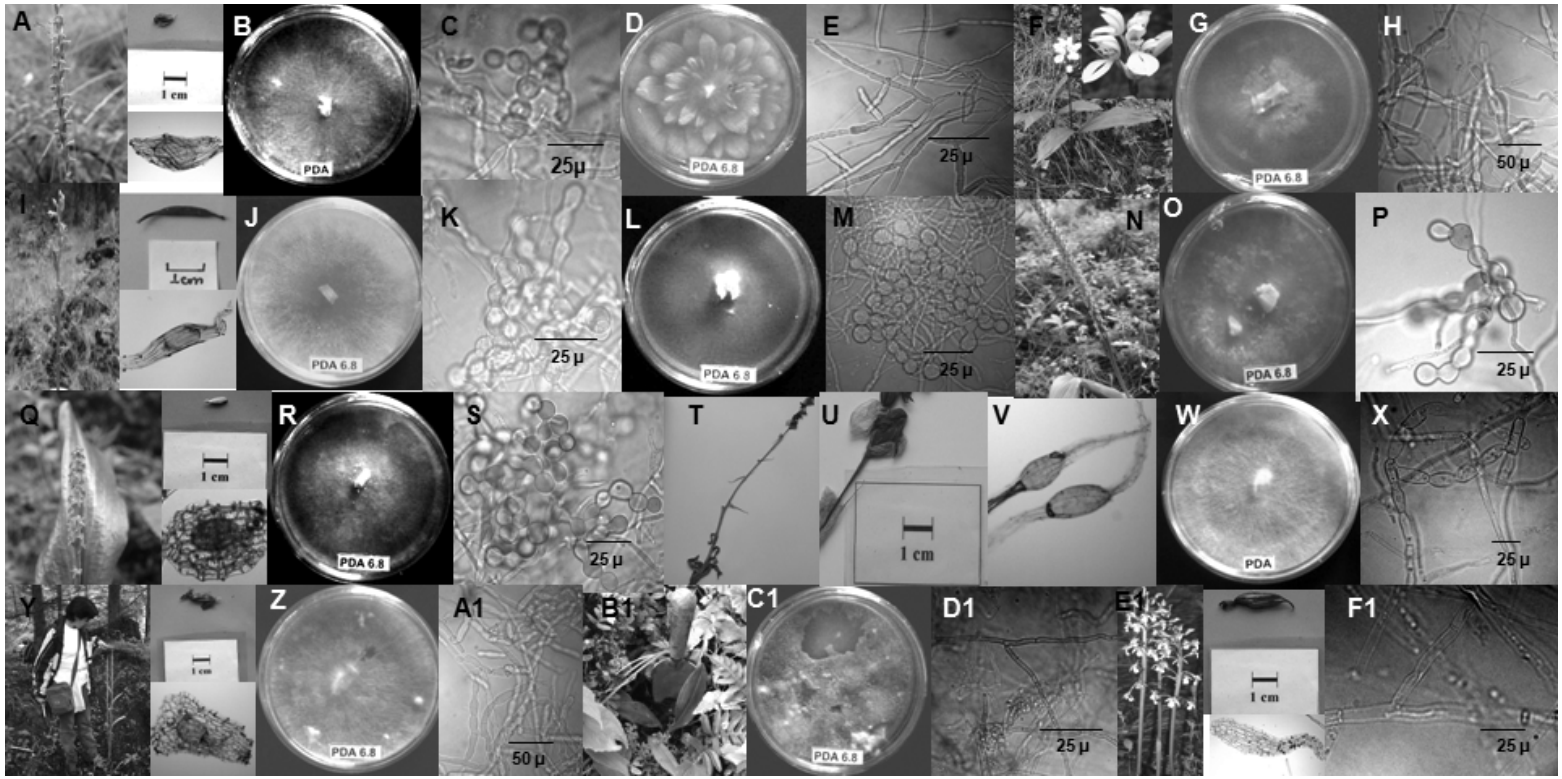

Fig. 2. **A.** *Galeottiella sarcoglossa* (left), capsule (upper right) and light microscopy seed (100 x) (lower right). **B**, **D.** Colonial morphology. **C**, **E.** Microscopic morphology of monillioid cells and mycelium. **F.** *Govenia liliacea.* **G.** Colonial morphology. **H.** Microscopic morphology of monillioid cells. **I**. *Habenaria novemfida* (left), capsule (upper right) and light microscopy of seed (100 x) (lower right). **J, L** Colonial morphology. **K, M.** Microscopic morphology of monilioid cells. **N.** *Malaxis* sp. 1, **O.** Colonial morphology. **P.** Microscopic morphology of monilioid cells. **Q.** *Malaxis* sp. 3 (left), capsule (upper right) and light microscopy of seed (100 x) (lower right). **R.** Colonial morphology. **S.** Microscopic morphology of monilioid cells. **T**. *Schiedeella eriophora* (herbarium) **U.** Herbarium flower. **V.** Light microscopy of seeds. **W.** Colony morphology. **X**. Microscopic morphology of monilioid cells. **Y**. *Platanthera volcanica* (right), capsule (upper right) and light microscopy of seed (100 x). **Z.** Colony morphology. **A1.** Microscopic morphology of monilioid cells. **B1**. *Spiranthes* sp. 1. **C1.** Coloby morphology. **D1.** Microscopic morphology of mycelium. **E1.** *Coralorrhiza maculata* (left), capsule (upper right) and light microscopy of seed (100 x). **F1.** Microscopic morphology of hyphae. Illustrations by Rangel.

soil conditions and if soil seed storage has high probability to find compatible fungus partner in natural conditions.

Additional to the studies of symbiotic and asymbiotic germination *in vitro* and *in situ*, we have also conducted studies of reintroduction, in the Ecological Reserve el Pedregal (see Ortega-Larrocea and Rangel in the same volume), showing the relevance of mycorrhizal fungi in the development and survival of orchids. This is the first Mexican report that uses a combination of strategies (*e.g*., germplasm preservation, fungal isolation, database recording) to promote orchid conservation both *in situ* and *ex situ*. The main aim of this project is to initiate a global conservation program of symbiotic fungi diversity where a collection of seeds is necessary to test the symbiotic effectiveness of the isolates. With the reintroduction results, we intend to attire the attention of the main organisms promoting orchid conservation in Mexico and present convincing evidence that mycorrhizal fungi are necessary for any realistic conservation project. We will conduct future research to demonstrate that habitat degradation decreases the biodiversity functions of soil and fungi potential and in consequence, the ability of orchids to survive in nature. We are conscious that this will be a long-term project and at the present, we attempt only easy *in vitro* bioassays with the recently collected material testing seed and fungi viability, isolate effectiveness and isolate diversity and specificity.

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