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AN INTEGRATED APPROACH TO CONSERVATION OF A CRITICALLY ENDANGERED ORCHID

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ABSTRACT. This study applied principles of an integrated conservation approach to a nationally listed, critically endangered orchid (*Caladenia huegelii)* of the metropolitan Perth area. In-situ ecological studies found a sparse distribution of the mycorrhizal fungi associated with *C. huegelii* in field sites, using recently developed in-situ and ex-situ seed baiting methods. Symbiotic germination assays were undertaken to identify levels of specificity between different populations of *C. huegelii* with fungal isolates obtained from different sites and other closely related *Caladenia* species. A narrower level of specificity was found for *C. huegelii* than for other *Caladenia* species. Mycorrhizal efficacy did not vary between populations. Genetic analysis using amplified fragment length polymorphism (AFLP) of adult plant tissue found a distinction between the two populations sampled. A similar range in genetic diversity was found to exist within and among fungal isolates obtained from different sites. The presenter reported on this study, which represents significant advances for the practical conservation and recovery of *C. huegelii.* The study suggests that a recovery program based on research outcomes is required for the effective conservation of temperate terrestrial orchids.

Key words: AFLP, mycorrhizal fungi, Australia, *Caladenia huegelii*

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CONSERVATION AND CARPOLOGY OF ORCHIDS

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ABSTRACT. The immense variation seen in orchid flowers and pollination mechanisms is well known. Variation is the reason why this plant group captivates the mind of those interested in natural history and hence the ultimate reason for the wish to conserve orchids. The next step in the life cycle after pollination is that involving orchid fruits, which have been much neglected in orchid research. Orchid fruits are much more variable than generally imagined, and their basic structure remains inadequately described and understood. Yet structure and function of orchid fruits are important for many aspects of orchid conservation and may contribute significantly to the understanding of orchid phylogeny. Carpological studies of orchids are part of the ongoing research of the monocot phylogeny research group at the University of Copenhagen.

Key words: Pollination mechanisms, orchid fruits

Conservation Grants Program of the San Diego County Orchid Society

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Abstract. In 1991, the San Diego County Orchid Society (SDCOS) began a program of awarding grants to institutions or individuals working toward orchid conservation. Funds for the grants are raised each year through the sale of donated orchid plants at two San Diego orchid shows. SDCOS currently advertises for applications once a year, generally with a late summer deadline. The ads request a proposal with a budget near $2500 for a 1-year grant. The interests of the Society are first, to protect orchids in the wild; second, to establish and maintain organizations that protect orchids and orchid habitat; third, to conduct studies related to orchid conservation; and fourth, to educate the public about conservation in general and orchid conservation in particular and to encourage public participation in conservation. In 2003, from 24 applications received, the Society awarded six grants. Since 1991, the Society has raised nearly $100,000, of which ca. $90,000 has been distributed in grants. Members of SDCOS are pleased and grateful that the American Orchid Society awarded them the 2003 AOS Orchid Conservation Award for these efforts.

Key words: AOS Orchid Conservation Award, grant funding

Costa Rican Zygopetalinae: Conservation Assumptions from Taxonomic Information

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Abstract. The subtribe Zygopetalinae (Orchidaceae: Maxillarieae) in Costa Rica encompasses 70–80 orchid species, pertaining to 12 genera, some of which are now being re-evaluated on the basis of new phylogenetic data from molecular analyses. Particularly rich in species are the genera *Chondrorhyncha* s.l., *Chondroscaphe, Dichaea,* and *Kefersteinia.* Systematic revision of the group in Costa Rica and comparison with Zygopetalinae taxa from other countries in Mesoamerica have revealed the existence of new species that allow critical reconsideration of many old names, traditionally lumped into broad and ill-defined species complexes. This is especially true for species of the genera *Chondroscaphe, Dichaea,* and *Kefersteinia.* In the last few years, these genera received new attention from systematic botanists, who considerably broadened our understanding of the diversity of these taxa. Orchid taxonomists traditionally were obliged to rely on scanty material for their studies, and their aversion to old names, often based on poor descriptions and insufficient field data, is somewhat proverbial. The recent development of a generation of botanists working in species-rich tropical areas represents a new opportunity to fill the gaps of our knowledge about species distribution and circumscription with a flow of data based on field observations and a broader perception of natural variation. Are the new taxonomic views generated by these data useful for conservation purposes? Several examples illustrated their potential. In the case of Costa Rican Zygopetalinae orchids, former assumptions based on broadly defined taxonomic concepts produced questionable decisions in public conservation policies. A good taxonomic system, including appreciation of natural var-

iation based on a large-scale sampling of biodiversity, should be welcome as the sound basis for the development of conservation strategies in biologically rich areas.

Key words: Orchidaceae, Maxillarieae, conservation strategies

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DETECTING TERRESTRIAL ORCHID FUNGI IN SOILS

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ABSTRACT. Two seed-baiting techniques were developed to detect mycorrhizal fungi compatible with terrestrial orchids. Ex-situ baiting with field-collected soil incubated in the laboratory demonstrated that coarse organic matter (more than 2 mm) in the litter and topsoil was a key reservoir of inoculum for orchid fungi. In-situ baiting with multi-chambered seed packets buried in orchid habitats during the growing season tested the germination of ten orchids simultaneously. These studies used Western Australian terrestrial orchids in the genera *Monadenia (Disa), Microtis, Caladenia, Pterostylis,* and *Diuris.* Trials were located in areas of high or low weed cover at Bold Park, a large urban bushland remnant with *Banksia* and *Eucalyptus* trees. Both methods detected compatible fungi for all species, but common orchids germinated more frequently than uncommon species in the park. Seeds germinated in soil from high or low weed cover areas, even though orchids were rare in weedy areas. The two methods varied in applicability depending on the situation where they were used. The ex-situ method, however, allowed the time-course of germination to be observed, resulting in the production of more protocorms, and it facilitated isolation of mycorrhizal fungi from protocorms. The presenter discussed these techniques as valuable new tools to assist orchid research and conservation.

Key words: mycorrhizal fungi, ex-situ baiting, Western Australia, orchid conservation

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ECOLOGY, CONSERVATION, AND MYCORRHIZAL ASSOCIATIONS OF ORCHIDS FROM THE KIMBERLEY REGION OF WESTERN AUSTRALIA

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ABSTRACT. The tropical Kimberley Region of Western Australia covers an area of 300,000 km^2 with habitats ranging from dry rain forest to arid deserts. A total of 17 orchid species are recorded for this region (two epiphytes and 15 terrestrials, including three saprophytes). Of these, three apparently are endemic and currently not formally named. The Kimberly Region is largely unmanaged wilderness, although still subject to weed invasion, feral animals, and regular broad-scale fires, each of which provides threats to orchid habitats. Research into the ecology of these orchids has spanned the past two decades. Recently, fungal endophytes have been isolated from most of these species. Diversity of these fungal endophytes has been assessed, using pectic zymograms and rDNA sequences. The rDNA sequence data have shown that most of these fungi actually are *Fusarium* species (sensu lato) rather than *Rhizoctonia* species (sensu lato), as previously assumed. Fungal specificity between species is high, even within a genus. Several orchid species have more than one species of mycorrhizal fungus associated with them.

Key words: mycorrhizal fungi, endophytes, *Fusarium, Rhizoctonia*

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EX-SITU ESTABLISHMENT AND GERMPLASM STORAGE FOR TERRESTRIAL ORCHIDS

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ABSTRACT. This study evaluated the establishment of temperate terrestrial orchids, native to southwestern Western Australia (WA), in natural habitat. Orchid seed germinated well when sown into soil inoculated with mycorrhizal fungi in field sites but failed to develop tubers that survived summer dormancy. Seedling survival, however, was improved when actively growing symbiotic seedlings or dormant tubers were transferred to natural habitat during the growing season. This study highlights the benefit of using optimized methods for seedling production by symbiotic germination and nursery growth to produce advanced seedlings or tuberized plants to maximize the survival of plants transferred to field sites. An analysis of the impact of seed drying and seed storage was undertaken for a selection of temperate terrestrial orchids. Orchid seed with lowered moisture content was found to be suitable for long-term preservation in liquid nitrogen (LN). This simple and economical method for seed banking orchid seed provides benefits that include long-term storage, capacity to store large numbers of seed, potential for little change in genetic structure of the stored tissue, and efficient use of space. The long-term cryopreservation of orchid germplasm and mycorrhizal fungi has the potential to preserve many orchid taxa. For example, the Millennium Seed Bank Project (Royal Botanic Gardens, Kew, UK) is assisting Kings Park and Botanic Garden to store seed and fungi of more than 66 rare Western Australian terrestrial orchids. Although preservation of orchid germplasm may be possible for extended periods, the ex-situ preservation of material is no substitute for the in-situ conservation of existing orchid populations.

Key words: terrestrial orchid, seed storage, propagation, soil establishment

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HISTOLOGICAL DEVELOPMENT OF SYMBIOTIC *BLETIA URBANA*: AN ENDANGERED TERRESTRIAL ORCHID FROM MEXICO

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ABSTRACT. Information is scarce on histological changes during germination and development of symbiotic terrestrial orchids in Western Hemisphere ecosystems. Histological studies that provide information about the dynamics of the symbiosis can be useful in managing endangered species, especially when implementing reintroduction. The authors demonstrate the complete symbiotic development of an endangered terrestrial orchid, *Bletia urbana* Dressler, endemic to the south of Mexico. Fungal isolate was obtained from the same species growing in its natural habitat, an Ecological Reserve in Mexico City. The study described development of germinating seeds after 3 days of symbiotic incubation, 2-week-old protocorms, seedlings after 3–4 weeks, and roots developed at week 5. Structures were fixed in Navashin solution for 24 hr. and preserved in 70% ethanol. Dehydration was achieved with terbutilyc alcohol, infiltration, and inclusion with paraffin at 58–60°C. De-paraffination was made with xilol-alcohol solutions and stained with fast green-safranine. A number of 15 μm slides were obtained from a rotation microtome, and permanent slides were mounted in synthetic resin. Seed material stored for more than 14 years and recently collected showed characteristic germination and symbiotic development patterns. Microscopic observations revealed that, previous to cellular division of the seed embryo, the fungi had started to colonize suspensor cells, forming the first hyphal coils. In the early protocorm stage, the apical meristem began to be defined. Fungal coils were distributed along the central tissue cells of the protocorm, where reserve substances are accumulated, as they are in the periphery of the basal cells, where abundant rhizoids formed. Foliar primordium developed starting from the apical meristem, and different physiological stages of hyphal coils were observed. Leaves continued to develop after 3 weeks of in-vitro culture, as did their initial rootlet. Hyphal coils continued to be central and basal, until their migration into the radical zone during the seedling stage (week 4). Vascular tissue formed beginning with the procambium and developed a central cellular line versus the root and apical zone of the protocorm. At week 5, the root was completely developed with hairs and an apical meristem but without coils, in contrast to the different degenerated stages of these structures found in the root base. Several physiological stages of coils degradation were recorded during the germination and protocorm stages, showing that developing orchids depend on fungal nutrition. Hyphal re-growth from rhizoids into the medium showed different entrance and exit fungal patterns during exploration of the substrate. In contrast to symbiotic plants, mycorrhizal protocorms develop abundant rhizoids, when the first protocorm cells form. External morphology of asymbiotic protocorms differs in growth and appearance, perhaps because they are artificially induced and difficult to find under natural conditions. In-vitro studies revealed that seedlings with functional symbiotic roots could be reintroduced into their natural habitat after 5 weeks of culture. Field germination observations can provide more information about the soil dynamics of this species to address conservation management practices.

Key words:: Histology, Orchidaceae, mycorrhizal fungi

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How Do Red Lists Help Plant Conservation? A Case Study of the Endemic Orchid Flora of Ecuador

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Abstract. In Ecuador 250,000 ha are deforested each year, which explains why one out of every three species of endemic orchids is threatened and could disappear in the near future. In 2000, Ecuador became the first neotropical and megadiverse country to evaluate the conservation status of its endemic flora using the new IUCN criteria. The evaluation of each species was based mainly on the analysis of its distribution profile and habitat destruction, combined with the frequency and dates of the plant collections. This information was obtained from herbaria specimens. The endemic orchids of Ecuador, because of their high species diversity, exemplify ideally the general trend in the conservation status of all Ecuadorian endemic flora. Even though the orchid family has representatives in all habitats, individual species commonly present restricted distributions, marked habitat preferences, and different levels of tolerance to disturbance. Nearly 70% of threatened endemic orchids occur in Ecuador in low and high Andean forest (1000–3000 m), and only 13% are protected in the National System of Protected Areas. Based on the 2000 proposal, Ecuador has been able to define priorities for plant conservation. Legal reforms already had been initiated to protect plants, and since publication of the IUCN Red List 2000, research projects studying endemic plants have increased exponentially. Will the next edition of the IUCN Red List (2005) update the data (ca. 600 new orchid species in Ecuador) and integrate information from these projects to meliorate the promise of the Red List?

Key words: RAMAS, endemic flora, IUCN criteria, herbaria specimens

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How to Maintain Orchid Diversity in a Highly Urbanized Country Such as The Netherlands

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Abstract. The Netherlands ranks among the most densely populated countries in the world, with an average of 400 inhabitants per km^2. Such a large human population in a small area is the result of the easy accessibility of this flat country, which varies in height from 8 m below sea level to 320 m above sea level. The country is highly industrialized; and, moreover, agricultural lands are among the most productive in the world. In spite of development, some 35 terrestrial orchid species are considered indigenous to The Netherlands, and most of them are still present today. Within this species assortment, a surprising variety of life-history strategies can be distinguished, such as short- and long-lived species, early and late flowering in the vegetational season, nectar rewarding and non-rewarding species, and autotrophic and heterotrophic series. Most orchid species occur in so-called semi-natural vegetation created by several centuries of human interference. These include coppiced woodlands, grazed chalk grasslands, and dry and moist heath lands. Since the majority of these areas have lost their natural ecosystems to modern agricultural activities, different kinds of specific small-scale nature man-

agement have to be applied to maintain the attractive native orchids in this part of the world. An appropriate management method for native orchid sites, however, does not always guarantee long-term conservation, because habitat fragmentation combined with high atmospheric nitrogen input are serious threats to orchid species diversity in The Netherlands.

Key words: Conservation, semi-natural vegetation, habitat fragmentation, orchid management

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Integrated Conservation of Australian Orchids: A Local and Global Perspective

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Abstract. Orchid conservation is at a crossroads. Never before has the global diversity of the estimated 25,000 species of orchids been under such threat. The science of conservation that emerged from the conservation crises of the late 1980s has delivered a broad raft of new technologies in the molecular, propagation, and restoration sciences. Orchids with their high public profile have fortunately been at the forefront of these research areas. For example, the Orchidaceae were one of the first families of plants to be comprehensively DNA sequenced, which led to new insights into the phylogenetic relationships of the genera with important consequences in developing conservation priorities. Equally, research initiated by Australian scientists into the symbiotic role of orchid mycorrhiza in germination and development of terrestrial species has led to significant conservation advantages for orchids. Further developments in conservation science in seed banking and understanding orchid population dynamics, particularly in relation to the specificity of the orchid-fungus relationship, have provided new understanding of the way in which the habitat can be manipulated to maximize orchid management and aid in translocation programs. In Western Australia, recovery of a number of orchids has been achieved through the integration of a raft of scientific approaches. The carousel spider orchid (*Caladenia arenicola*) has been used as a flagship species to test the principles of integrated conservation in orchids. This species has been the subject of extensive genetic testing to establish both the principles of species boundaries but also to ensure the genetic fidelity of plants used in translocation programs. In addition, the species has been seed banked using cryogenic approaches that achieved long-term storage of seed. The mycorrhizal agents essential for propagation have been genetically tested and only highly efficacious isolates have been used in propagation and translocation programs. Finally the inoculum potential of wild site fungi and the degree to which artificial supplementation is essential for growth and survival of the orchid have been established. The end result is the sustainable translocation of an orchid to a wild site as part of conservation and restoration programs. The challenge for orchid scientists and conservation practitioners is to apply the principles established for the carousel spider orchid to a broader range of species, especially those under threat.

Key words: terrestrial orchid, integrated conservation

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METAPOPULATION DYNAMICS IN AN EPIPHYTIC HERB: VARIATION IN EXTINCTION AND COLONIZATION RATES AND DISTANCE FROM CLOSEST OCCUPIED PATCH

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ABSTRACT. Dispersal is a fundamental component of classic metapopulation and may influence population persistence. The authors are studying a naturally fragmented orchid metapopulation, where dispersal may occur in the form of seeds that have a very low probability of establishing. They collected 5 years of data on the orchid *Lepanthes rupestris,* an epiphytic and lithophytic orchid of Puerto Rico. In 1998, they permanently identified 250 occupied sites to evaluate extinction probabilities and 750 occupied orchid sites to evaluate colonization probabilities. Since then, they have sampled twice a year, increasing the sample size as new sites are discovered. The orchid is reproductively active throughout the year. The populations are scattered in a more or less linear fashion on trees and rocks along both sides of a river. Median population sizes on the first survey were 23 individuals with a range of 1–700 individuals. Dispersal appears to be very limited, and extinction is habitat dependent. Conditions for metapopulation dynamics appear to be present in this species of orchids, suggesting that consequently a paradigm shift in orchid conservation may be necessary for managing some orchid species in the wild.

Key words: population dynamics, colonization, population extinction, Caribbean National Forest, Puerto Rico

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MYCORRHIZAL ASSOCIATIONS OF TERRESTRIAL ORCHIDS: BRIEF ENCOUNTERS AND LASTING RELATIONSHIPS

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ABSTRACT. The diversity of mycorrhizal fungi associated with two contrasting orchids, *Disa bracteata,* a weedy introduced species, and *Pyrorchis nigricans,* an Australian native species, were investigated. Ribosomal DNA sequencing was used to separate orchid fungi into phylogenetic groups within the *Rhizoctonia* complex. Fungi associated with *D. bracteata* were distinct from most fungi utilized by Australian native orchids. No clear trends were evident between the phylogenetic groups and the geographic origin of isolates from *D. bracteata* and *P. nigricans.* Symbiotic germination assays were undertaken to identify the levels of fungal specificity of *D. bracteata* and several common Australian native orchids. Degrees of specificity varied with orchid species, from relatively broad associations formed by some weedy orchid species to highly specific associations demonstrated by other common Australian native orchids. Some fungal relationships formed by orchids displaying broad specificity, however, were brief encounters that resulted in initial germination but did not progress to produce autotrophic seedlings. Only those associations that resulted in substantial plants with green leaves were deemed to be indicative of a compatible and successful symbiotic relationship.

Key words: Western Australia, *Rhizoctonia,* symbiotic germination

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MYCORRHIZAL CONTROLS ON ORCHID ABUNDANCE ALONG FOREST SUCCESSIONAL GRADIENTS IN UPSTATE NEW YORK: IMPLICATIONS FOR MANAGEMENT IN FRAGMENTED LANDSCAPES

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ABSTRACT. All native orchid species in New York State are ranked as exploitably vulnerable and protected under state law (NYS DEC Env. Cons. Law §9-1503). In upstate New York, terrestrial orchid populations are patchily distributed within a mosaic of human land use and ecological conditions, wherein species may be favored or limited by historic or ongoing disturbance. Although numerous studies have shown that terrestrial orchids respond favorably to natural disturbance, these relationships are poorly understood. Better understanding of rare orchid distribution and abundance in managed forests and successional communities is needed to ensure the long-term persistence of populations in fragmented landscapes. Because all orchid species are obligate myco-heterotrophs during seed germination and development, mycorrhizae play a significant role in the ecology and distribution of orchids and may explain, in part, their

patchy distributions and often-extreme rarity. We lack, however, a fundamental understanding of what controls seedling establishment. Recently developed techniques for in-situ "dust" seed germination and identification of mycorrhizal associates via genetic sequencing now offer the opportunity to examine the habitat conditions that facilitate mycoheterotrophic interactions and orchid seedling establishment. The authors propose to apply these recent advances in the study of mycoheterotrophic plants toward the conservation and management of six orchid species in New York State. The first of these is an achlorophyllous species, the striped coral root, *Corallorhiza striata* Lindl. In addition, the study will feature the following five green orchids: the pink ladyslipper, *Cypripedium acaule* Ait.; the showy ladyslipper, *Cypripedium reginae* Walt.; the large round-leaved orchid, *Platanthera orbiculata* (Pursh) Lindl.; the greater round-leaved orchid, *Platanthera macrophylla* (Goldie) P.M. Brown; and the three-birds orchid, *Triphora trianthophora* (Sw.) Rydb. ex Britt. The six species selected represent a hierarchy of current understanding of the plant-fungus interaction—from well-documented plant-fungus associations (coral root) to associations with a single fungal family in orchids with minimal photosynthetic tissues and subterranean habits (round-leaved and three-birds orchids) to unknown but presumably generalist associations in orchids with relatively abundant photosynthetic tissues (ladyslipper orchids). The goal of this research is to assess the relationship between the habitat associations of terrestrial orchid species and their mycorrhizal fungal symbionts. Specific objectives are (1) to identify the mycorrhizal fungi associated with each orchid species; (2) to determine whether these fungal associates occur in occupied and unoccupied habitats; and (3) to assess micro-scale habitat characteristics correlated with the occurrence of these fungi to inform the management and conservation of native orchid species in New York State and throughout their ranges.

Key words: Conservation, seedling establishment, plant-fungus association

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WILD LOVE AFFAIR: ESSENCE OF FLORIDA'S NATIVE ORCHIDS

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ABSTRACT. At the closing banquet of the Second International Orchid Conservation Congress, a light visual-lecture exploration of native Florida orchids and their swamp and prairie habitat dispensed scientific material with a spoonful of sugar. The jazz guitar sounds of Santana and the vocals of Dave Matthews singing, "Love of My Life," wowed the audience, as the music merged with orchid images. Via digitized programming on iPlay Scala, the soundtrack and visuals took the orchidists on a fast-forward ride through the wilderness. Reading from her book, *Wild Love Affair: Essence of Florida's Native Orchids,* the presenter merged poetic prose with flowing, dancing images. The visual-lecture closed with a foot-stomping ride through the swamps to the sound of Cindy Hackney's "Gator in the Slough." Throughout, the presenter brought an artist's eye to the work of probing the wilderness within to find equally magnificent places of repose, as she enchanted the orchidists with that wild beauty and perfection (FIGURE 1) they seek to conserve.

Key words: Everglades, iPlay Scala, Florida native orchids

FIGURE 1. The Fakahatchee Strand State Preserve at the edge of the Everglades is renowned for the largest number of native orchid species in the United States, among them the following. **A.** The bee-swarm orchid (*Cyrtopodium punctatum*). **B.** The clamshell orchid (*Prosthechea cochleata*). **C.** The ghost orchid (*Dendrophylax lindenii*), which was selected as the logo for the Second International Orchid Conservation Congress. Photos © 2004 Connie Bransilver.