DIVERSITY AND RARITY IN AN ANDEAN CLOUD FOREST: DECEIT POLLINATION

AND FRUIT SET IN THE ENDEMIC ORCHID, *CHONDROSCAPHE EMBREEI*

by

ALEXANDER A. REYNOLDS

Under the direction of C. Ronald Carroll

ABSTRACT

Diversity and rarity of orchid species were assessed in an Andean cloud forest, the Maquipucuna Reserve, Ecuador (00°02'-08'N; 78° 35'-38'W). Over 322 species are reported with 56 endemic species, an increase of 117 species over the last published list. General mechanisms for diversity and rarity of orchids discussed include pollinator relationships, biogeography, and microclimatic variation. During a one-year monitoring program, interseasonal euglossine bee population dynamics were surveyed using chemical baits, and the pollination ecology of an endemic orchid, *Chondroscaphe embreei* was determined by observing the phenology of three subpopulations. Pollination success rates, 7.3% (male) and 1.0% (female) were as low as recorded for any tropical orchid. *C. embreei* is deceit pollinated by a male euglossine bee, *Euglossa trinotata*. Low fruit set results from low pollinator visitation, a natural consequence of its non-rewarding strategy. Potential conservation strategies for endemic species are discussed, including micropropagation, reintroduction, and environmental education.

INDEX WORDS: Orchid, Diversity, Euglossine bee, Pollination ecology, Deceit pollination, Maquipucuna, Ecuador, Conservation, Rarity, Micropropagation, Reintroduction, Fruit set

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B.S., The University of Georgia, 2000

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004

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August 2004

ACKNOWLEDGEMENTS

I would like to thank anyone who has helped nuture, support, or even endure my fascination with tropical ecology over the last few years. First, I'd like to thank my graduate committee: Ron Carroll, Jim Affolter, and Ted Gragson, whose guidance over the last two years has been invaluable; the Institute of Ecology and all its students and staff for all the support and comments on my work as it developed into this present form; and equally important, Rodrigo Ontaneda, Rebeca Justicia, and everyone with Fundación Maquipucuna who have provided whatever I needed to carry out the projects involved, especially Juan Carlos Unda, Francisco Prieto, Fernando Echeverria, Mariel Araujo, and the Niño Naturalista program which provided the fragrances for the first chemical baiting experiments. My field assistant Patricio Oña deserves special thanks for carrying out the orchid monitoring while I could not be in the field. I am deeply indebted to the Ron Determann and Ron Gagliardo and the Atlanta Botanical Garden for first inspiring my interest in orchids and continuing to support my work in every way and to Larry Wilson at Emory University who encouraged me to visit the tropical rain forest for the first time. To all the other orchidologists that have supported my work – at Florida: Mark Whitten, Norris Williams, Robert Dressler, and Mario Blanco; at Selby Gardens: Cal Dodson; at PUCE: Lorena Endara; at USC: Robert Raguso – as well as Lou Jost, Alex Hirtz, and Eric Hágsater for orchid identifications. To Jim Carr at National Geographic for the euglossine bee footage. And finally to my family and friends that have supported me and will continue to support me in all my attempts long before and long after this work is completed – you have my love and gratitude. Thanks...

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INTRODUCTION: ORCHIDOLOGY 101

Overview and Purpose of the Study

Orchids comprise one of the largest flowering plant families in the world (Madison 1977, Dressler 1981, Kress 1986). Atwood (1986) and Dressler (1981) provide counts of 19,128 and 19,192 registered species, respectively, however, due to a large number of recently described and as-yet-undescribed species, the accepted general range is between 25,000-30,000 species (Dressler 1981) or many more (Gentry and Dodson 1987) worldwide. Regardless, it is commonly cited that one out of every ten flowering plants (10%) is an orchid (Gentry and Dodson 1987). Orchids inhabit every vegetated continent, but they are most closely associated with the tropical regions of the world, specifically Central and South America and Southeast Asia and Indonesia (Koopowitz 2001). Over 10,500 orchid species, or 35-42% of the world's orchid flora, are registered for the Neotropics (Dodson *et al*. 2003). An estimated 78% (6,472 out of 8,257 total) of South American orchids are found in the Andes, especially in the lower montane forests and cloud forests between 1000 and 2800 meters above sea level (msl).

Habitat heterogeneity and microclimatic variation, the same factors promoting species diversity in the Neotropics, promote species rarity as well. Rare species usually occur in low frequencies due to natural causes, such as strict habitat requirements or pollination mechanism, or are threatened by anthropogenic causes, such as deforestation. Endemism is a common measure of rarity, but often carries multiple definitions. In a scientific sense, it usually refers to either a commonly occurring, but range restricted species, or a widely distributed species that occurs in low densities. Dodson and Gentry (1991) estimated that 20% of all orchids in Ecuador

were endemic, but Pitman *et al.* (2000) show that approximately 26% of all native plants in Ecuador are endemic, while for orchids, in particular, the rate of endemism is double that estimate (40%; Endara and Jost 2000). Moreover, one out of every three endemic plants, or 33% of the country's total endemic flora (1,319 out of 4,011), is an orchid (Pitman *et al.* 2000). In Ecuador, the Andean region contains 75% (2,965) of the country's total endemic species. With such high rates of regional endemism, it is not surprising that the Tropical Andes is considered one of the top biodiversity 'hot-spots' of the world (Mittermier *et al.* 2000).

Despite its relatively small geographical size (273,000 km^2), Ecuador is the most orchid diverse country in the world with 3,792 species (Dodson *et al.* 2003) with more species named every year. This species diversity is primarily due to its equatorial location, offshore ocean currents, and especially the geographical dominance of the Andes, which creates a microhabitat rich landscape, well-suited for orchids and other epiphytic vegetation. However, Ecuador also has one of the highest deforestation rates in the Neotropics, with an estimated 53.6% loss in forested lands between 1950 and 1992 (Koopowitz 2001; see Figure 1.1). In 1990, a report on deforestation in western Ecuador, showed that only 21.6% of the 102,000 km^2 region is still forested; however, outside of the major reserves of the region, such as Cotacachi-Cayapas and Awa Reserves, deforestation pressures have only increased in the last 15 years (Dodson and Gentry 1991). Thus, an improved understanding of the mechanisms promoting orchid diversity and rarity in the Neotropical Andes is vital to a better understanding of orchid conservation and strategies for management. This introductory chapter will provide a brief overview of orchid morphology, ecology, and evolutionary history with emphasis on the causes and consequences of diversity and rarity in the family. The chapters that follow will provide a general overview of diversity and rarity of species at a reserve in the Andean cloud forest and the specific factors
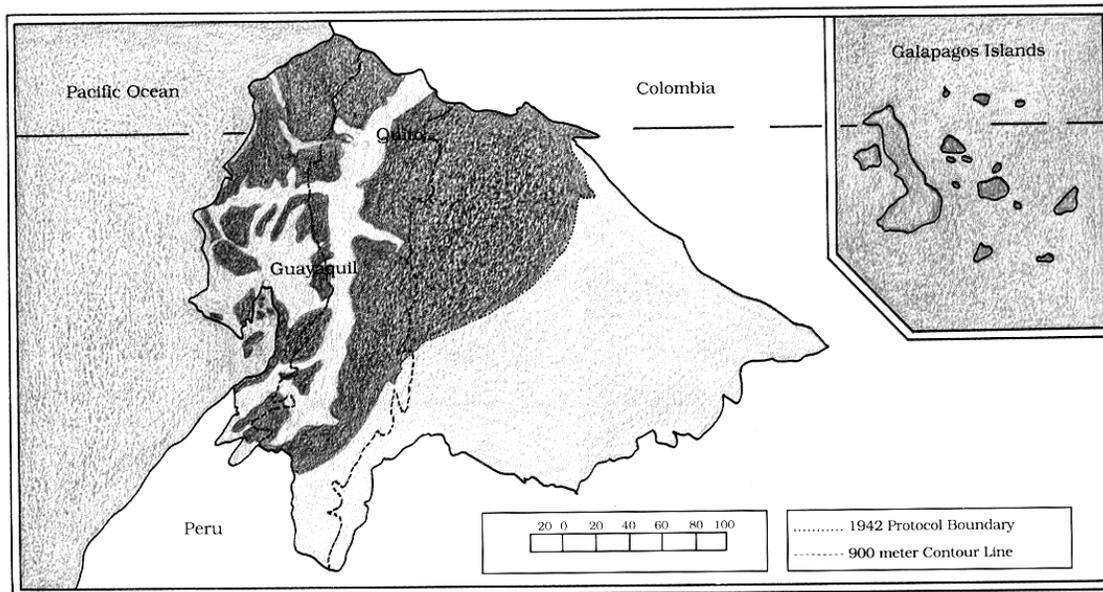
(a) Forest cover in 1945
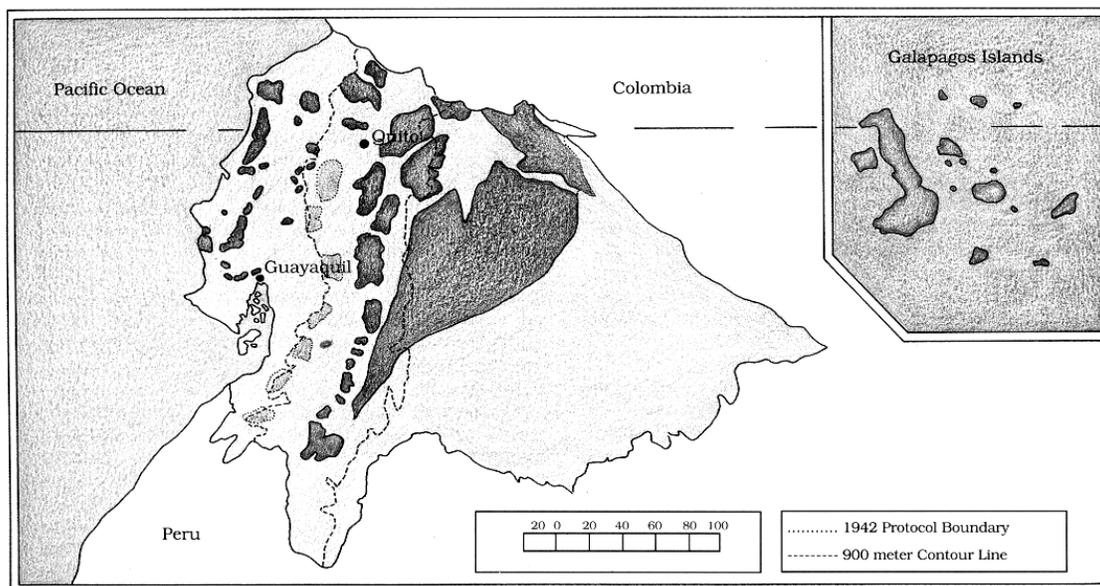
(b) Forest cover in 1992

Figure 1.1. Deforestation in Ecuador since WWII. Forest cover in Ecuador in 1945 (top) and 1992 (bottom) is shown by the dark shaded areas. Deforestation rates in Ecuador are among the highest in the Neotropics, especially in western coastal and Andean regions. Map used with permission from Dodson *et al*. 2003.

influencing one endemic species, the deceit pollination of *Chondroscaphe embreei*. The concluding chapter will review specific orchid conservation strategies being implemented in the region of study, including environmental education and research, opening responsible markets through micropropagation for the sale of ornamental orchids, as well as the potential for local reintroduction of rare species.

Orchidaceae – One Big, Happy Family

Orchids are considered one of the most evolutionarily advanced taxa in the plant kingdom, primarily due to their high level of specialization with their pollinators (Pilj and Dodson 1966). Despite the great diversity within the family and adaptation to even the most restricted habitats, there are several characteristics that link all orchids, primitive and advanced:

1) the stamens, usually only one fertile, but in primitive terrestrials, up to two or three, are all located one side of the flower, instead of whorled symmetrically;

2) the partial union of male and female sexual parts (stamens and pistil) or complete fusion into a single reproductive structure called the column;

3) microspermy, or microscopically small seeds, often lacking an endosperm;

4) the pollen is packaged into large masses called a pollinia, which are attached to a sticky pad called the viscidium (derived from part of the stigma lobe called the rostellum) by a membranous stipe to form a unit called the pollinarium which carried away in its entirety by the pollinator; and

5) a specialized petal called the labellum, or lip, which is often specialized in shape to either attract pollinators or facilitate pollination by serving as a landing platform through a 180 degree twist of the flower called resupination (Dressler 1981; see Figure 1.2).
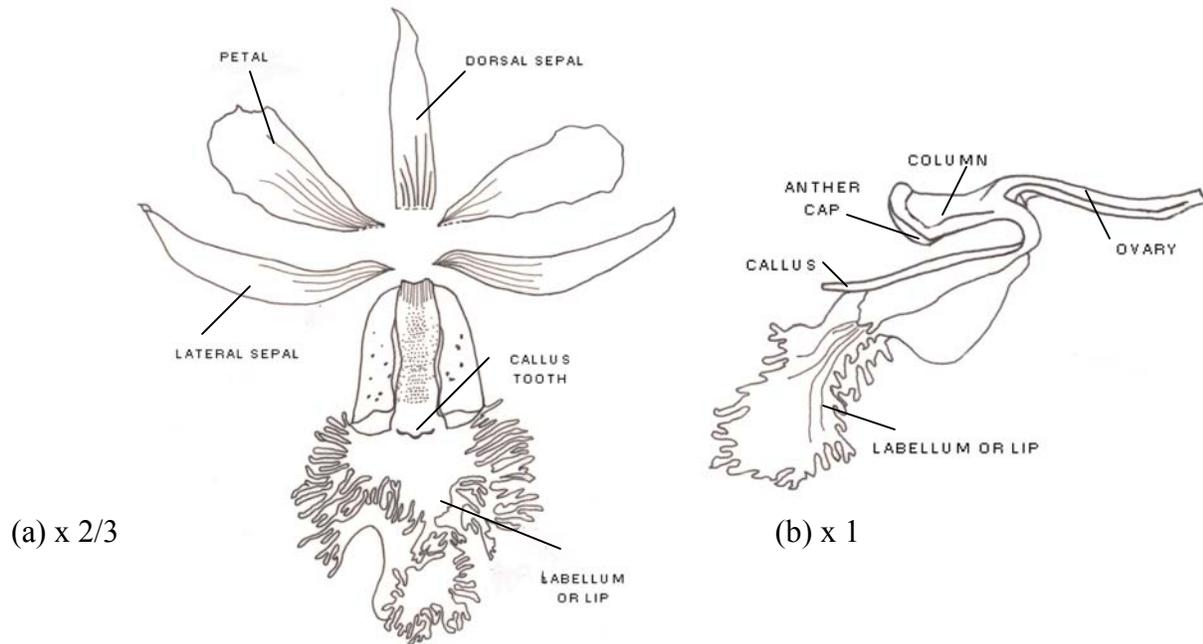
Figure 1.2. Floral dissection of *Chondroscaphe embreei* flower. Floral anatomy of the typical orchid flower is labeled from the flattened dissected view (a) and side view without sepals or lateral sepals (b). The callus tooth is a key taxonomic feature of the subtribe to which *C. embreei* belongs. Original drawings by the author.

While none of these adaptations are unique to orchids – several other liliaceous species have stamens on one side, some gingers have a column-like structure, and many families show microspermy, though orchids do produce many more than any other group – it is their combination, especially the first three listed above, that separate orchids from all other plants (Koopowitz 2001). The latter two are commonly associated with orchids, but usually function in specialized pollination mechanisms, are not restricted to orchids, and do not define the family. These morphological adaptations have ecological consequences that have helped the orchids radiate into such a diverse group, namely through specialized pollination, long-distance dispersal, and the epiphytic habit.

Ecological Considerations: Pollination and Dispersal

Several morphological adaptations, primarily the evolution of a united column with a rostellum, pollinaria, and a specialized labellum, suggest that pollination mechanism provides a strong selective pressure in orchids (Pilj and Dodson 1966). Though many orchids are self-compatible, their floral structure generally prevents autogamy; thus, they rely on specialized pollinator relationships for successful outcrossing (Darwin 1862, Dressler 1981). These adaptations to attract pollinators are necessary to ensure survival of the species due to the hyperdispersed nature of most orchid populations (Benzing and Atwood 1984, Ackerman 1986). In the tropical orchids, floral visitation and successful pollination events are rare, thus low fruit set is common, but is compensated for by high fecundity, with large numbers of reduced seeds in an orchid capsule (Dressler 1981). The adaptation of pollinaria allows for more precise pollen delivery and higher rates of ovule fertilization and higher seed set (Gentry and Dodson 1987). A single seed capsule often contains hundreds, thousands, or even millions of seeds, all of which are dependent on mycorrhizal fungi for seed germination (Stoutamire 1964, Koopowitz 2001). In fact, small seed size may be correlated with degree of mycorrhizal host dependence (Ackerman 1983a).

In Darwin's first treatise on pollination ecology, *On the Various Contrivances by which Orchids are Fertilized by Insects*, he mused that if a slightly larger fraction of orchid seeds "were not largely destroyed, any one of them would immediately cover the whole land" (1862). During that time, it was unknown that orchid seeds, as do many plants, require mycorrhizal fungi to germinate (Dressler 1981). Though it represents a potentially strong selection pressure, this stage in orchid biology has received little attention, apart from studies of physiology and potential specificity (or lack of) between orchids and mycorrhyzae (Warcup 1975). This is likely due to

the difficulty of studying germination of microscopic seeds in the wild. While orchid root symbionts have been isolated from many species, even the idea that orchids need mycorrhizae to germinate has been challenged due to advances in micropropagation techniques, in which agar gels provide the necessary nutrients (Curtis 1939, Arditti 1984). This field requires much more research, especially concerning how it affects conservation strategies, such as rare species reintroduction. For example, one recent study of the relationship between mycorrhizal fungi and *Lepanthes* seedlings concludes simply that some fungus species are beneficial while others are detrimental to orchid growth (Bayman *et al*. 2002).

In any given flowering season low-fruit set likely results from pollinator limitation, however, there is evidence the reproductive costs of producing millions of seeds in a single capsule limit flowering from year to year (Ackerman and Montalvo 1990, Calvo 1993, Melendez-Ackerman *et al*. 2000). Through hand pollination trials, Primack and Hall found that the temperate lady slipper orchid, *Cypripedium acaule*, fruits two seasons successively before not flowering the third (1990). Likewise, in some tropical orchids, such as *Aerangis verdickii* and *Paphiopedilum sanderianum*, it has been noted that only two-thirds of the mature plants flower each year, suggesting that on average, they 'rest' every third year (Koopowitz 2001). For other tropical orchids, which are more strongly limited either by pollinators or resources, this number of resting plants may be much higher in the population, only further isolating individual conspecific flowers from each other.

It has been noted that high fecundity of wind-dispersed seeds in orchids represents a random selection event through dispersal to suitable microhabitat, introducing the potential for a non-Darwinian selective pressure rarely mentioned in orchid literature (Atee 1937). This hypothesis was long abandoned in favor of possible Darwinian explanations of seedling survival

in orchids (Benzing and Atwood 1984). However, the 'transilience', or high genetic variability, of orchid seeds has been related to long-distance dispersal founder events, which are linked to diversification of closely related populations and subsequent speciation (Gentry and Dodson 1987). Due to low germination rates in nature, this variability could lead to genetic drift, as has been implicated in differentiation of highly dispersed, low density populations in the genus *Lepanthes* (Tremblay and Ackerman 2001) or cause population bottlenecks (Ackerman and Zimmerman 1994). Tremblay and Ackerman (2003) used Wright's F statistics to assess the potential for genetic flow in orchid species and found that 31% of species (18 of 58) studied had under one migrant per generation, indicating that in small effective population sizes ($N_e < 50$), genetic drift could lead to population differentiation. They also conclude that in populations of intermediate gene flow (36%, 21 out of 58), high variability in migration can also cause sufficient genetic drift to lead to speciation. However, in large populations ($N_e > 50$), natural selection likely dominates the evolutionary process. Thus, there are multiple potential evolutionary paths in orchids. Hyperdispersed, low-density orchid populations create a unique selective environment where morphological adaptations promote both r-selected seed dispersal and specialization to specific pollinators. Under these conditions, random speciation events can be reinforced by diversification of closely related species through pollinator switching (Gentry and Dodson 1987). This 'leap-frog' speciation, or abrupt evolutionary divergence caused by a shift in primary pollinator in response to altered floral morphology or genetic variation, may be characteristic in the orchid diversification and no other family (Dodson *et al*. 1969, Benzing 1987). Clearly more research is needed on the effects of the relationship between seed dispersal, pollinator specificity and low population densities and how the comparative effects of selection versus genetic variability affect orchid diversity and rarity.

Epiphytism: Ecological Constraints and Adaptations

In montane cloud forests, epiphytes predominate; however, individuals of any one species are often widely scattered (Webster 1995). Epiphytes grow on other plants in a commensalistic, non-parasitic relationship, though their better access to light and nutrients in the canopy is often referred to as 'nutritional piracy', which may indirectly harm the host tree (Benzing and Seeman 1978). Also, epiphytosis, or the subsequent fungal infection of the host tree through its host orchid, can indirectly cause problems for the host tree, but may have also selected for chemicals in their bark that inhibit germination of some orchid species (Frei and Dodson 1972, Dressler 1981). The high diversity of epiphytic and hemi-epiphytic (e.g., climbing vines) vegetation has a strong influence on ecosystem function and structure. Despite only constituting only 2% of dry weight biomass, epiphytes represent up to 30% of their foliar biomass. Epiphytes also contain approximately 45% of foliar mineral content of montane cloud forests, a large percentage of which are more labile and turnover more rapidly than tree biomass (Nadkarni 1984). While epiphytes collect litter in their vegetative mats, their litterfall comprises up to 10% of total fine litterfall, however its nutrient dynamics are distinct, generally with slower decomposition and more sporadic occurrence (Nadkarni and Matelson 1992). Epiphytes also alter the humidity, temperature, and precipitation chemistry in the forest and thus have been implicated as indicators of potential climate change scenarios in the Tropics (Benzing 1998, Nadkarni and Solano 2002). Increased shade and microhabitats found in epiphyte vegetative mats in the canopy support a large diversity of organisms ranging from birds (Nadkarni and Matelson 1989) to spiders and ants to insects and invertebrates to frogs and salamanders that rarely appear on the forest floor (Lowman and Nadkarni 1995). Some tree species even

opportunistically root into the canopy humic layer accumulated by epiphytic vegetation, in a mutualistic relationship between epiphytes and host species (Nadkarni 1981).

An estimated 29,000 species of plants spend part of their life as epiphytes, and approximately two-thirds (73%, Atwood 1994) of these are orchids. Moreover, orchids contain approximately ten times as many species as the second and third largest epiphytic families, the aroids and bromeliads (Gentry and Dodson 1987). Epiphytic orchids have evolved a large number of morphological and ecological adaptations to life aboveground, such as: 1) thick, waxy leaves or cuticles to conserve water loss; 2) a thick, fleshy organ at the base of the stem called a pseudobulb, which serves for water and nutrient storage; 3) roots containing velamen, or thickened outer layers of epidermal cells, which aid in water and nutrient uptake, preventing dessication, and structural support (Koopowitz 2001). These adaptations are common among epiphytes of lowland, wet seasonal and tropical dry forests, though, interestingly, many epiphytic orchids of the high montane cloud forests lack these adaptations (Dressler 1981). The constant high humidity of the cloud forests has allowed a large radiation of species in this group. Some of the largest groups of orchids in these forests are in the subtribe Pleurothallidinae, specifically the genera *Pleurothallis*, *Lepanthes*, and *Stelis*, which are partially characterized by lack of pseudobulbs (Dodson *et al*. 2003).

While dispersal may introduce random chance into the survival of orchid seeds, the ecological limitations resulting from its evolutionary adaptations serve as strong selective pressures that have promoted speciation through geographic and ecological isolation (Pilj and Dodson 1966, Dressler 1981). Epiphytism often limits plant and flower size, limiting plant visibility to its pollinator and thus fruit set (Calvo 1990); however, life above the ground also makes many epiphytes more accessible to their pollinators and certainly less susceptible to

herbivory (Dressler 1981). Microhabitat on the host tree is an important factor for both orchid germination and survival and plant mortality, since branchfalls are more common in the outer portion of a tree crown (Heitz 1997). Other trees have physical mechanisms of shedding epiphytes, such smooth bark, bark exfoliation or limb breakage. Due to space and resource limitation, the post-germination juvenile phase likely represents an important competition phase between conspecifics and other orchids as well as between other epiphytes.

While the origins of the orchid family are still debated, radiation into the epiphytic habit initiated much of its evolutionary success (Garay 1972; Ackerman 1983a; Benzing and Atwood 1984; but see Robinson and Burns-Balough 1982). High population densities are difficult to maintain in the canopy or on tree trunks, thus providing a selective pressure for specialized pollinator relationships, to ensure success despite rare encounters with pollinators (Pilj and Dodson 1966, Benzing 1990). These adaptations occurred ancestrally in scattered populations of terrestrial orchids, as seen in some of the bizarre pollination mechanisms found in *Ophrys* and other terrestrials, such as *Paphiopedilum rothschilianum* (Benzing and Atwood 1984, Atwood 1985), but allowed orchids to be especially successful in the epiphytic habit. Pollinator specializations maintain long-distance gene flow between scattered populations and microspermy allows for successful dispersal along the three dimensional scale of forest canopies. However, it is interesting to note that with the exception of achlorophyllous (leafless) orchids, epiphytic orchids generally have the weakest specificity for their mycorrhizal mutualists (Benzing and Atwood 1984). Instead of parasitizing fungi like many saprophytic temperate orchids, many Neotropical epiphytes are autotrophic shortly after germination and may not even require mycorrhizal associations in mature plants (Dressler 1981). Epiphytic orchids rely on pollination mechanisms that often result in low fruit set (Neiland and Wilcock 1998), but

compensate with microspermy as mentioned above, which despite low germination, allow most orchids to persist in low density, scattered populations.

This study attempts to understand orchid diversity and rarity on a regional scale as well as a local population level. The following chapter will discuss hypotheses on the hyperdiversity and high rates of endemism seen in the orchids of the Ecuadorian Andes and then report the diversity of orchids at the Maquipucuna Reserve in the northwestern province of Pichincha, Ecuador, while later chapters will treat specifically with the pollination ecology of an endemic species, *Chondroscaphe embreei*. The results of this research emphasize that successful conservation strategies of orchid species cannot occur without consideration of species natural history, including pollination and habitat requirements. The high diversity of orchids in the Neotropics offers endless potential for combinations in ecological interactions that encourages careful consideration of a species needs before recommendation of any particular conservation strategy, especially reintroduction of rare species. Despite the persistence of external threats, such as deforestation or overcollection, orchids may also be resource, habitat, and/or pollinator limited; indeed, despite the desire to augment and manipulate populations, with some species it may be okay to be rare. However, in the context of high levels of endemism and external threats, preservation of montane cloud forests is one of the world's highest conservation priorities (Gentry 1995).

CHAPTER TWO

ORCHID DIVERSITY AND RARITY IN AN ANDEAN CLOUD FOREST

The morphological adaptations to diverse habitats of the Andes and specialized pollinator mechanisms in orchid populations have created both geographical and biological isolation mechanisms leading to high diversity of species (Dressler 1990, Dodson 2003). The Central Andes of Ecuador, formed approximately 2.5 million years ago, represent an extreme geographical barrier to orchids and other species, isolating populations through the Pleistocene to the present (Neill 1999). Many plant species are unable to disperse over the high Andean peaks and the relatively dry inter-Andean valley. This isolation has resulted in distinctly different eastern and western Andean floras, including the orchids and other epiphytes (Gilmartin 1973, Dodson and Gentry 1991). Rates of orchid endemism (~20-27%) are equally high on either side of the Andes, indicating that on a local level within the Andean landscape, geographical barriers and microclimatic conditions strongly affect orchid biogeography (Dodson *et al.* 2003).

An estimated one out of every ten flowering plants is an orchid (Gentry and Dodson 1987). Several biological factors, including high seed production, wind dispersal of seeds, and the epiphytic habit, have a significant impact on orchid diversity. Several additional hypotheses for why the orchids have developed into such a diverse plant family include: coevolutionary relationships; biogeographical considerations; and microclimatic variation. The possibility of coevolutionary relationships within the orchids has been the classic argument for orchid diversity in the tropics, going back to Darwin original observations in the nineteenth century. However, only a select group of orchids can be considered under this category because

many orchids do not have such specialized pollinator relationships, thus between- and within-habitat diversity must be considered as significant isolation mechanisms. These three hypotheses will be considered briefly in the context of the Ecuadorian Andes before progressing to a specific treatment of the orchid flora of the Maquipucuna Reserve in northwestern Pichincha on the western cordillera.

Coevolution

In his essay, "The Panda's Thumb," Steven J. Gould comments that Darwin immediately followed his controversial *Origin of the Species* with his 'most obscure work,' *The Various Contrivances by which Orchids are Fertilized by Insects* (1980). However, this book was a groundbreaking treatise on how adaptations for cross-pollination influence the evolution of species and led to a century of pollination research based on the premise that nature "abhors perpetual self-fertilisation" (Darwin 1862). If the 'coevolutionary arms race' promotes diversity through continual adaptation by insects to plant defenses, then the orchids take a much less militaristic approach, offering flowers with attractive displays and sweet rewards to lure their pollinators into visiting only their flowers, ensuring that pollinaria from one flower is received by another of the same species. Darwin's initial observations also sparked much research concerning potential coevolutionary relationships in orchids (Pilj and Dodson 1966, Dressler 1990, Peakall and Beattie 1996). 'Strict coevolution', or reciprocal change in orchids and their species-specific pollinators, as defined by Janzen (1980), has not been demonstrated clearly, though potential models, including the euglossine-syndrome and pseudocopulation, will be discussed later in the text. In general, however, there is strong evidence that 'diffuse coevolution' between groups of orchids and pollinators has significantly affected orchid diversity (Feisinger 1983).

Orchids have developed a variety of ways of attracting, rewarding, and occasionally deceiving their floral visitors so that they are pollinated. Attractants vary from large inflorescences, spectacular color and ultraviolet displays, and nectar guides to sweet fragrances and stinking odors (Koopowitz 2001). Rewards can be anything from food – nectar, pollen, and pseudopollen, that is, infertile, but protein-rich pollen – to the fragrances themselves, such as in the euglossine bee pollination syndrome that will be discussed in more detail later (Pilj and Dodson 1966). In fact, Dodson *et al*. (1969) argued that the mixture of active chemical components in the fragrances of *Stanhopea tricornis* and *Gongora* aff. *quinquenervis* were so pollinator-specific, they could be the major reproductive isolating mechanism even in sympatric populations. Because euglossine bees pollinate approximately 10% of orchid species, the role of fragrances as an isolating mechanism could strongly influence orchid diversity (Pilj and Dodson 1966). However, no reciprocal change in euglossines has been demonstrated, despite the hypothesis that sexual selection affects male bees through changing sensitivity and individual variation in fragrance collection behavior and resulting female choice during mating (Dodson 1975, Schemske and Lande 1984). The 'coevolution hypothesis' could also be supported by 'pollinator tracking,' or the synchronicity in flowering time of sympatric orchid species and peak abundance of their specific pollinator (Ackerman 1983b), however it has also been shown that some orchid flowers are out of phase with their pollinator's population dynamics; euglossines, for example, peak in abundance in phase with food plant availability and only opportunistically collect orchid fragrances (Zimmerman *et al*. 1989, Roubik 2004).

However, the relatively high prevalence of deceit pollination in orchids is an important aspect of understanding orchid pollination ecology (Pilj and Dodson 1966, Ackerman 1986). According to basic coevolutionary theory, these 'tricksters,' opportunistic non-rewarding flowers

that deceive pollinators looking for the advertised reward should be selected against due to low pollination success and pollinator constancy, or learned avoidance of non-rewarding flowers (Dodson 1962, Dafni 1984). However, up to one-third of all orchids, possibly 10,000 species, use deceit in some way to achieve successful pollination and some of the most common forms of deceit, such as pseudocopulation, only occur within the orchids (Ackerman 1986). In fact, Dodson (2003) recognizes pollinator specificity through deception as a major component of orchid diversity. Non-model Batesian mimicry of rewarding flowers, such as false nectaries or colorations indicating a non-existent nectar reward, is arguably the most common type of deceit in orchids, while others mimic specific sympatric rewarding flowers, especially nectariferous Leguminosae and Malpighiaceae. Other orchids capitalize on reproductive instincts of pollinators, such as orchid flowers imitating female flies or wasps, which trick unwitting pollinators in very successful ways. Many of these sexual deceit flowers actually have relatively high pollination success through highly specialized relationship with their pollinators, which could indicate a diffuse coevolutionary relationship (Peakall and Beattie 1996). Other species use their pollinator's territorial defense instincts by mimicking rivals of the same sex (Dodson 1962). Their lack of investment in a reward is compensated for by investment in color reflectance patterns (Nilsson 1983) or highly specialized lip architecture that ensures pollinator fidelity.

The relative prevalence of non-model mimics is more difficult to attribute to coevolution (Stoutamire 1971, Ackerman 1983, Roubik 1989). These flowers are dependent on 'chance pollination', high variation in floral characteristics, and even rarity itself for their success. Pollinators can learn to avoid non-rewarding flowers (Roubik 1989, Michener 2000), so their persistence depends on high variation in color or fragrance to prevent learned avoidance or in the lack of this population variation, low densities to prevent high visitation (Dafni 1984). The

coevolution model of orchid diversity alone does explain why deceit orchids persist in the natural environment because there is no mutualistic gain for the pollinators and may indirectly harm them through wasted energetics (Heinrich 1979). Pollination in these mimicry systems should be frequency dependent and subject to negative feedback mechanisms in pollination success depending on pollinator abundance (Dafni 1984). Specific deceit pollination syndromes and their consequences for orchid pollination will be discussed in greater detail in Chapter 3, due to the non-model mimicry of *Chondroscaphe embreei*.

<u>Biogeography</u>

Since many Pleurothallids, the largest subgroup of the orchid family, are only found in high elevation cloud forests, perhaps habitat specificity could explain their evolutionary success. The geographical juxtaposition of the equator, high Andes and offshore confluence of warm and cold ocean currents, and the resulting climatic variation is commonly cited as a primary causal factor for Neotropical orchid diversity (Dodson and Gentry 1991). As already mentioned, an estimated 78% of Neotropical orchids are found on the slopes of the Andes, between 300 msl and 3000 msl (Dodson *et al*. 2003). The Ecuadorian coast spans a precipitation gradient from almost desert near the Peruvian border to one of the rainiest tropical forests on Earth in the Ecuadorian Chocó bioregion, where estimates as high as 8,800 mm/year are common (Webster and Rhode 2001). The confluence of the cold Humboldt current with the warm southward California current just north of the equator is principally responsible for this strong gradient, and the earth's seasonal oscillation affects seasonality of this precipitation (Dodson 2003). This trend continues onshore, though less dramatically and the steep slopes of the western Andes are particularly affected. Lower montane forests are characterized by heavy precipitation, while clouds provide 'horizontal precipitation' as the elevation continues to rise, fostering a rich

diversity of plants. What these forests lack in tree diversity is compensated for by the eternal green of pteridophytes (ferns), mosses, and other epiphytic life, including orchids (Webster 1995).

Andean cloud forests are dominated by epiphytic vegetation, that is, plants growing on trees or other plants (Webster 1995, 2001). In western Ecuador, Gentry and Dodson found 33% of all species in 0.1 ha forest plots consisted of vascular epiphytes and hemiephytic climbers (1987). Wet, mid-elevation, tropical regions, especially in northwestern Ecuador showed the highest diversity of epiphytic species in the world, and they further demonstrated that 0.1 ha plots in the cloud forests of Ecuador have more plant species than any forest in the world, even when the trees are excluded (Gentry and Dodson 1987). In these areas, the explosion of epiphytes likely accounts for the disproportionately high species diversity of the Neotropical montane forests. This would suggest that the factors that influence epiphyte diversity in general also favor orchid diversity. These large-scale habitat differences make it apparent that diversity and biogeography are linked, however, extreme heterogeneity within the large Andean bioregion and local endemism is also an important factor influencing orchid diversity.

Microclimatic Variation

The macro-scale biogeographic explanation for diversity in the Andean cloud forests is further elaborated by the microclimatic variation prevalent in these habitats. Steep slopes combine with rugged mountain topography to produce a high degree of habitat heterogeneity. Cloud cover, temperature, humidity, and light can have a significant influence on the suite of orchid species present (Dressler 1990). Tree architecture within the forest structure is also important, with the majority of orchid species preferring thick limbs and large trunks, though some groups, known as 'twig epiphytes' specialize on the precarious thin limbs of young trees

and the outermost branches of adults. In a Mexican cloud forest, plants living on outer branches survive an average of 1-3 years and suffer a disproportionate percentage of mortality due to branchfall, while those nearer to the trunk on thicker branches rarely die from branchfall (Hietz 1997). An estimated 4% of orchid species inhabit this outermost part of the canopy, while 76.2% inhabit the inner two-thirds of branches, and only 10.9% colonize trunks (Dressler 1981). Many orchid species are found only in specific microhabitats within the tree canopy.

Disturbance dynamics of Andean forests is another contributing factor to high local orchid diversity. The region is characterized by intense geologic activity, which can be devastating on a large scale as well. The eruption of Mt. Chalupas, approximately 80,000 years ago, left most of central Ecuador covered in 30 meters of ash (Dodson 2003) and ash from this eruption has been uncovered as far as the Galapagos islands (Hirtz, *pers. comm.*). More recently, Mt. Pululahua (see Figure 2.2) erupted, covering the entire province of Pichincha in 1.5 meters ash (Dodson 2003). Seismic activity can also severely impact on the landscape. In 1987, an earthquake measuring 7.2 on the Richter scale hit near El Reventador, near Baeza, causing a 40,000 ha landslide (Dodson 2003). This is a dramatic example of a common occurrence in the Andes. Landslides of varying size occur throughout the rainy season, as heavy precipitation compromises the integrity of steep slopes made of volcanic ash, increasing habitat heterogeneity across the landscape (Dodson and Gentry 1991). Many orchids, along with other plant species colonize these areas, because light and nutrients are more readily available, creating a temporal microhabitat (Dodson 2003). In fact, in the absence of natural fire and high winds, landslides and associated treefalls, provide a major disturbance mechanism on the local scale.

The epiphytic habit itself may contribute to diversity of orchids by opening up new potential habitats that are easily isolated. Epiphytic populations may proceed along

evolutionarily divergent paths due to specificity to tree canopy microhabitats, leading to reproductive isolation, whether through pollinator relationships or morphological adaptations (Dodson 2003). As previously discussed, orchids were especially well-adapted for radiation into the epiphytic habit, due to their r-selected reproductive strategy of mass production of small seeds, whose germination is facilitated by a mycorrhizal association (Benzing 1981). Pseudobulbs are another morphological adaptation of many larger epiphytic orchids that must withstand prolonged dry seasons or higher temperature and humidity extremes in the canopy (Gentry and Dodson 1987). Other canopy species, such as the Pleurothallids, do not have pseudobulbs and are commonly found in areas, such as cloud forests, with a less pronounced dry season or fewer daily fluctuations in temperature and humidity. While life in the canopy allows better access to light and nutrients, certain species are better suited to particular microhabitats than others, allowing for diversification of species even within a single tree canopy. As mentioned above, some orchid species have adapted to the precarious 'twig epiphyte' habitat (e.g., *Psygmorchis, Ionopsis*, and *Rodriguezia)*, with a small habit, quick flowering phenology, and shortened lifespans (Dressler 1981).

Altitudinal effects are important, not only due to precipitation and humidity, but also their effects on the availability of pollinators (Pilj and Dodson 1966). High cloud forest species, especially the speciose Pleurothallids are often pollinated by non-specific, diverse fly populations because few other effective pollinators thrive at such high elevations. While specific pollinator relationships do occur at such high elevations, primarily through deception, it is probable that microhabitats created by the geography of the steep Andean slopes, especially variation in temperature and humidity due to shading influence speciation through isolation of populations. Vertical stratification of habitat through the epiphytic habit provides even more

available microhabitats, with additional opportunity for adaptation and isolation. Epiphytism also increases the success of wind seed dispersal, especially when copious amounts of seed are produced. Mycorrhizal relationships further affect microhabitat specialization. Orchids are unique in achieving this combination of a generalized r-selected seed dispersal syndrome and specificity in microhabitat and pollinator relationships, leading to their mass diversification in the Andean cloud forests (Benzing 1981, Gentry and Dodson 1987).

Rarity and Endemism – Potential Mechanisms

Increased diversity of orchids is paralleled only by the exceptional increased prevalence of rare and endemic orchid species. In Ecuador, approximately 30% of all orchids are considered endemic and one out of every three endemic plants (33%) is an orchid (Pitman *et al.* 2000), thus endemism is not only high within the family, it is disproportionately so. Despite all the arguments previously mentioned for the diversity of orchids in the tropics, there are fewer explanations for why there are so many rare species in the family. Endemism is a measure of rarity, at least on a spatial scale, though in many cases it does not accurately reflect the causal mechanisms for rarity in a species' population dynamics. An endemic species can be widely rare or locally common, with threats to a population's existence varying from local deforestation to widespread pollinator declines (Koopowitz 2001). It can also simply be a measure of inadequate collections of a species since herbarium collections are the recognized form of documenting species existence; many endemic orchids in Ecuador are known from only one or a few specimens (Endara and Jost 2000).

For the following discussion, however, we are most interested in a biological definition of endemism, which includes geographic range restriction and limited population size. Gentry provided a definition of 'locally endemic' species, that is, species geographically limited in range

to 75,000 km^2, which includes almost any species limited to the 80,000 km^2 region of western Ecuador (Dodson *et al*. 2003). In reality most endemic species are much more range limited, often inhabiting a narrow elevational range or even a single ridgetop. Dodson and Gentry (1991) detail the case of Centinela, a 100 ha ridgetop in western Ecuador, where approximately 10% of the flora was strictly endemic to the site. In this case, the endemics were threatened in a real sense, through deforestation pressures, and by 1988, only 11 years after being described, nearly 100 endemic species were extirpated through complete landscape conversion of the Centinela ridge to agriculture, and most are presumed extinct. The previous discussion of Ecuadorian endemic species defines any species restricted to a range of less than 20,000 km^2 as at least vulnerable (Pitman 2000); thus high rates of endemism include many range-restricted species that may or may not face immediate threats to existing populations.

It is commonly argued that rare species represent either newly isolated species that will expand their range through dispersal or those doomed to eventual extinction due to habitat fragmentation and low effective population sizes due to pollinator limitation (Koopowitz 2001). Rarity also can be systemic in the population dynamics of a species. This has been demonstrated in many rewardless orchids, through low fruit sets in deceit pollinated orchids (Ackerman and Montalvo 1990). In fact, most tropical orchids show low fruit set whether rewarding or not when compared to temperate orchid species (Neiland and Wilcock 1990). Low fruit set may be a consequence of ineffective pollination mechanism, few pollinator visits, or high reproductive costs of producing capsules year after year, as previously discussed. While low fruit set is implicated in species rarity, it is a natural consequence of resource or pollinator limitation, so it cannot be stated clearly that the population is declining without long-term studies of population

dynamics. Due to the high costs of fruit production, high seed production may be adaptive to ensure that low fruiting frequency does not limit seed dispersal.

The preceeding discussion has developed the general arguments for diversity and rarity in the orchid family that will be developed further in Chapters 3 and 4 with specific reference to euglossine bee diversity and deceit pollination of an endemic orchid in an Andean cloud forest. These experiments were conducted at the Maquipucuna Reserve, a montane cloud forest reserve in the northwestern Province of Pichincha, Ecuador. A brief description of the Reserve follows, with particular emphasis characteristics influencing its orchid diversity. The chapter concludes with the results of an orchid diversity assessment, also conducted during the course of the orchid monitoring experiments.

Site Description: The Maquipucuna Reserve, Ecuador

The Maquipucuna Reserve is a 6,500 hectare (ha) cloud forest reserve privately protected by the Maquipucuna Foundation, an Ecuadorian, not-for-profit non-governmental organization founded in 1988 as a steward for the Reserve (see Figures 2.1 and 2.2). The reserve protects one of the last intact forests on the western Andean cordillera between 1000msl, southwest of the town of Nanegal, and 2800msl, at the peak of Cerro Montecristi, between 00°02'-08'N and 78° 35'-38'W. The Reserve is surrounded by several other protected areas including the Protected Forest of the Upper Guayllabamba Watershed (13,880 has) and the Mindo-Nambillo Protected Forest (19,200 has), which are vital to the watershed management of the Guayllabamba River Basin (Sarmiento 1995). This basin feeds the Esmeraldas river, the second largest South American river emptying into the Pacific Ocean. Despite protected status, these reserves are still under severe pressures through landscape conversion, habitat fragmentation, and the resulting threat to biodiversity, especially due to its close proximity to Quito (Webster 1995).
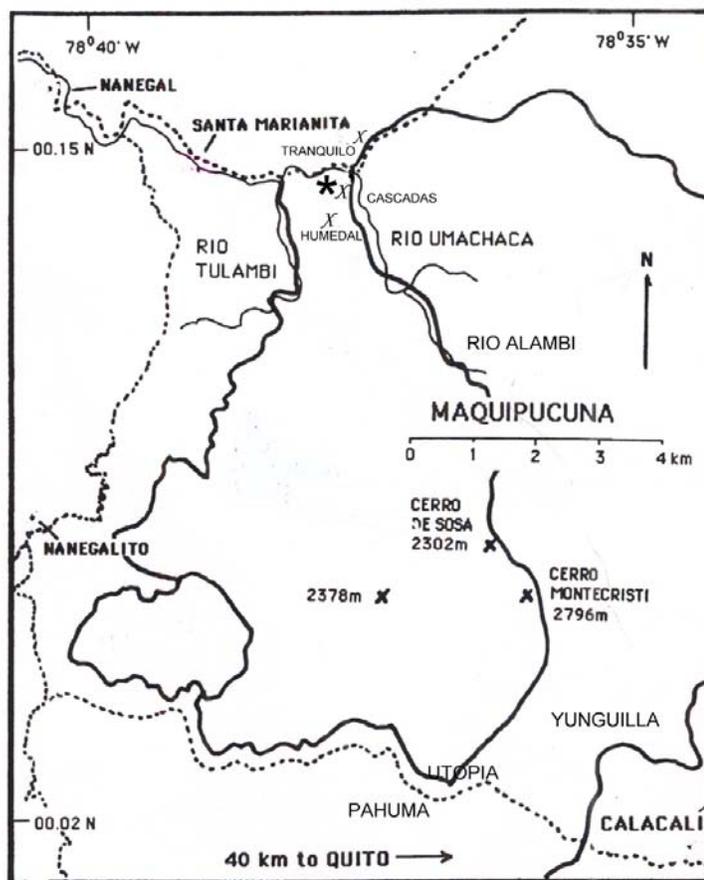
Figure 2.1. Map of the Maquipucuna Reserve, Pichincha, Ecuador. The Thomas H. Davis Scientific Station is marked with an asterisk. Orchid populations were monitored on the Humedal, Tranquilo, and Cascadas trails, as shown. Euglossine bee surveys were performed on the Humedal trail as well. Other major collection areas, such as Yunguilla, Utopia, and Pahuma are shown as well. Solid lines mark the borders of the Maquipucuna Reserve and Protected forest of the Upper Guallyabamba Watershed, thin solid lines represent rivers, and dashed lines indicate roads. Adapted with permission from Raguso and Gloster 1993.

The Maquipucuna Reserve preserves both montane rain forest between 1000msl and 2400msl and upper montane cloud forest above 2400msl to 2800msl. Steep mountain slopes (up to 60-70°) characterize the higher elevations and approximately 80% of the Reserve above 1500msl is considered 'pristine' forest as there is no historical evidence of logging and tree
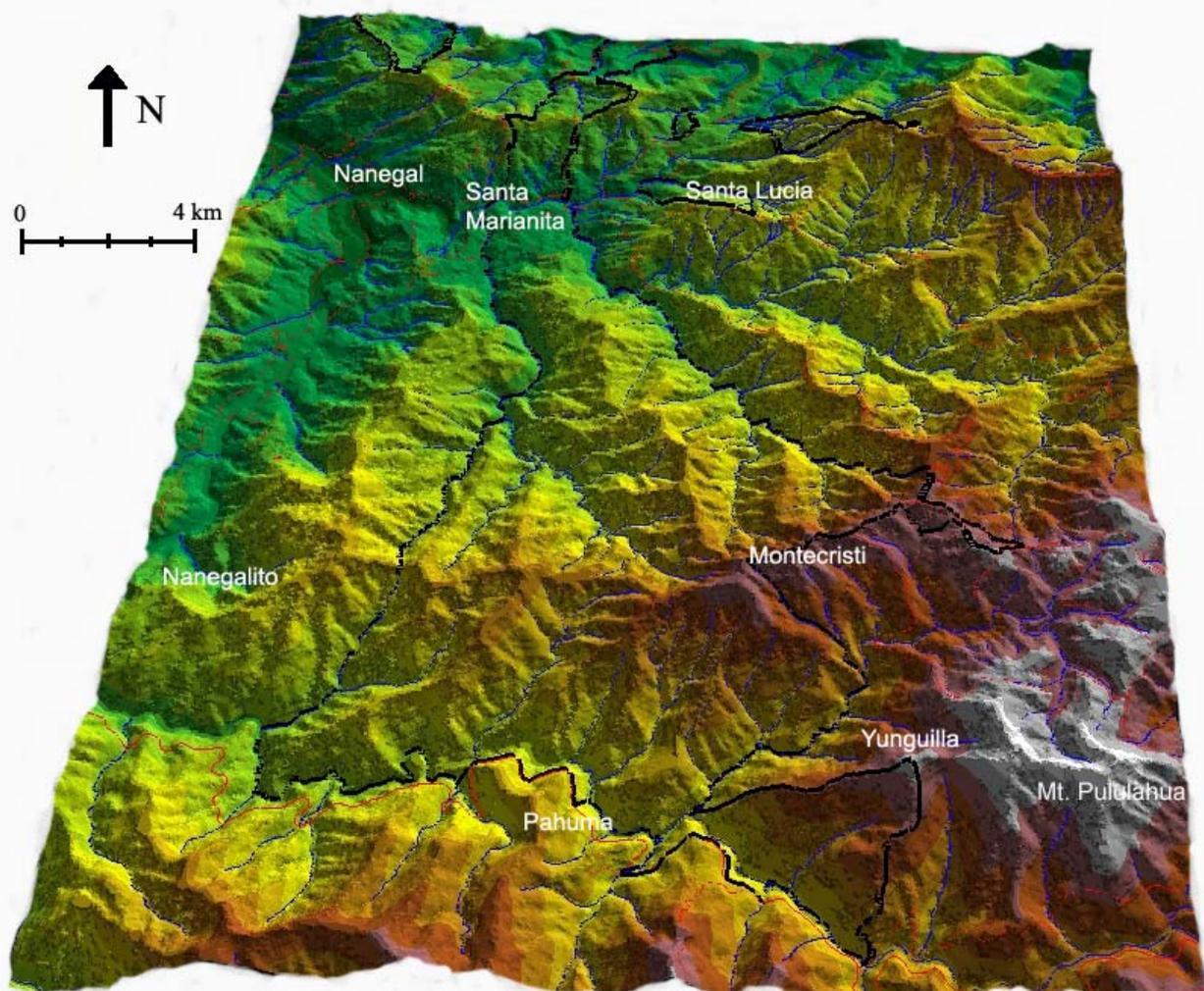
Figure 2.2. Relief map of the Maquipucuna Reserve. Note the steep Andean topography of the region, ranging from 1,000-3,000+ msl (dark to light coloration). The highest peak shown, Mt. Pululahua, is 3,356 msl, while the Thomas H. Davis Scientific Station at the Reserve, in the northern third is at 1235 msl. Due to the rugged terrain, approximately 80% of the Reserve is primary forest. Note recent land acquisitions to Reserve compared to Figure 2.1.

diameters are large (Justicia, *pers. comm*.), while below 1500msl, much landscape has been

converted to agricultural land and cattle pasture (Webster 2001). The Reserve follows a

longitudinal precipitation gradient due to the altitudinal effects of the Andes. The areas around

the Thomas H. Davis Scientific Station in the northwestern corner of the Reserve have a mean

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
☐ Nanegalito	334.3	233.6	531.1	568.4	450.7	240.3	128.4	63.3	149.1	207.3	204.8	250.3
■ Nanegal	215.2	209.3	589.3	479.5	461.6	182.1	162.3	75.7	115.3	184.6	268.6	254.9
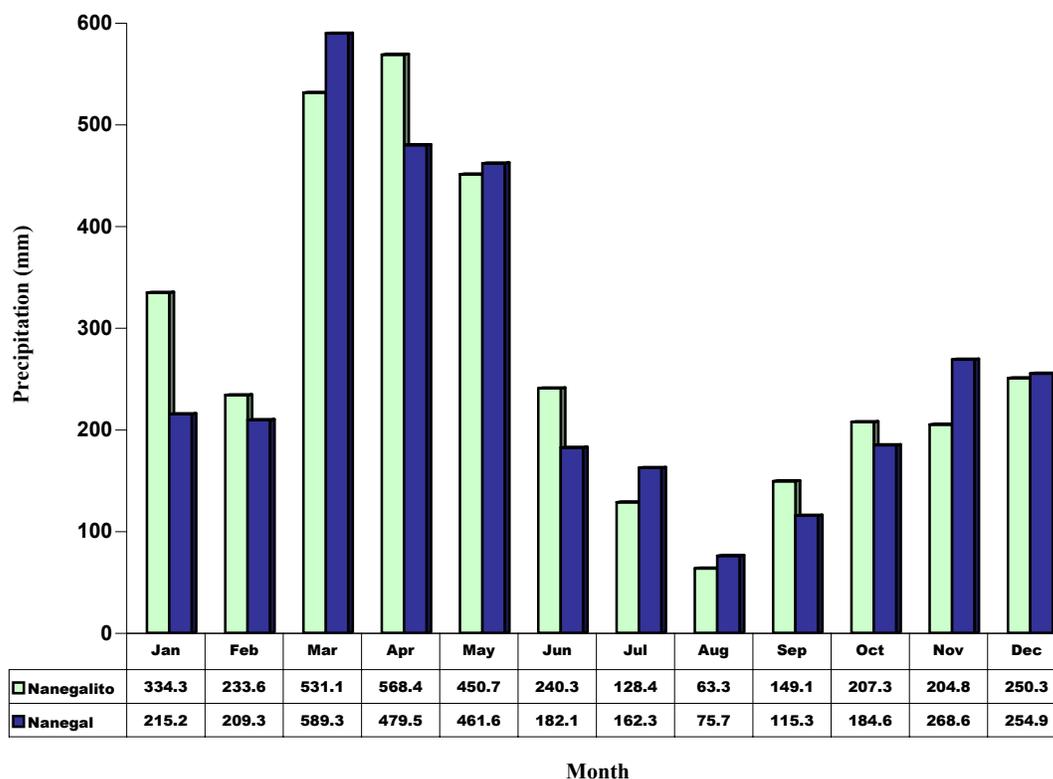
Month

Figure 2.3.   Monthly precipitation in Nanegal (1200msl) and Nanegalito (1630msl).   These towns are on the north and southwestern sides of the Maquipucuna Reserve. Note strong dry season between June and September. Adapted from Sarmiento 1995.

temperature of 18°C (Svenning and Balslev 1998, Webster and Rhode 2001). Due to the equatorial location, the daily fluctuations in temperature are higher than the seasonal variation (Webster 1995). Annual precipitation for Nanegal (1200msl) in 1987 was recorded as 3198.4 mm, while Nanegalito to the southwest of the Reserve at 1630msl received 3361.6 mm in the same year, with both areas experiencing a short, but strong dry season between June and September (see Figure 2.3).   The eastern side of the Reserve receives more precipitation and cooler average temperatures (mean of 10 °C) due to higher altitude (Webster and Rhode 2001) before the rain shadow effect in the Interandean Valley dominates, creating dry valleys with primarily xeric vegetation (Sarmiento 1995). Interannual variation in precipitation, such as

increased frequency of both drought and the El Niño phenomenon, also likely affects vegetation on the Andean slopes (author, *pers. obs*.).

The Maquipucuna Reserve is 'conservationally significant' due to its well-preserved, mid-elevation montane and cloud forests with high rates of endemism typical for the Andean region (Gentry 1991). Gentry performed a series of rapid assessment plots (0.1 ha) for vegetation at the Reserve, near 1600msl, and concluded that the forest is similar in tree diversity (123 spp. > 2.5 cm DBH) and large tree composition (80 inds. > 10 cm DBH) to intact coastal lowland forest of Ecuador. The Reserve also contains many more hemiepiphytic climbers, which is typical of lowland Chocó forests (Gentry 1995). However, this data does not include any information concerning the vascular epiphytes and pteridophytes (ferns) of Maquipucuna. Webster and Rhode (2001) indicate that the most species rich taxa of the Reserve are the ferns (262 native species) and epiphytes, especially the orchids (205 spp), aroids (74 spp), and bromeliads (57 spp). Ferns and epiphytes combined constitute approximately 42% of the native flora species list of the Reserve. The authors also note that orchids are by far the largest flowing family at Maquipucuna (205 spp vs. 87 for Asteraceae, the next closest family in species richness; Webster and Rhode 2004). Based on this information, I focused on documenting as many orchid species as possible during my studies at the Reserve. What follows is a report on my 2004 updated orchid list for the Maquipucuna Reserve and how these additions affect the overall orchid flora.

Methodology: Orchid Diversity Surveys

The surveys to document orchid species in the Maquipucuna Reserve occurred between June-July 2001 and January 2003-March 2004. Species were digitally photographed and their location, habitat, and elevation were recorded. These surveys were done throughout the Reserve and surrounding forests, primarily following existing trails, but several key survey areas are

marked on Figures 2.1 and 2.2. A taxonomic expert in the specific species subgroup verified every addition either in the field or through photographs, as noted in the list (see Appendix I). All species (28 spp) observed in flower during March 2004 were collected, pressed and deposited in the herbarium at the Pontificia Universidad Católica del Ecuador (QCA). Also during January 2003-March 2004, flowers were collected and kept in alcohol (70% ethanol) and over 100 species are included in the private collection located at the Choco-Andes Orchid Conservation Center at the Reserve. Many species additions were also cultivated and are maintained as living specimens in the Maquipucuna orchid garden. Many of the newly reported species for the Reserve still need to be collected in future surveys to officially document their presence in national herbaria.

Results: Maturation of a Flora

The full results of the surveys, combined with the previous existing orchid list from Webster and Rhode (2001) is provided in Appendix I. Table 2.1 lists the species additions, totaling 119 species and 11 newly reported genera. The table does not include new names based on taxonomic revisions or reduction to synonomy. It also does not include any new species found outside of the original study range of Webster and Rhode (2001), defined as between the equator and 00º10'N and 78º35W-41'W, which includes the entire Maquipucuna Reserve and some of the Upper Guayllabamba Watershed Protected Forest (also approximately the area covered by Figures 2.1 and 2.2). These species are listed in Appendix I in brackets and represent an additional 23 species potentially located within the Reserve. Table 2.1 includes species documented by the author during the diversity survey that are represented in the digital imagery database (see Figure 2.4 for representative photos), pickled flower collections, or as a living

Table 2.1. Species additions to the Maquipucuna orchid list. Additions made during orchid surveys by the author. Species representing new genera are marked with an asterisk. For complete revised list of Webster and Rhode 2001, see Appendix I.

Species Name	Species Name	Species Name
Ackermania cornuta (Garay) Dodson & R.Escobar*	*Cyrtochilum williamsianum* (Dodson) Dalström	*Epidendrum quitensium* Rchb. f.
Ada elegantula (Rchb.f.) N.H. Williams	*Cyrtochilum* sp. 1	*Epidendrum rostratum* Garay & Dunst.
Altensteinia virescens Lindl.	*Dracula felix* (Luer) Luer	*Epidendrum scharfii* Hágsater & Dodson
Anguloa virginalis Linden ex B. S. Williams*	*Dracula vespertilio* (Rchb.f.) Luer	*Epidendrum trachysepalum* Hágsater
Campylocentrum polystachyum(Lindl.) Rolfe	*Dracula wallisii* (Rchb.f.) Luer	*Epidendrum trachysepalum* Hágsater
Campylocentrum polystachyum(Lindl.) Rolfe	*Dressleria fragrans* Dodson*	*Epidendrum* sp. 3
Cranichis sp. 2	*Dryadella simula* (Rchb. f.) Luer	*Eriopsis rutidobulbon* Hook*
Crossoglossa tipuloides (Lindl.) Dodson	*Elleanthus oliganthus* (Poepp. & Endl.) Rchb. F.	*Erythrodes* sp. 1
Cryptocentrum latifolium Schltr.	*Elleanthus petrogeiton* Schltr.	*Eulophia alta* (L.) Fawc. & Rendle*
Cryptocentrum latifolium Schltr.	*Elleanthus* sp. 2	*Eulophia alta* (L.) Fawc. & Rendle*
Cryptocentrum lehmanni (Rchb. f.) Garay	*Elleanthus* sp. 3	*Govenia tingens* Poepp. & Endl.
Cryptocentrum lehmanni (Rchb. f.) Garay	*Epidendrum blepharistes* Barker ex Lindl	*Kefersteinia ocellata* Garay
Cryptocentrum sp. 1	*Epidendrum blepharistes* Barker ex Lindl	*Kefersteinia taurina* Rchb. f.
Cryptocentrum sp. 1	*Epidendrum embreei* Dodson	*Lepanthes acarina* Luer
Cyrtochilum cimiciferum (Rchb.f.) Dalström	*Epidendrum macroöphorum* Hágsater & Dodson	*Lepanthes ballatrix* Luer
Cyrtochilum cimiciferum (Rchb.f.) Dalström	*Epidendrum macroöphorum* Hágsater & Dodson	*Lepanthes biloba* Lindl.
Cyrtochilum macranthum (Lindl.) Kraenzl.	*Epidendrum mancum* Lindl.	*Lepanthes kuijtii* Luer & Hirtz
Cyrtochilum macranthum (Lindl.) Kraenzl.	*Epidendrum microcarpum* Hágsater et Dodson	*Lepanthes magnifica* Luer
Cyrtochilum ramosissimum (Lindl.) Dalström	*Epidendrum microcarpum* Hágsater et Dodson	*Lepanthes pelyx* Luer & Hirtz
Cyrtochilum ramosissimum (Lindl.) Dalström	*Epidendrum nanegalense* Hágsater & Dodson	

Table 2.1 (cont'd). Species additions to the Maquipucuna orchid list.

Species Name	Species Name	Species Name
Lepanthes villosa Lojtnant	*Oerstedella medinae* (Dodson)	*Pleurothallis linguifera* Lindl.
Lockhartia chocoensis Kraenzl.	Hágsater*	*Pleurothallis restrepioides* Lindl.
Lycaste ciliata (Ruiz & Pav.)	*Oncidium* sp. 1	*Pleurothallis ruberrima* Lindl
Lindl. ex Rchb. f.	*Otoglossum anixopterum*	*Pleurothallis tripteranthum*
Lycaste sp.	(Rchb.f) Garay & Dunst.	Rchb. f.
Lycomormium ecuadorense	*Pleurothallis antennifera* Lindl.	*Pleurothallis* sp. 1
Sweet	*Pleurothallis bicruris* Lindl.	*Pleurothallis* sp. 2
Malaxis cf. *andicola*.*	*Pleurothallis bivalvis* Lindl.	*Pleurothallis* sp. 3
Masdevallia sp. 3	*Pleurothallis cordifolia* Rchb. f.	*Pleurothallis* sp. 4
Masdevallia sp. 4	& Wagener	*Pleurothallis* sp. 5
Maxillaria aggregata (Kunth)	*Pleurothallis crossota* Luer &	*Pleurothallis* sp. 6
Lindl.	Dalström	*Polystachya concreta* (Jacq.)
Maxillaria lehmannii Rchb. f.	*Pleurothallis crucifera* Luer &	Garay & H.R. Sweet
Maxillaria	Hirtz	*Scaphosepalum ophidion* Luer
pseudoreichenheimiana	*Pleurothallis deflexa* Luer	*Scaphosepalum swertiifolium*
Dodson	*Pleurothallis dibolia* Luer	(Rchb. f.) Rolfe
Maxillaria sp. 1 (sp. nov)	*Pleurothallis ensata* Luer	*Scaphyglottis prolifera* Cogn.*
Maxillaria sp. 2	*Pleurothallis epiglottis* Luer	*Schlimia stevensonii* Dodson*
Maxillaria sp. 3	*Pleurothallis erythrium* Luer	*Sigmatostalix picta* Rchb. f.
Maxillaria sp. 4	*Pleurothallis gelida* Lindl.	*Sobralia atropubescens* Ames &
Odontoglossum armatum Rchb.f	*Pleurothallis lacera* Luer	C. Schweinf.
Odontoglossum sp. 1		*Sobralia lancea* Garay

Table 2.1 (cont'd). Species additions to the Maquipucuna orchid list.

Species Name	Species Name	Species Name
Stelis allenii L.O. Williams	*Stelis* cf. *lindenii* Lindl.	*Trichosalpinx memor* (Rchb.f.)
Stelis argentata Lindl.	*Stelis* cf. *mucronata* Lindl.	Luer
Stelis calotricha Schltr.	*Stelis striolata* Lindl.	*Xylobium foveatum* (Lindl.) G.
Stelis columnaris Lindl.	*Stelis triseta* Lindl.	Nicholson
Stelis concinna Lindl.	*Stenorrhychos speciosum* (Jacq.)	*Xylobium* sp. 1
Stelis eublepharis Rchb.	Rich. ex. Spreng.	*Zootrophion dayanum* (Rchb. f.)
Stelis flacca Rchb.f.	*Telipogon steinii* Dodson & R.	Luer
Stelis hirtzii Luer	Escobar*	*Zootrophion hypodiscus* (Rchb. f.)
Stelis jamesonii Lindl.	*Trichopilia steinii* Dodson	Luer

specimen in the orchid garden. It also includes the results of a literature search of a database of orchid collections in Ecuador provided by Dodson *et al*. (2003); however, only species with locality information specific enough (e.g. GPS coordinates) to include in the study range or with Webster, UC-Davis collection numbers are included.

The revised orchid list contains 327 species within the range of study (see Appendix I). This is an increase of 122 species over the last update for Webster and Rhode (2003). An additional 8 species that were not documented by these survey results have also been added since 2003 from new records gleaned from the TROPICOS database (Rhode, *pers. comm*.). The newly reported genera are represented by: *Ackermania cornuta*, *Anguloa virginalis*, *Campylocentrum polystachyum*, *Dressleria fragrans*, *Eriopsis rutidobulbon*, *Eulophia alta*, *Malaxis* sp., *Oerstedella medinae*, *Scaphyglottis prolifera*, *Schlimia stevensonii*, and *Telipogon steinii*. Each of these is only represented by a single species. The largest percentage of the new species additions (35%) are included in three speciose genera in the subtribe Pleurothallidinae:

Pleurothallis (17 identified and 6 unidentified additions), *Stelis* (13 additions), and *Lepanthes* (7 additions). The genus *Epidendrum*, with 11 new species, also makes up a significant portion of the revisions. This maintains *Epidendrum* as the largest genus of orchids at the Reserve with 41 species of the 327 total (12.5%) now recorded, while *Pleurothallis* and *Maxillaria* follow with 37 (11.3%) and 20 (6.1%) species, respectively. Other major taxonomic changes include replacing all *Encyclia* spp. with *Prosthechea* spp., separating many *Oncidium* spp. into *Cyrtochilum*, and some rearranging of the Secundum and Paniculatum subgroups within the genus *Epidendrum* (Hágsater in Dodson *et al*. 2003).

The original list provided by Webster and Rhode contained 39 endemic species, or 19% of the total orchid flora. These are defined as endemic in either the Catalogue of the Vascular Plants of Ecuador (CVPE) or the Libro Rojo de Plantas Endémicas del Ecuador 2000 (Jorgenson and León-Yañez 1999, Pitman et al. 2000). These endemics are further classified by the IUCN as 'vulnerable' (23 species), 'nearly threatened' (9 species), and 'least concern' (4 species). The revised list adds 12 endemic species, 8 vulnerable (all *Pleurothallis* and *Lepanthes* spp.), 2 nearly threatened, and 2 of least concern. The 8 species added from the TROPICOS database also included a single endemic species (*Elleanthus aristatus*), for a revised total of 52 endemic species, or 15.9%, which is slightly lower than previously reported (see Table 2.2). It should be noted that none of the 48 unidentified species listed for the Reserve are included in this statistic; it is very likely that at least several of these unidentified species will be endemics or even new taxonomic determinations, in which case, the type specimen is by definition endemic to the Reserve. While these cannot be quantified as of yet, when unidentified species are removed from the original and revised lists (leaving 166 and 279 spp., respectively), the rates of endemism

Table 2.2. Endemic species at the Maquipucuna Reserve, Ecuador. The IUCN classification is included where possible, however species listed as endemic in Jorgenson and León-Yañez (1999), but not Endara and Jost (2000), are listed simply as 'Endemic' because IUCN classification is not known.

Species Name	IUCN classification	Species Name	IUCN classification
Chondrorhyncha embreei Dodson & Neudecker	Nearly threatened	*Epidendrum tandapianum* Dodson & Hágsater	Vulnerable
Chondrorhyncha thienii (Dodson) Dodson	Vulnerable	*Erythrodes jamesonii* (Garay) Dodson	Vulnerable
Crossoglossa nanegalensis Dodson	Vulnerable	*Govenia sodiroi* Schltr.	Nearly Threatened
		Lepanthes cassidea Rchb. f.	Nearly Threatened
Dichaea sodiroi Schltr.	Nearly Threatened	*Lepanthes effusa* Schltr.	Nearly Threatened
Dracula dodsonii (Luer) Luer	Vulnerable	*Lepanthes kuijtii* Luer & Hirtz	Vulnerable
Dracula navarroörum Luer & Hirtz	Vulnerable	*Lepanthes magnifica* Luer	Vulnerable
Dracula sodiroi (Schltr.) Luer	Vulnerable	*Lepanthes pecunialis* Luer	Least Concern
Elleanthus petrogeiton Schltr.	Vulnerable	*Lepanthes pelyx* Luer & Hirtz	Vulnerable
Elleanthus vernicosus Garay	Least Concern	*Lepanthes rhodophylla* Schltr.	Vulnerable
Epidendrum aristatum Ackerman & Montalvo	Vulnerable	*Lepanthes zygion* Luer	Vulnerable
Epidendrum brachystele Schltr.	Nearly Threatened	*Lycomormium ecuadorense* Sweet	Vulnerable
Epidendrum caloglossum Schltr.	Vulnerable	*Macroclinium perryi* (Dodson) Dodson	Vulnerable
Epidendrum diothonaeoides Schltr.	Least Concern	*Masdevallia ophioglossa* Rchb. f.	Nearly Threatened
Epidendrum marsupiale F. Lehm. & Kraenzl.	Least Concern	*Masdevallia parvula* Schltr.	Endemic

Table 2.2 (cont'd). Endemic species of the Maquipucuna Reserve.

Species name	IUCN classification	Species name	IUCN classification
Maxillaria nanegalensis Rchb. f.	Vulnerable	*Porroglossum amethystinum* (Rchb. f.) Garay	Vulnerable
Pelexia ecuadorensis Schltr.	Vulnerable		
Pleurothallis crossota Luer & Dalström	Vulnerable	*Scaphosepalum ophidion* Luer	Endemic
		Scelochilus chiribogae Dodson	Vulnerable
Pleurothallis crucifera Luer & Hirtz	Vulnerable		
		Scelochilus heterophyllus Rchb. f.	Vulnerable
Pleurothallis deflexa Luer	Least Concern		
Pleurothallis dibolia Luer	Least Concern	*Scelochilus jamiesonii* Lindl.	Vulnerable
Pleurothallis ensata Luer	Nearly Threatened	*Stelis morganii* Dodson & Garay	Endemic
Pleurothallis epiglottis Luer	Vulnerable		
Pleurothallis erythrium Luer	Vulnerable	*Telipogon steinii* Dodson & R. Escobar	Vulnerable
Pleurothallis gelida Lindl.	Vulnerable		
Pleurothallis lacera Luer	Vulnerable	*Trisetella vittata* (Luer) Luer	Vulnerable
Pleurothallis macra Lindl.	Least Concern	*Zootrophion hirtzii* Luer	Vulnerable
Pleurothallis stevensonii Luer	Vulnerable		

increase to 23.5% and 18.6% respectively. These high rates of endemism are considered normal for orchids, but are lower than Ecuador's overall floral endemism rates (26%) and much lower than Ecuador's rate of orchid endemism (~40%, Endara and Jost 2000).

<u>Discussion: Orchid Diversity and Rarity in an Andean Cloud Forest</u>

In a region as orchid rich as the Maquipucuna Reserve, it is not at all surprising that an informal diversity survey would discover a significant new percentage of the flora. Webster and Rhode (2001) state that their count of 205 orchids species likely represented scarcely half of the overall orchid flora. The Maquipucuna Reserve is one of the better-sampled flora in northwestern
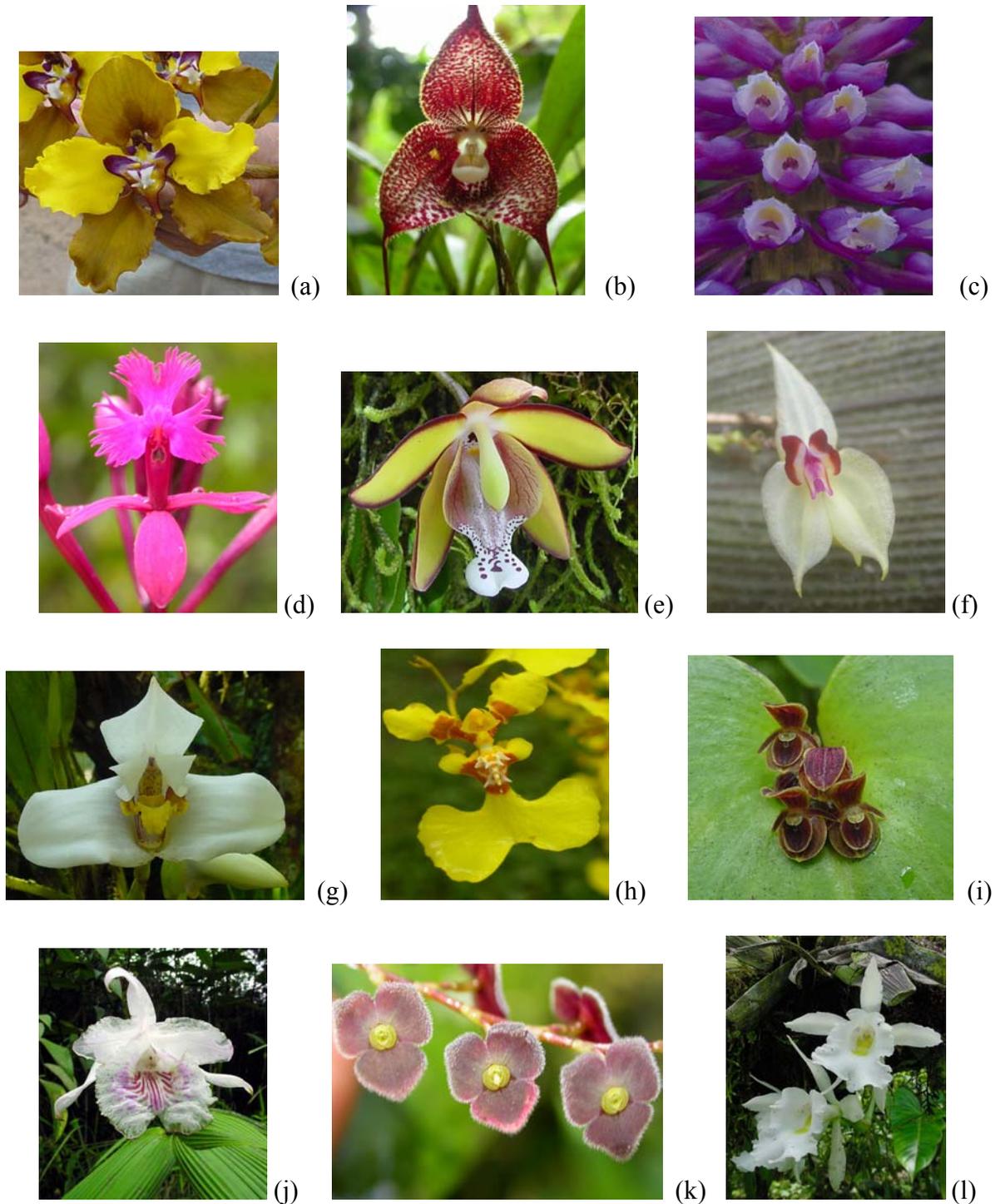
Figure 2.4. Photos of representative orchid genera at Maquipucuna. *Cyrtochilum macranthum* (a); *Dracula wallisii* (b); *Elleanthus robustus* (c); *Epidendrum quitensium* (d); *Eriopsis rutidobulbon* (e); *Lepanthes magnifica* (f); *Maxillaria lehmanni* (g); *Oncidium klotzcheanum* (h); *Pleurothallis crossota* (i); *Sobralia pulcherrima* (j); *Stelis argentata* (k); and, *Trichopilia fragrans* (l). Note the extreme variation based on the basic floral morphology in the family. Photos by the author, not to scale.

Ecuador, but despite this fact, preliminary results of a diversity assessment focused on orchids added 122 species within the study range of Webster and Rhode (2001), a 60% increase in the orchid diversity of the Maquipucuna Reserve. An additional 26 species were added whose range bordered the study area, indicating that these are likely found within range as well (see Appendix I). While it is possible that other species-rich groups at the Reserve, such as the ferns or bromeliads are equally under-represented, these taxa have only increased by 60 (30 %) and 22 (63 %) species, respectively. This orchid survey is the largest species addition within any family to the Maquipucuna checklist since its publication in 2001.

The results mark the maturation of a flora, allowing for a better comparison of the Maquipucuna orchid flora to other cloud forests. The updated species list for Maquipucuna puts in a similar category of species diversity as the Chocó Province (335 species), but still significantly below Monteverde, Costa Rica (393 species), though more species are still being documented in all of these forests (Webster and Rhode 2001). In Ecuador, the most orchid rich locality is Lita, with 306 species in 10 km^2, however, a similar study range at Maquipucuna could have as many species, making it one of the most orchid diverse areas in Ecuador (Dodson and Escobar 1996). Research will continue documenting the diversity of orchid species at the Reserve and surrounding forests, especially in relation to causal factors, such as microclimatic and biogeographic variation by overlaying existing collection data with GIS mapping data.

The large representation by the genera *Pleurothallis*, *Lepanthes*, and *Stelis* is not surprising, since the Pleurothallids combined contain an estimated 1,650 species in Ecuador alone (43% of the total orchid flora, Dodson 2003). The most notable change in the revised list is the addition of many *Stelis* species, whose names were previously unidentified; Webster and Rhode (2001) list only three *Stelis* species, while the new list contains fifteen documented

species, making it one of the top five speciose orchid genera at the reserve. Much taxonomic revision of this genus has occurred since 2001 and the new list accounts for much of this change (Endara, *pers. comm.*). It is especially interesting to note the high degree of endemism within the Pleurothallid genera *Lepanthes*, *Pleurothallis,* and *Stelis*. Half of the species in the genus *Lepanthes* recorded for the Reserve (8 of 16) are considered endemic, and over half of those are classified as vulnerable by the IUCN (5 of 8). Almost one-third of *Pleurothallis* species recorded at Maquipucuna (11 of 36) are endemic, while 7 of these are vulnerable. Meanwhile, as previously discussed, out of the 16 Stelis spp. now included in the list, only *Stelis morganii* is considered endemic and this is not even listed by Endara and Jost because it has since been found in Costa Rica (2000).

Many of these species are well-adapted to the high cloud forests of Maquipucuna and surrounding areas, and are found in low-density populations and limited in range to only those forests. Thus, due to natural range restrictions and population limitations, they are endemic by definition. While protected by the Reserve, they may or may not be immediately threatened due to deforestation; however, these populations (often individual plants) should be monitored in the future to better understand the ecological dynamics of endemism and formulate strategies for the conservation of the species, if necessary. The importance of epiphytic flora, especially orchids, at the Maquipucuna Reserve is significant and should be incorporated into regional conservation strategies and environmental education programs; example of these efforts will be detailed in the concluding remarks of this thesis. The following two chapters will deal with specific results of monitoring experiments of euglossine bee pollinators and an endemic orchid, *Chondroscaphe embreei*, as an example of the often complex relationships between the dynamics affecting diversity and rarity in the Andean cloud forest.

CHAPTER THREE

EUGLOSSINE BEES (APIDAE: EUGLOSSINI) OF THE

MAQUIPUCUNA RESERVE, ECUADOR

Introduction

Euglossine bees, also known as orchid bees, are important orchid pollinators with a widespread distribution throughout the Neotropics (Mitchener 2000). These rapid flying, often brilliantly gold, green, and coppery colored bees are most common in wet lowland tropical forests up to 1500 masl, but are occasionally observed in high mountain passes (2000+ masl) on sunny days (Dodson 1962). Due to their rapid flight and the solitary nature of many species, euglossines are rarely directly observed, except though collection methods; however, in some forests, they may account for up to one-quarter (25%) of the total bee community (Roubik 2004). The males and females have quite distinct life histories, with females primarily collecting nectar and pollen, as well as resins used in constructing brood cells, while males are characterized as 'vagabonds', visiting flowers for nectar and special fragrances, which are used in attracting females for copulation (Dodson et al. 1969) and occasionally sleeping in flowers, thus beginning foraging from a different locality every morning (Dodson 1966). However, it has also been reported that males and females 'trapline' when available nectar sources are scarce. Traplining refers to a foraging behavior where the bee follows a fixed route of highly rewarding floral sources. Thus, site fidelity in euglossines may be dependent on available food or fragrance resources (Janzen 1971, Kroodsma 1975, Ackerman 1982). Both males and females visit a variety of food resources in more than 30 families, including Fabaceae, Bigoniaceae,

and Malphigiaceae, and are often important pollinators. It is believed that euglossines prefer tubular nectar flowers and more often remove pollen from these flowers than from flat, disc-like flowers (Zucchi *et al*. 1969, Williams and Dodson 1972); this is likely due to the abnormally long-tongue that characterize the Euglossini subtribe, which is held flat against the body, not coiled, as in other nectivarous insects. Male euglossines are also known to collect fragrances from Araceae, Solanaceae, and Gesneriaceae, in addition to orchids. Female nesting habits are described in great detail in several sources and will not be discussed here for the sake of brevity (Zucchi *et al*. 1969, Kimsey 1982, Roubik 2004). The complex behavior of euglossine bees, especially the chemical collection of the males, is not yet fully understood, and makes them one of the most evolutionarily advanced groups of invertebrates (Zucchi *et al*. 1969).

The tribe Euglossini consists of 184 known species in five genera (a previous sixth genus *Euplusia* is synonymous with *Eufriesia*, Kimsey 1979): three solitary or social/eusocial genera *Euglossa*, *Eulaema*, and *Eufriesia* and two parasitic genera *Aglae* and *Exaerete* (Williams 1982). *Euglossa* consists of small to medium sized bees (8-18 mm), which are usually brightly metallic colored, and is by far the largest genus, with 103 described species. *Eufriesia* are medium to large (14-26 mm), hairy, and often brightly colored with bluish or green faces. This genus currently has 58 species, but is much more seasonal than *Euglossa* and likely still under represented in collections. *Eulaema*, with 16 species is also hairy, but is usually black without the face colorations and has alternating yellow bands on the abdomen. These bees are some of the largest (20-30 mm) euglossines and are the most common, widespread species of euglossines. *Exaerete* and *Aglae* are both nest parasites of other euglossines. *Exaerete* contains six species, is large (15-28 mm), shiny metallic, usually blue-green in color
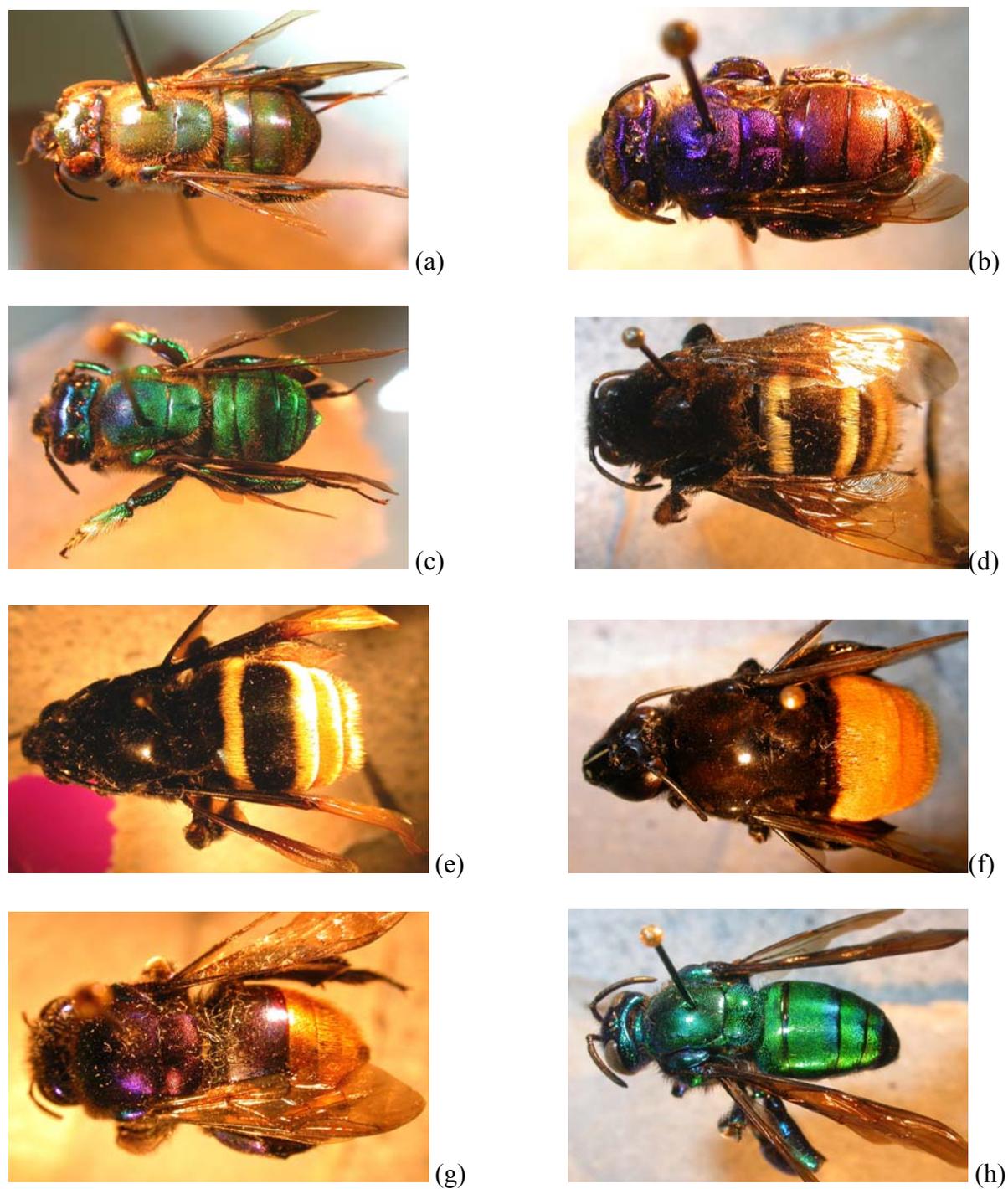
Figure 3.1. Representative euglossines of each genus at the Maquipucuna Reserve. *Euglossa trinotata* (a), *Euglossa mixta* (b), and *Euglossa nigropilosa* (c) are small to medium size (8-18mm) and metallic. *Eulaema boliviensis* (d), *Eulaema bomboides* (e), and *Eulaema polychroma* (f) are large (20-30mm), usually black and hairy. *Eufriesia chrysopyga* (g) is medium to large (14-26mm), hairy and brightly colored. *Exaraete smaragdina* (h) is a parasitic genus, large (15-28mm) and metallic. Photos by the author, not to scale.

and wasplike in form. The monotypic *Aglae caerulea* is also large (23-25 mm), but is darker blue with a flattened body (Dressler 1982, Kimsey and Dressler 1986, Roubik 2004). Lists of known species of euglossines have been provided by Moure (1967), updated by Kimsey and Dressler (1986), and recently by Roubik (2004). A current review of the phylogenetic relationships within the tribe has also been provided by Cameron (2004). It should be noted that euglossine males are generally better known than females because the majority of collections are made using fragrance baits which attract only male bees. Recent advances using artificial nests, have also increased the comparative knowledge of females (Kimsey 1982, Garófalo *et al*. in Cameron 2004).

Euglossine bees are reportedly long-lived, with lifespans ranging from six weeks to three months and three to six months in males and females, respectively (Kimsey 1980, Ackerman and Montalvo 1985). In addition, both males and females are strong flyers and have been reported to fly many kilometers in a single foraging trip at rates of up to 20 km/hr (Janzen 1971) and cover 80-90 km in a week's flight (Williams and Dodson 1972). These characteristics enable euglossines to have large foraging areas (up to 23 km^2 for a female *Eulaema surinamensis*, Janzen 1971) and because of this, they are implicated in long distance gene flow in the orchid species they pollinate (Williams and Dodson 1972). Due to the lack of nesting responsibilities, male euglossines may even have a larger foraging area than female. Janzen (1981) comments that males probably forage for nectar, fragrances, and females in separate habitats, especially in an area with high seasonal variation. This also suggests that male and female habitats only overlap in mating areas and for a short period of time. Because of their importance to orchid pollination and the fact that males and females pollinate different species groups in distinct habitats, euglossine bees have been classified as a keystone hymenopteran; in other words,

declines in euglossine abundance can influence the entire web of unrelated plant groups that they pollinate, including orchids (Gilbert in LaSalle and Gauld 1993).

The use of fragrance baits to attract and collect male euglossines led to an explosion in euglossine bee taxonomy as well as a better understanding of their population dynamics, foraging ecology, and potential coevolutionary relationships with orchids (Dodson *et al*. 1969, Williams and Dodson 1972), while at the same time improved methods of fragrance detection have led to a better understanding of the mechanisms of orchid pollination (Williams and Whitten 1983, Kaiser 1993). The baits consist of known chemical components of the orchid flowers visited by euglossines, such as cineole, eugenol, or methyl salicylate, as well as other sources, such as skatole, which is collected from rotting wood and decaying organic matter, not orchid flowers (Dodson *et al*. 1969, Ackerman 1983b; see Figure 3.2). Some bee species visit only specific chemical baits, while others are more generalist (Ackerman 1989), but males of most known euglossine species have been attracted to baits, making this an effective method of assessing the local populations of bees (Roubik and Ackerman 1987, Roubik 2001). However, local heterogeneity of food and fragrance resources and resulting small-scale population structure of male euglossines can result in sampling error (Armbruster 1993). Seasonal variation in foraging behavior may also be important. Many euglossine species show peak abundance during early or mid to late-wet season (Ackerman 1983b, Roubik and Ackerman 1987) and a smaller peak during the middle dry season (Pearson and Dressler 1985). It has been reported that population densities are lower in open pasture areas and forest fragments, due to elevated temperature and lower humidity (Janzen *et al*. 1982, Powell and Powell 1987). However, though it has more recently been demonstrated that recent deforestation may adversely affect euglossine

Figure 3.2. *Euglossa trinotata* at dimethoxybenzene bait. Euglossines use their front tarsal brushes to collect the bait and then transfer it to their hind tibial organ. This individual is collecting fragrance that dripped off of the bait (upper-right corner) and onto the moss below.

abundance, long-standing forest fragments have stable populations and there is no significant difference between euglossine abundance in forest fragments and intact forest (Becker *et al.* 1991, Tonhasca *et al*. 2002a, 2002b). Furthermore, fragmentation does not significantly effect euglossine dispersal between patches in a disturbed forest, even when separated by a kilometer or more of deforested area (Tonhasca *et al*. 2003). In fact, despite within and between habitat variation and long-term temporal changes in species abundance, euglossine bees appear to be one of the most stable insect populations in the Neotropics (Roubik and Ackerman 1987, Roubik 1989). Furthermore, Roubik reports that in 21 years of continuous baiting in Panama, there was no aggregate change in species abundance or species richness, and biodiversity increased slightly (2001).

Euglossine Pollination of Orchids

Euglossine bees are the primary pollinator of an estimated 10% of all orchids, a significant proportion, considering that they are limited to the Neotropics in distribution (Pilj and Dodson 1966). More detailed studies show that approximately 650 species particularly rely on a

unique behavior of the male euglossines, the collection of aromatic substances, and offer no nectar or pollen reward in their pollination syndrome (Williams 1982, Roubik 2004). The intricate relationship between male euglossine bees and orchid flowers was first described by Cruger in 1865 and later publicized by Darwin in his classic book on orchid pollination (Dressler 1968). Cruger's observations led to the erroneous belief that euglossines 'gnaw' on orchid flowers for almost an entire century. In the 1950's, Vogel contributed several hypotheses that were later disproven, including pseudocopulation and mimicry of female brood cells by orchid flowers, but it was not until 1966 that he published a hypothesis that bees were possibly collecting pheromones to be later modified or emitted (Zucchi *et al*. 1969). Meanwhile, Dodson and Frymire reported that euglossines scratching the surfaces of fragrant orchids, such as *Stanhopea*, *Gongora*, and *Catasetum*, became intoxicated and as a result were easily manipulated in the complex pollination mechanisms in these flowers in a way that a 'sober' bee would easily avoid (Dodson 1962, Williams 1982). Dodson *et al*. (1969) later relegated this 'intoxicated' behavior to a response by the bees of both sexes to collecting fragrances and resins, where the bee became much less wary and was easily captured. It is now clear that euglossines collect volatile chemicals from osmophores of flowers of certain orchid groups and that the flower is usually pollinated in the process, sometimes through highly modified systems of pollinaria delivery, such as forcible ejection in *Catasetum* or a 'slip-and-slide' mechanism in *Stanhopea* and *Gongora* (Pilj and Dodson 1966). The euglossine pollination syndrome has been detailed in many studies and reviews (Williams 1982, Dressler 1982, Roubik 1989, 2004) and will be only briefly discussed here because of its general importance to Neotropical orchids and to contrast it with the deceit pollination using a euglossine bee of *Chondroscaphe embreei*. It should also be
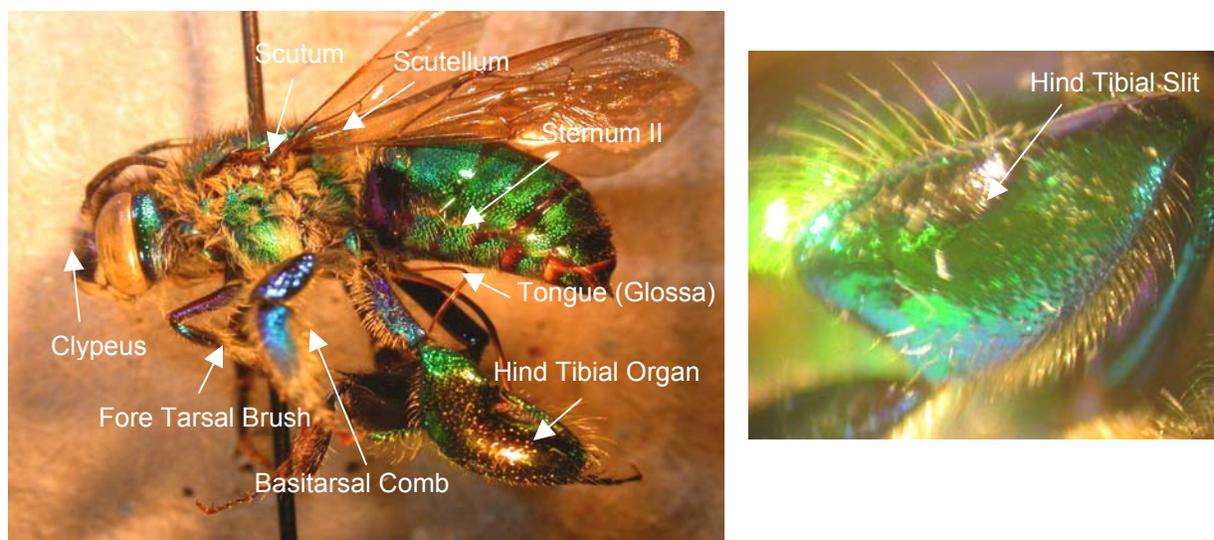
Figure 3.3. General euglossine anatomy. a) Photo of *Euglossa* sp nov. 1 (Glossurella subgroup), showing key taxonomic features and b) a close-up photo of the hind tibial organ of *Euglossa trinotata*. Euglossines collect volatile chemicals using their fore tarsal brush and then transfer it using the basitarsal comb into the hind tibial organ.

noted that females do pollinate some nectar producing orchids, such as *Sobralia*, but are not included in the syndrome described below (Dressler 1981).

The male bees collect fragrances using their tarsal brushes and store them in a specially adapted inflated pouch on the hind tibia, called the hind tibial organ, which is characteristic in all euglossines (Figure 3.3; Zucchi *et al*. 1969, Kimsey 1982, Roubik 1989). Gas chromatography analysis of chemicals from this organ shows that these chemicals are not metabolized in the HTO and age correlated accumulation of fragrance in this organ indicate that they are stored and do not volatilize (Eltz *et al*. 2003). Euglossines have also been observed collecting toxic chemicals, such as DDT, which supports the idea that chemicals are stored and not metabolized (Roubik 2004). The function of the fragrances collected is not clear, though it is believed that they serve in attracting females and increasing copulatory success (Eltz 1999). It was also suggested that these fragrances influenced male vitality and were required nutritionally, since individuals

deprived of them did not survive long in captivity (Bennett 1965, Dodson 1966), however this idea has been disproven by later experiments rearing euglossines in captivity, which can survive several months without fragrances (Ackerman and Montalvo 1985). Other plausible hypotheses for fragrance collection have been presented including: 1) long-range attraction of females for mating (Vogel 1966, Williams and Whitten (1983); 2) chemical stimulus for male congregation and 'lek' formation (Dodson *et al*. 1969); and 3) accumulation of a qualitatively significant chemical bouquet, which affects female choice (Whitten *et al*. 1989, Roubik 1989). The first hypothesis is weak, considering that females are not attracted to these fragrances and no evidence for metabolism of the fragrances into a female attractant exists (Ackerman 1989, Roubik 1989). Furthermore, while it is believed that males mark their territories through mandibular secretions, and it has been observed that females are attracted to excised heads of male euglossines, more recent evidence shows that cephalic lipids from labial glands in males simply act as nonpolar solvents, increasing fragrance collection efficiency (Willliams 1982, Whitten *et al*. 1989, Roubik 1998). In contrast quite sufficient evidence exists that male euglossines form 'leks', defined as an aggregation of individuals, each defending territorial sites that are used for mating and not feeding, that allows for female choice of mate (Kimsey 1980). Because females are not attracted to the fragrance itself, they may respond to visual or auditory signals resulting from the congregation of excited males (Peruquetti 2000). Other males are attracted to the fragrances collected by conspecific males, as evidenced by necrophagy of dead euglossine males (Dodson 1975, Roubik 1998), suggesting that accumulation of fragrances may also be species specific. Peruquetti reported male lek formation resulting from one male secreting substances on a guava limb near a bait, which attracted two females (2000); however, males generally often react aggressively to each other, especially in mating territories, thus leks

may be facultative in certain species depending on lack of available territorial sites in an area (Kimsey 1980). Schemske and Lande (1984) demonstrated that cineole collection increased territorial display in *Euglossa imperialis*, though no functional mechanism for this behavioral response was found. Thus, while chemicals may affect a response in conspecific male euglossines, true lek formation is still debated, primarily because it is still unknown how the fragrance collection behavior and the quantitative and qualitative composition of its bouquet affects female choice. The third hypothesis is increasingly popular, but also suffers from a lack of direct evidence. Eltz *et al*. (2003) detailed a flight cage experiment with *Euglossa hemichlora* and concluded that male display rate positively influenced copulation success, but that quantity or quality of chemical composition had no significant effect. These results may have been anomalous however based on abnormally homogenous fragrance quantity in the males observed and a low number of observed copulations. Furthermore, the extreme difficulty of observing euglossine copulations in the wild has left this issue unresolved. In conclusion, euglossine bees may form leks and female choice may be an important factor in copulatory success, but the extent of the role of collected fragrances in male aggregation or any individual's mating success is still unknown.

As already mentioned, due to the chemical specificity of some euglossine bees, this pollination syndrome is thought to promote sympatric speciation of some orchid species through variation in chemical composition of their fragrance bouquet (Dodson *et al*. 1969). Data from chemical baiting supports this idea, since mixtures of fragrances are much more selective than single compounds and a single admixture can result in a different visitor (Hills *et al*. 1968, Williams and Dodson 1972) and recent studies using gas chromatography (GC) with electro-antennographic detection (EAD) show that certain chemical compounds may actually deter floral
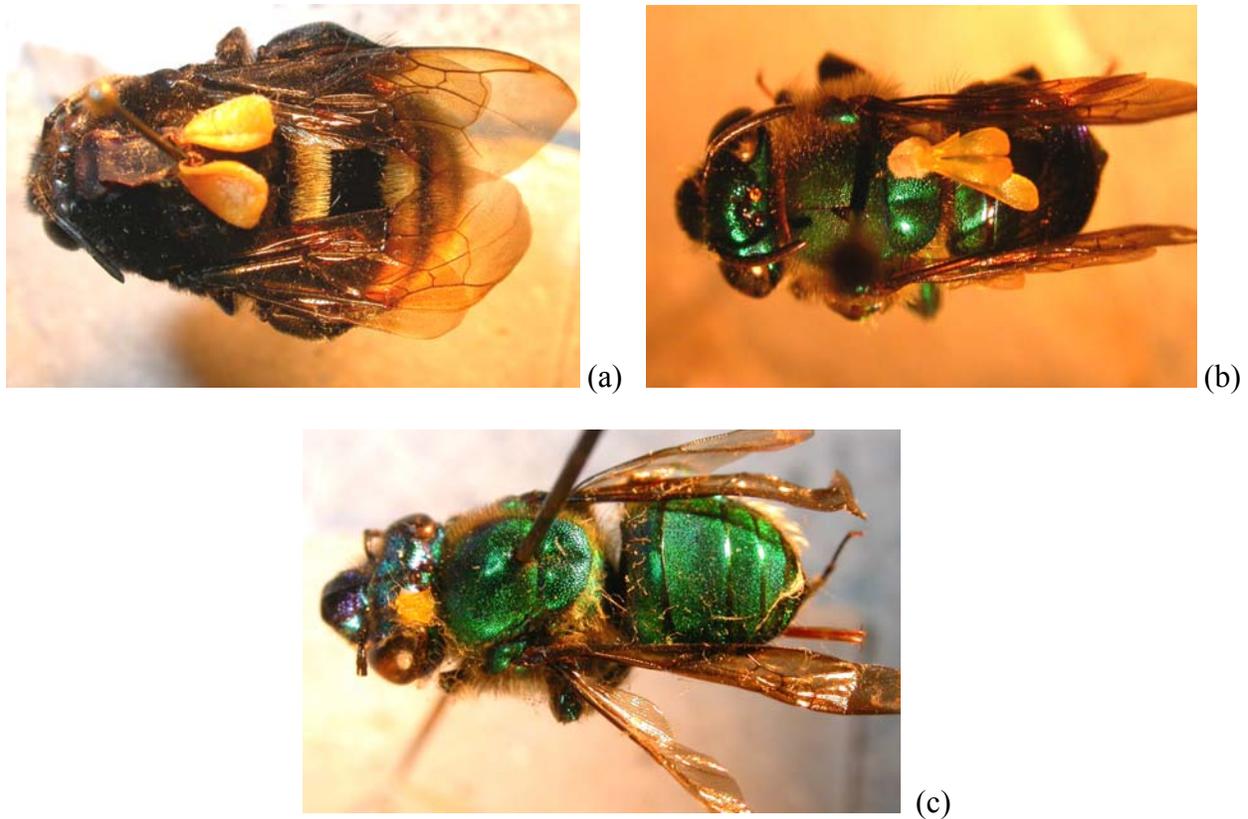
Figure 3.4. Pollinaria placement on several euglossines. Different orchids place their pollinaria on separate parts of the bees: a) *Catasetum* sp. on the scutellum of *Eulaema cingulata*, b) *Chondroscaphe embreei* on the right metasoma of *Euglossa trinotata*, and c) remnants of an unidentified Zygopetalinae pollinarium on the head of *Euglossa* sp nov. 2 (Glossurella subgroup).

visitors, while others attract them (Schiestl and Roubik 2003). Even when chemical specificity is not strong enough to provide a barrier, sympatric orchid species often share euglossine pollinator through mechanical isolation, such as species-specific pollinarium placement on different parts of the bee (see Figure 3.4; Pilj and Dodson 1966, Dressler 1981). This is an important adaptation, because long term data shows that an estimated 90% of euglossines use multiple pollinators and up to half use at least two genera (Roubik 2004). However, despite this abundance of fragrance data affecting pollinator selection, it does not appear to be a reliable indicator of orchid evolutionary history (Williams and Whitten 1999). Most convincingly, recent data from

molecular phylogeny in *Stanhopea* refutes the strict coevolution of orchid species and euglossines. Bootstrap values of molecular phylogeny were reduced when combined with those of fragrance-based clades. Thus, while analysis of fragrance composition in orchid species is an important tool for orchid pollinator ecology and variation may affect orchid speciation, it should not be interpreted as a coevolutionary pressure and does not result in a reciprocal euglossine speciation (Janzen 1980, Roubik and Ackerman 1987).

The preceding discussion of euglossine biology and its effects on orchid pollination and evolution emphasizes the importance of understanding diversity and stability of pollination systems in the context of orchid diversity and rarity. These orchids are dependent on stable euglossine populations for the long distance gene flow between populations and usually have higher pollination success per flower produced than other pollination syndromes because of the strong instinct of male euglossines to collect fragrances (Dodson *et al*. 1969, Ackerman 1983b). However, this description of euglossine pollination does not apply to all flowers pollinated by euglossine bees. After observing the pollination of *Cochleanthes lipscombiae* by a euglossine bee, Ackerman (1983b) hypothesized many gullet-shaped flowers of orchids in the subtribe Zygopetalinae were either non-model mimics of nectar flowers or direct mimics of sympatric nectarifarous Leguminosae. This system will be further discussed in Chapter 4 because *Chondroscaphe embreei* is in the same subtribe and closely resembles the deceit syndrome described. Thus, in order to better understand the effects of pollination syndrome on this endemic orchid, the local euglossine bee populations at the Maquipucuna Reserve were surveyed for population dynamics, including variation in abundance and interspecific composition across seasons.

Chemical Baiting Methods

The chemical baiting occurred during three four-day sessions (15 hrs total each) at different times of year -early dry season (June 25-28, 2002), early rainy season (February 18-21, 2003), and late rainy season (May 12-15, 2003) - to assess the affects of interseasonal variation on euglossine abundance and species composition. The methodology closely follows that of Dodson et al. (1969) and Ackerman (1986). Six chemicals – 1,8 cineole (C), eugenol (E), methyl salicylate (S), methyl transcinnamate (M), benzyl acetate (B), and 1,4 dimethyoxybenzene (D) - known to be strong euglossine attractants were pipetted onto 2.5" x 2.5" squares of herbarium blotter paper, which were placed on tree trunks approximately 2 m from ground level and 10 m apart from one another. Two chemicals, methyl transcinnamate and dimethoxybenzene were crystalline and were dissolved in 95% ethanol before application to the blotter paper. Cineole, methyl transcinnamate, and dimethoxybenzene baits were replenished halfway through each 4-hr period due to volatilization. Baits were monitored by walking the transect approximately every 10 minutes between 8:30 am and 12:30 pm each day of the survey. This four hour period was when first light cleared the ridge and usually before afternoon rains regardless of season. Baiting only occurred on sunny or partly cloudy days, since euglossines are not active in rainy or cloudy conditions and baiting had to be stopped early twice due to rains, resulting in 15 hrs/survey, instead of the expected 16 hrs.

Baits were placed along the southern side of the swamp along the Humedal trail at the Maquipucuna Reserve (see site description in Chapter 2), at approximately 1250 masl. The baits were generally linear in arrangement along the trail and overlapped the area of the Humedal subpopulation of *C. embreei* (as described in Chapter 4). This part of the forest is a heterogeneous mixture of regenerating and secondary forests due to natural disturbance and the

presence of the swamp nearby, which creates a significant light gap. A large treefall occurred adjacent to the baiting transect in November 2003, after the baiting trials were completed, but reopened a gap that had been created by an earlier medium size treefall previous to baiting.

Bees visiting the baits were captured when possible, identified, and released; however, taxonomically new individuals, pollinaria carriers, and bees that were difficult to identify in the field were placed in a killing jar and kept as specimens. Field notes, including bee species, attracting bait, time of visit, and general comments, were kept for each survey period, in order to determine species diversity and abundance, as well as chemical specificity for species in each of the three seasons. A small-scale mark-recapture study was performed over the last three survey dates in June 2002 to determine site fidelity of baited bees. This was done by capturing bees and placing a large white mark on their scutal patch between the wings; once the mark dried, the bee was released in the same area and marked when reobserved. The mark was visible from a distance and remained so, even when faint, as when the individual obviously had attempted to groom itself clean. All bee specimens and attached pollinaria were identified by Dr. Robert Dressler at the University of Florida Museum of Natural History. Type specimens of the new species were also deposited in this collection (FLMNH).

Results of Euglossine Bee Surveys

A total of 373 bees were observed over 45 hours of chemical baiting, of which 23 specimens were kept, representing 11 species in 3 genera, two of which are undescribed. Table 3.1 lists the euglossine species from the Maquipucuna Reserve, their attracting chemical, and any pollinaria identified from individuals. This list includes all bees caught in the three study periods, as well as additional specimens caught in a separate baiting period using different baits (January 29-30 2004) performed by the author and Dr. Gunter Gerlach and bees captured by Dr. Robert

Table 3.1. Results of euglossine bee surveys at Maquipucuna Reserve. A total of 271 visits to baits were observed, with 14 species in four genera. Total number of individuals per season are listed in this order: early rainy (Feb 2003), late rainy (May 2003), and then dry season (June 2002). Attractive baits are abbreviated as in text. Bees visiting another source (O) are discussed in the text. *Eulaema polychroma* visited beta-ionone (I) in a separate baiting trial; *Euglossa* sp. nov. 1 was caught by R. Raguso in 1991; *Exaraete smaragdina* was caught in preliminary trials at the same site. Six individuals of four species were caught with orchid pollinaria attached and are listed also.

Euglossine spp.	# of Inds – total	Attractive baits	Pollinaria attached
Euglossa deceptrix	12	C, M, E	
Euglossa ignita	23	C, D, M, B, S, O	
Euglossa mixta	2	M, S	
Euglossa nigropilosa	47	B, C, M, E	unidentified Zygopetalinae (1)
Euglossa trinotata	162	C, B, D	*Chondroscaphe embreei* (3)
Euglossa sp. nov. 1 (Glossurella)	14 - 30 Nov. and 2,4,5 Dec (early rainy season)	C, M	
Euglossa sp. nov. 2 (Glossurella)	6	C	unidentified Zygopetalinae (1)
Eulaema boliviensis	107	E, B, C, D, O	
Eulaema bomboides	1	B	
Eulaema cingulata	5	M	*Catasetum* sp. (1)
Eulaema polychroma	1 – 5 Feb (early rainy season)	I	
Eufriesia chrysopyga	1	C	*Stanhopea impressa* (1)
Eufriesia sp nov.	9	M, B, D, O	
Exaerete smaragdina	2 - 16.June (early dry season)	S, O	

Raguso in a previous baiting experiment performed in 1991, which are reported here for the first time. These other reports account for an additional two species of euglossines at Maquipucuna, including an additional undescribed species in the Glossurella subgroup of Euglossa. A species in a fourth genus, *Exaerete smaragdina* was also collected by the author in preliminary baiting trials on June 16 at methyl salicylate and is also included in Table 3.1.

An average of 8.35 bees/hr were seen across all seasons combined, within the range of 4/hr to 10.67/hr. Bees were most frequently observed in the early rainy season (140), despite the abnormally high abundance of *Euglossa trinotata* in the late rainy season, and least frequently in the early dry season (109). There was some interspecific seasonal variation, though it was more notable in some species than others (see Figure 3.5, Table 3.2). Nine species were attracted to the baits in the early rainy season, eight in the late rainy season, and seven in the dry season. *Eulaema boliviensis* was most abundant in both the early rainy season and dry season, with 40% and 32% in each, respectively, but *Euglossa trinotata* accounted for 75% of total individuals in the late rainy season. In general, most other euglossines decline in abundance during the late rainy. season. Only *Euglossa deceptrix* and *Euglossa mixta* were more common in the dry than in both rainy season surveys. Intergeneric variation was also significant. *Eufriesia* and *Eulaema* preferred the early rainy season; both *Eufriesia* were absent and only 17 *Eulaema* individuals (15 *Eulaema boliviensis*) were observed in the late rainy season.

All of the fragrance baits attracted at least one bee, though some were much more attractive than others. Cineole and dimethoxybenzene attracted at least 75% of all observed bees in all three baiting surveys, while methyl salicylate and eugenol only occasionally attracted euglossines (see Table 3.2). Certain species were attracted to specific baits, though most (except the most rarely observed) were seen at multiple baits. Six species are recorded from only one

Seasonal changes in euglossine diversity
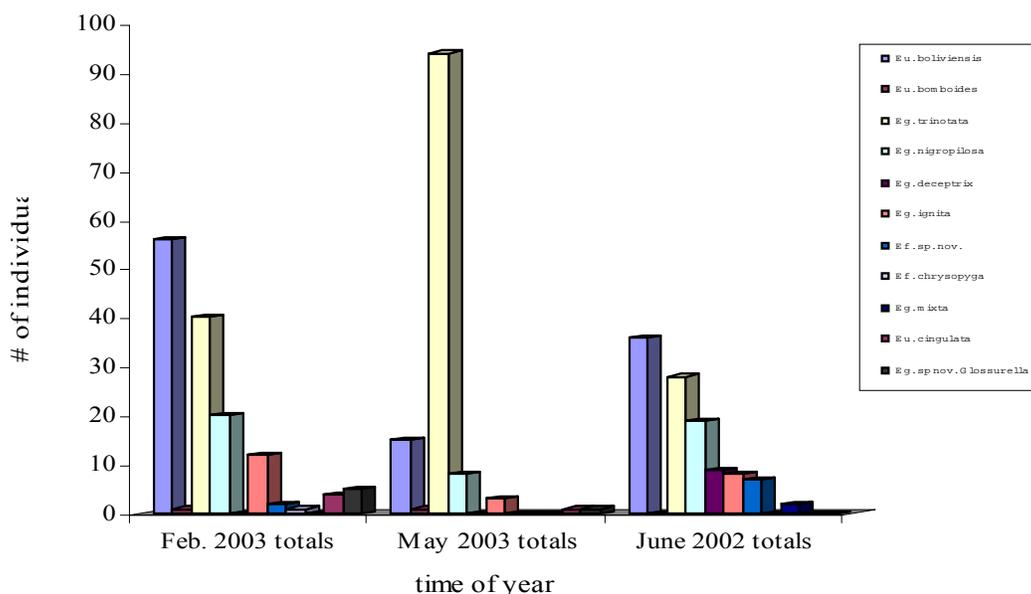
Figure 3.5. Seasonal changes in euglossine diversity. Both species abundance and diversity are highest in the early rainy season. Abundance of *Eulaema boliviensis* is notably less in the late rainy season (May 2003), while *Euglossa trinotata* abundance is much higher than either other season. *Euglossa nigropilosa* and *Euglossa ignita* are the only other significantly abundant species across all three seasons.

bait, but all of these were only observed five times or less overall (Table 3.1). Notably, this includes three of the four *Eulaema* species (all except *Eulaema boliviensis*), *Exaerete smaragdina*, *Eufriesia chrysopyga*, and one of the unidentified *Euglossa*s. *Euglossa ignita* was the least fragrance specific, appearing at five of the six baits, while *Eulaema nigropilosa* and *Eulaema boliviensis* visited four of them. It can also generally be seen that *Euglossa* preferred cineole while *Eulaema* visited other baits, such as benzyl acetate and dimethoxybenzene. Of all the *Euglossa*s, only *Euglossa trinotata* visited dimethoxybenzene frequently, with 78 of 162 recorded visits (48%), apparently switching preference from cineole during the rainy season, since none of these visits occurred in the dry season. In fact, cineole was visited more by most

55

Table 3.2. Interseasonal euglossine bee abundance at specific chemical baits. Only the 11 species attracted during the baiting experiment are included. The seasons are abbreviated early rainy (e), late rainy (l), and dry (d). A total of four observations were observed not at baits, as indicated by an asterisk. The majority of observed bees in any season were attracted to cineole or dimethoxybenzene, primarily due to the preferences of *Euglossa trinotata* and *Eulaema boliviensis*.

Euglossine sp.	C			E			S			M			B			D			Totals		
	e	l	d	e	l	d	e	l	d	e	l	d	e	l	d	e	l	d	e	l	d
Eulaema cingulata	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	4	1	0
Eulaema bomboides	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Eulaema boliviensis	0	0	4	1	0	0	1	0	0	0	0	0	7	1	8	46	14	24	55	15	36*
Eufriesia chrysopyga	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Eufriesia sp nov.	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	3	1	0	6*
Euglossa deceptrix	0	0	5	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	9
Euglossa ignita	4	0	5	0	0	1	3	0	0	2	3	2	2	0	0	1	0	0	12	3	8*
Euglossa mixta	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	2
Euglossa nigropilosa	19	5	14	1	1	2	0	0	0	0	1	0	0	0	3	0	1	0	20	8	19
Euglossa trinotata	13	40	28	0	0	0	0	0	0	0	0	0	3	0	0	24	54	0	40	94	28
Euglossa sp. nov. 2 (Glossurella)	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0
Totals	42	46	56	2	2	3	4	0	1	5	5	10	14	3	11	71	67	27		369	

species in the dry season. In contrast, only 2 of the recorded 107 observations (2%) of *Eulaema boliviensis* occurred at cineole and this was the only *Eulaema* observed at cineole. As expected, there was little interseasonal variation in bait attractivity, with the notable exception of dimethoxybenzene, which attracted 138 individuals combined during the rainy season (71 early

and 67 late) and 27 during the dry season, only 16% of the total seen at this bait. It should be noted that despite the overlap of baiting area with the Humedal subpopulation of the monitoring of *C. embreei*, only one bee, *Euglossa trinotata*, was observed probing visiting a flower, at 12:20 pm in a preliminary baiting session at the Humedal site on June 16, 2002. Unfortunately, the bee was seen leaving the flower and was not collected, so no further details are available. Another *Euglossa trinotata* was observed probing a *Kohleria* sp. (Gesneriaceae) in flower in close proximity to the dimethoxybenzene bait on May 13, 2003 at 10:00 am, but did not appear to pollinate it. Three species, in a total of four observations, visited the author's backpack, which had a mixture of fragrances inside; these are counted in the total observations because they involved fragrance attraction, but are not included in Table 3.2 because they were not attracted to a specific individual chemical bait.

The mark-recapture experiment took place over the first three days of the June 2002 baiting period. Seven bees were marked June 25, 15 on the 26th with one marked bee observed, and 8 on the 27th with 2 marked bees observed, and a fourth marked bee was observed the final baiting day. Only 4 of 30 marked bees, or 13%, were observed again, relatively low site fidelity. Applying this data to a simple population equation: N/M=n/R, or N=nM/R, where N is the total population in the area, M is the initial number of individuals marked, n equals the number of individuals recaptured and R represents the number of marked recaptures, results in population estimates of 128 and 72 male euglossines locally in the area for each of the 4-hour baiting periods (Southwood 1978). No mark-recapture was performed during the rainy season, so for this study, it is assumed this rate of site fidelity is not seasonally dependent. On these two days, 32 and 26 bees were observed at the baits, an efficiency of 25% and 36%, respectively, based on the calculated euglossine populations. Moreover, assuming that 13% of bees observed were the

same individual, the number of individuals observed would be reduced to 325 total over the three

baiting periods – 122, 108, and 95 for early and late rainy season and dry season, respectively.

In the author's opinion, this is a reasonable set of estimates for each season's local euglossine

population and is not significantly different from the calculated dry season population.

<u>Discussion: Population Dynamics and Orchid Diversity</u>

The objective of monitoring the population dynamics of the euglossine bees at the

Maquipucuna Reserve was to determine the role of pollinator limitation in the euglossine

pollinated orchids of the Reserve. Though euglossines are often found as transients as high as

2000 msl, most are found below 1000 msl and since most euglossine pollinated orchids are found

at lower elevations (>1000 msl), it could not be assumed a priori that euglossines are found in

abundance at 1250 msl, where the study site is located (Roubik 2004). Indeed, out of the 322

documented species at Maquipucuna, only a few, such as *Stanhopea impressa*, *Lycomormium*

ecuadorense, *Anguloa virginalis*, and *Dressleria fragrans*, are fragrant and fit the euglossine

pollination syndrome, though several others are euglossine pollinated. The 373 observations in

45 hours of baiting in this study represents the euglossine abundance of a lower montane forest

and is much lower than most euglossine studies, which often collect by the tens of thousands

(Dodson *et al*. 1969, Ackerman 1986, Roubik 2001). For example, Janzen (1981) performed a

mark-recapture experiment on 407 *Eulaema polychroma* males visiting one *Catasetum*

maculatum plant at Santa Rosa National Park in Costa Rica and estimated a local population

between 931 and 4208 of that one species. However, it is common for orchid pollinators to occur

in low frequencies (Montalvo and Ackerman 1987) and some orchids may be adapted to this

pollinator limitation (Ackerman and Montalvo 1990). Despite this fact, in most fragrant

euglossine pollinated species at Maquipucuna, fruit set is quite high for Neotropical orchids

(author, *pers. obs*.). This is likely due to the innate ability of euglossine pollinators to locate chemical resources even in a spatio-temporal heterogeneous environment, such as the forest canopy (Folsom 1994). For the purpose of this study, however, overall low euglossine abundance is important, as are comparative rates of success for other euglossine pollinated orchids to demonstrate that the deceit pollination mechanism of *Chondroscaphe embreei* plays an important role in limiting its fruit set. This will be discussed in further detail in the following chapter.

Interseasonal variation of pollinator abundance likely plays an important role in the phenology of euglossine pollinated orchids and may provide temporal isolation for sympatric conspecifics. The author has observed that the majority of orchids at Maquipucuna flower in the early rainy season and again in the late rainy season. This is likely related to the abundance of resources available at this time of year, which would also influence euglossine foraging success. Euglossine abundance was higher during the early rainy season, which coincides with flowers of *Stanhopea*, *Lycomormium*, and *Dressleria*, but 'pollinator tracking' may be a reflection of a more generalized trend of resource availability for both plant and pollinator (Zimmerman *et al*. 1989). Many species, such as *Dichaea potamophila* in Colombia, flower year-round, as well, either in a 'steady state' flowering strategy that can increase chances of visitation through constant pollinator reward (Folsom 1994), or a deceit strategy that opportunistically invites naïve bees or experienced bees that switch resource base when food availability is low, such as *Cochleanthes limpscombiae* (Ackerman 1983b). Pollination success and fruit set in these flowers depends on the presence and quality of the reward, but is often quite low (Ackerman and Montalvo 1990). The fact that baiting occurred in only one location in all three seasons might account for some of the variation due to site specificity; Armbruster (1983) showed that simultaneous multiple baiting provides a better representation of euglossine species composition

in an area. It is also significant to note that chemical specificity appears to influence interseasonal variation of euglossine abundance. *Euglossa trinotata* was seen more frequently at dimethoxybenzene in the rainy season, while it clearly preferred cineole in the dry season. This may reflect seasonal pollinator switching of fragrance resource base depending on availability, but this is only speculative. Another baiting period should have been performed in the late dry season, but was not logistically possible. Finally, though not measured in this study, it has been reported that age structure can significantly affect chemical specificity, and these temporal changes in age structure may account for some interseasonal variation (Ackerman 1989, Zimmerman and Madriñan 1988)

It is uncertain how interspecific competition affects the dynamics of euglossine populations because aside from nesting and territorial defense, which usually occurs between conspecifics (Zucchi *et al*. 1969, Kimsey 1980), few observations have been published. Out of the 14 species observed at Maquipucuna, there are five species that dominate, with *Euglossa trinotata* and *Eulaema boliviensis* representing 72% of the total bees observed. The large increase in *Euglossa trinotata* during the late rainy season may correlate to the decline of other species, or may represent repeated observations of the same individuals, since no marking occurred in this survey; regardless, it was the dominate species in the late-rainy season survey. *Eulaema boliviensis* abundance declined dramatically in the late rainy season, a trend observed in most male euglossines (Roubik and Ackerman 1987), though again, this is probably a reflection of forest resource availability, instead of interspecific competition or floral tracking. As shown by the mark-recapture, site fidelity is low, at least in the dry season. Certainly, the transient nature of the males affects interspecific competition by maintaining low densities in any localized area and preventing overlapping use of food resources. It has been suggested that

necrophagy of males is evidence for intraspecific competition for chemical fragrances (Roubik 1998); however, this could also simply reflect the opportunistic nature of male euglossine fragrance collection, since they also collect from non-floral sources such as decaying wood. The results of the mark-recapture generally support the 'vagabond' hypothesis for male euglossine behavior, and further emphasize that long-range pollen flow is an important consequence of euglossine pollination.

All of the chemicals used were all well documented euglossine attractants (Dressler 1982, Ackerman 1989, Roubik 2004). Cineole is produced in the fragrance mixtures of over 60% of all euglossine pollinated orchids and attracts approximately 70% of euglossine males, and is by far the most universal attractant in chemical baiting (Dodson *et al*. 1969). Morever, Dodson et al. show that in western Ecuador at a lower elevation than Maquipucuna, 121 individuals were observed in 13 species (avg 30/day), of which 117 were attracted to cineole. These numbers are remarkably close to this study (371 individuals in 14 species, avg 33.4/day), but do not account for the high attractivity of dimethoxybenzene to *Eulaema boliviensis* and *Euglossa trinotata*. The percentages of euglossines observed at each bait were generally within the expected range; for example, benzyl acetate, which is produced by approximately 25% of euglossine pollinated orchids, attracts about 10% of individuals, very similar to this study (Dodson *et al.* 1969). Cineole attracted much less overall (38%) than most reports, though Janzen *et al.* (1982) found that cineole was less attractive at higher elevations in Costa Rica (still only 300 msl). However, Roubik (2004) reports that euglossine abundance peaks at approximately 800 msl in Costa Rica and Panama, so this might not be a general trend. Dimethoxybenzene attracted more individuals than cineole during the rainy season and is an under-utilized chemical bait. The addition of this

chemical to future baiting programs is highly recommended to better understand its potential as a euglossine attractant in the Andean region.

Dressler (1976) notes that fragrance baiting of euglossine bees is a useful way of studying orchid pollination because bees carrying orchid pollinaria are most certainly pollinators of whatever species they are carrying. Several euglossine census studies have included pollinaria data (Roubik and Ackerman 1987) and both Williams (1982) and Roubik (2004) give summaries of euglossine pollinators of orchids identified using this method. The morphological specificity of many pollinaria characteristics allows them to be identified to genus and often species, which makes studying orchid pollination possible even without orchids. The pollinaria carriers caught in this study and the species they pollinate are listed in Table 3.1 and photographs of several are included in Figure 3.4. *Eufriesia chrysopyga* was caught carrying *Stanhopea impressa* and *Euglossa nigropilosa* and an unidentified *Euglossa* were carrying an unidentified pollinaria in the Zygopetalinae subgroup. Also, *Eulaema cingulata* was caught carrying a *Catasetum* sp. pollinaria. This pollinaria is unique and easily recognized, but interestingly, no species in this genus are known from the Reserve. Either *Catasetum* does occur at Maquipucuna and has not been seen yet, or this *Eulaema* carried its pollinaria a long distance (at least 10 km) before being observed at the bait; since *Catasetum* is quite distinct vegetatively, and euglossines are known to fly long distances, the second explanation is likely and provides strong evidence for the role of euglossines in long-distance gene flow in orchids (Williams and Dodson 1972). Finally, and most directly significant to this study, three *Euglossa trinotata* were caught with *Chondroscaphe embreei* pollinaria. This is the first recorded evidence of this orchid's pollinator, and confirms that it is pollinated by a euglossine bee, supporting the hypothesis that gullet-shaped flowers in the Zygopetalinae are pollinated by deceit, attracting unsuspecting euglossines by advertising a

food reward using false nectaries (Ackerman 1983b). This hypothesis and general significance of deceit pollination in orchids will be discussed in more detail in the following chapter.

In conclusion, the dynamics of euglossine bees are potentially important to the pollination success and fruit set of many orchids, whether nectar producing, deceit pollinated, or fragrance producing, as demonstrated in the euglossine syndrome. Despite its high elevation and general lack of euglossine pollinated orchids, there exists sufficient abundance of euglossine populations at Maquipucuna to affect pollination in orchids, though many of these are likely pollinator limited. Males attracted to chemical baits may be transients or locally foraging, though site fidelity is low in the dry season. Interseasonal and interspecific variation is significant and is likely influenced by available food resources, but in turn, influences orchid phenology and pollination. Pollinator dynamics is an important factor in the stability of plant populations (Buchmann and Nabhan 1996, Kearns *et al*. 1998) and especially in the context of orchids, euglossine bees are a keystone pollinator, with potential cascade effects on a variety of plants, from sympatric orchids pollinated by males and females to other food resources also pollinated by them. Furthermore, the variety of pollinator niches filled by euglossines makes them an integral part of orchid conservation strategies in the Neotropics.

CHAPTER FOUR

DECEIT POLLINATION AND FRUIT SET IN *CHONDROSCAPHE EMBREEI*

Deceit Pollination and Its Significance to Orchids

An estimated one-third of, or up to 10,000, orchid species utilize some form of deception in their pollination mechanism (Ackerman 1986). Deceit pollination in its most general sense, is defined simply as lack of pollinator reward, though in the Orchidaceae, it is often much more complex (Pilj and Dodson 1966) and includes both direct model mimicry and more general non-model systems (Dafni 1984). Direct model mimicry systems, also called Batesian mimicry, often occur between flowers, usually a nectar rewarding and nectarless flower. However, in orchids, this mimicry also often occurs between flowers and non-flowers, such as in *Dracula*, whose lip mimics a fungus to attract its fungus gnat pollinator (Ackerman 1986, Endara, *pers. comm.*). In extreme examples, orchid flowers even mimic animals, especially insect pollinators, including *Centris* bees, thynnine wasps, and female tachinid flies (Pilj and Dodson 1966, Dressler 1981, Ackerman 1986). Non-model mimicry systems, also includes convergence and Müllerian mimicry, and generally involve food or shelter mimicry; this category includes the development of nectar guides without nectaries or with false nectaries in many taxa, including the subtribe Zygopetalinae, to which *Chondroscaphe embreei* belongs (Ackerman 1983b). The natural history of this system will be discussed in greater detail following a brief overview of the other major deceit mechanisms. Ackerman (1986) summarizes the various categories of deceit as pseudocopulation, pseudoantagonism, brood-site selection, and general food and shelter mimicry.

Some of the most vivid examples of deceit involve pseudocopulation, a manipulation of the most basic of all animal instincts, reproduction. Mimicry of the female of the pollinator's species is surprisingly common in orchids and has proven so successful that it appears across phylogenetic lineages, through convergent evolution. In some groups, it also appears to provide a selective pressure strong enough to maintain tight phylogenetc linkages between pollinator and flower, a more convincing case for coevolution in orchid pollination than found in the euglossine syndrome (Mant *et al*. 2002). Several examples include: the well-known European terrestrial *Orphys* species imitating the female of an anthrophorine bee (Pilj and Dodson 1966); the Australian terrestrials *Caladenia* and *Chiloglottis* and their thynnine wasp pollinators (Peakall and Beattie 1996, Mant *et al*. 2002); the specialized appendix *Lepanthes*, an organ that enduces copulation in female flies (Blanco, *pers. comm*.); and the lip of *Stellilabium, Trichoceros,* and *Telipogon*, which have purple hairs that mimic female tachinid flies (Ackerman 1986). Pseudocopulation is thought to be unique to the orchids, but is successful enough in Australia to be one of the dominant pollination mechanisms (over 100 species across nine genera, Peakall and Beattie 1996). Incidentally, it has been proposed that this deceptive system could be harmful to its pollinators in different ways. Wong and Schiestl (2002) report that males responding to chemical cues from *Chiloglottis trapeziformis* learn to avoid these patches, to the detriment of female reproductive success in the area, but it has also been demonstrated that females can leave these areas, potentially minimizing this negative effect (Wong *et al*. 2003). Potential effects on males, positive or negative, have not been shown experimentally.

Pseudoantagonism is found in some *Oncidium* species, and is an adaptation to the strong territorial defense instincts of *Centris* bees. In this system, large displays of yellow and red flowers are produced in long panicles, which move freely in a slight breeze. The bees collect

oils that are secreted by the flowers, and have strong territorial instincts (Dressler 1981). The movement of the flowers in the wind apparently resembles an intruding bee, which is promptly attacked, and the flowers are pollinated in the process with the pollinarium placed on the face of the bee (Pilj and Dodson 1966). This type of deception is only reported from *Oncidium* and related genera, which incidentally are thought to directly mimic Malpighiaceous flowers, which are similar in size, predominantly yellow, and also provide oils as a reward for their pollinators (Roubik 1989). Interestingly, it is also hypothesized that this deceit pollination may actually be mutualistic, serving as important territorial practice for the defending bee (Ackerman 1986).

Brood-site selection is often described as a deception system, though the differentiation is much less clear, because although mimicry is involved, pollinators sometimes benefit from nectar collection or some other behavioral response. The most common form is probably 'fungus mimesis' as described above in *Dracula*; the flies are attracted in great numbers to the fungus-like lip and unpleasant odor, which stimulates mating courtship by the males and egg-laying by the females. The larvae, however, cannot eat the flowers and starve to death (Ackerman 1986). Similarly, *Cypripedium* and *Paphiopedilum* stimulate egg-laying in their fly pollinators (Atwood 1985). *Dracula* and related fly pollinated pleurothallid orchids have speciated explosively in the high cloud forests of Colombia and Ecuador, as have *Telipogon* and other fly pollinated deceptive orchids, indicating the unknown potential evolutionary consequences of these deceptive pollination systems.

The final type of deceptive pollination to be discussed here is the general food and shelter mimetism, prevalent through many groups of orchids. This type of deception includes both direct model and non-model systems, which are sometimes difficult to distinguish depending on availability of sympatric models. Ackerman (1983) describes the pollination system of

Cochleanthes lipscombiae by *Eulaema meriana* as non-model food deception, however, he also comments on the possibility of a model, a sympatric nectiferous legume, *Clitoris javacensis*. The orchid flowers have reflexed, tubular lateral sepals that resemble nectaries and distinct markings along the lateral edges of the lip, which act as nectar guides. The bees were observed approaching the flowers with tongue extending, a feeding behavior, and probing the lateral sepals for nectar. The tubular lateral sepals are believed to act as false nectaries and the flower is pollinated through deception. This fragrant nectarless orchid was visited several times by female *El. meriana*, eliminating the possibility of the male euglossine syndrome or fragrance collection. Ackerman (1983) hypothesized that several members of the subtribe Zygopetalinae, including *Chondroscaphe* are also pollinated this way. Williams (1982) suggested non-model deception as a pathway for the evolution of euglossine pollination: high cost of production of nectar in flowers with low visitation led to nectarless deceit flowers and then euglossine pollinators presented a selective pressure through their preadapted fragrance collection behavior for stronger fragrance production and bizarre morphological adaptations associated with these flowers.

Evolution of Deceit Mechanisms

Though pseudocopulation and brood-site mimicry are common among terrestrial orchids, it is thought that the epiphytic habit, and its consequence of low density, hyperdispersed populations, is especially well-suited for the selective adaptation of food mimicry. Many epiphytic and terrestrial orchids are both resource limited over time and pollinator limited within some seasons, compensating for the relatively high cost of fruit production with low fruit set (Calvo 1993). Most orchids are long-lived, yet individual plants only set fruit once or several times per lifetime, reducing the effects of resource limitation. This is even more prevalent in epiphytic orchids, which on average produce 150 times as many seeds per capsule than

terrestrials (Neiland and Wilcock 1998). It has been shown that nectar production, even in small quantity and low quality, increases pollinator visitation and fruit set over deceit pollinated mechanisms (Rodríguez-Robles *et al.* 1992, 1994, Smithson 2002, but see Smithson and Gigord 2001). However, resource limitation may play a significant role in the evolution of deceit pollination, especially in a pollinator limited system, where fruit set is already low so there is relatively low fitness cost associated with loss of reward (Montalvo and Ackerman 1987). Negative frequency dependent selection (FDS) is often applied to these deception system models, due to the density dependent fitness advantage of rarity and high morphological variation among floral mimics (Ackerman *et al.* 1997, Ferdy *et al.* 1998). Since pollinators quickly learn to avoid non-rewarding flowers, deceit pollinated flowers are dependent on slight variation within color or fragrance morphs to occasionally succeed in pollination. In fact, the potential for maintenance of stable color polymorphisms was demonstrated in an artificial flower experiment with bumblebees (Smithson and MacNair 1997). Artificial flower arrays of different corolla colors were arranged and bee visitation observed; when nectar rewards were provided, the most common color morph was visited more frequently, but when no rewards were offered, rare color morphs were disproportionately visited. The results of this experiment suggest that floral variation among populations can be maintained by deceit pollination, because pollinators will switch phenotypes when no reward is encountered. A similar mechanism may occur for negative FDS in deceit pollinated flowers in nature, but this has yet to be demonstrated experimentally for fragrance, food, or color in non-model food deceptive orchids (Ackerman *et al.* 1997, Aragon and Ackerman 2001, 2004).

A recent study of nectar addition in *Anacamptis morio*, a nectarless orchid, found that, as predicted, the addition of nectar increased pollen transport, but also significantly increased

geitonogamous selfing, or pollination of other flowers on the same inflorescence. The increased reward caused pollinators to spend on average, more time on each inflorescence (>18s) than it takes for the pollinaria to dry and bend into a position ready for contact with the stigmatic surface (Johnson *et al.* 2003), thereby allowing selfing. Since nectarless flowers are rarely visited for long enough to allow for pollinaria bending, this experiment provided new evidence for the evolution of deceit pollination to prevent geitonogamous selfing. Pollinaria bending has long been thought to be a primary anti-selfing mechanism in orchids (Darwin 1862), but Johnson *et al*. (2003) show that nectar production still increases geitonogamy despite the adaptation of pollinaria bending. They conclude that reduced inbreeding depression and increasing pollen carryover (pollen transport not lost to self flowers) between individuals may be a strong evolutionary selective pressure for deceit pollination.

This evidence reintroduces an alternative hypothesis to the resource limitation evolution of deceit in orchids, which are often pollen-limited, as opposed to other plant taxa, which also accounts for lack of strong frequency dependent selection in deceit pollinated orchids. The reduction of geitonogamous selfing by deceit pollination through lowered pollinator visitation time was previously hypothesized (Dressler 1981). However, since pollinaria are found in few plant families, the reduction of geitonogamous selfing through pollinaria bending may overcome the reduced fitness through inbreeding depression and pollen discounting normally associated with nectar production. The two hypotheses for the evolution of deceit (resource limitation and lowered inbreeding) are not mutually exclusive, since resource limitation likely still plays a role in limiting fruit production (Ackerman and Montalvo 1990) and inbreeding depression may not be a strong selective pressure in plants with low visitation (<10%). For these species, other possible evolutionary advantages of deceit pollination include simple cost-benefit reallocation of

resources to fruit maturation, or 'steady-state' flowering through lower flower investment. Low floral investment may also result in environments with consistent disturbance, as shown in the nectar-rewarding twig epiphyte *Comparettia falcata* (Chase in Ackerman 1992).

Natural History of *Chondroscaphe embreei*

What follows is a description of the natural history of an orchid endemic to the western Andean cordillera, *Chondroscaphe embreei* and its pollination ecology through the results of a year-long monitoring of pollination success and fruit set. Several hypotheses were tested: 1) due to deceit pollination, negative frequency dependence between density of individuals and fruit set would occur, which could be predicted by a measurable density threshold for fruit set; 2) plant fitness is related to phenology and fruit set; and, 3) introduced orchids would be equally successful in fruit set as existing plants in natural populations. The first two test potential mechanisms for diversity and rarity of orchid species in the Andes, while the third is a preliminary study on the effectiveness of plant reintroduction for conservation of threatened species. While it is important to determine what limits fruit set in endemic populations, it is also of utmost importance to understand how potential conservation strategies may interact with the population dynamics of the species.

Chondroscaphe embreei is a large epiphytic orchid with rather large and showy flowers. It has several fan-shaped, grass-like leaves, which overlap at the base. The longest leaves in healthy plants usually exceed 50 cm, but rarely 3 cm in width. Inflorescences are pendant and solitary, extending from the base of the plant with the cincinnus exposed, and a pedicel reaching approximately 5-10 cm in length, however multiple inflorescences often occur simultaneously. The pedicel has one or two flattened bracts. Flowers are creamy-white in color with a yellow

Figure 4.1. Line drawing of *Chondroscaphe embreei*. Note the long, grasslike leaves, solitary inflorescence, and lack of pseudobulbs, which is characteristic for the Zygopetalinae subtribe. *Chondroscaphe*, as a genus is separated morphologically by the 2-toothed callus (see Figure 2.2), non-reflexed sepals, and the fimbriate lip on the flower.

throat with many reddish-brown to black spots on the inside of the lip and are relatively large, reaching 8-10 cm across. The spots resemble the nectar guides described by Ackerman (1983), but the sepals are spreading and usually fimbriate, not tubular or reflexed. The lip is bilobed and parallel to the column for one-third of its length to form a gullet, tubular shaped, but spreading and flaring for the apical half. The lip is deeply and irregularly fimbriate, which is characteristic for this (sub)genus (see Figure 4.1). The pollinaria has four pollinia arranged in two pairs.

C. embreei grows in highly dispersed, low density populations in the humid lower montane forests of the western Andean cordillera at approximately 1000-1800 masl. It is an endemic, classified by the International Union for the Conservation of Nature (IUCN) as 'nearly threatened' (Endara and Jost 2000), but is locally common at the Maquipucuna Reserve. It is epiphytic, usually found between one and three meters off the ground on tree trunks, but has been seen as high as 15 m in a tree crown. While it apparently favors the humid, shady understory, it also occurs most frequently near disturbed areas, such as the borders of tree clearings and steep slopes. In fact, on very steep slopes (45-60°) it has been observed growing terrestrially, still with pendent flowers, indicating it is not obligately epiphytic, but that the terrestrial habit is occupied in steep areas. It flowers year-round with a notable increase in phenology during the mid-late rainy season. Flowers are solitary, with multiple inflorescences often open simultaneously. Individual flowers are open for an average of 6-9 days; however, the lip usually detaches one or two days before the flower falls, thus, flower longevity for effective pollination is shorter. Due to flower structure, the flowers are not autogamous in the wild, though hand pollinations showed that it is occasionally self-compatible. Six flowers in the Maquipucuna orchid garden were selfed and two set fruit (33%, one self and one geitonogamous

self), but no data is available on seed set in these fruits compared to hand-crossed pollination trials, which showed a higher fruit set (83%). My observations at the Maquipucuna reserve indicate that it is pollinated by a male euglossine bee, *Euglossa trinotata*, and no other pollinator has been observed. The only other observed floral visitor was a Curculionid orchid weevil, which often predated on flowers, including the column and petals, which accounted for a large amount of the bud abortion observed, especially in the dry season when fewer buds rotted due to high rainfall. This study confirms that pollinator visitation is low and fruits are rarely produced, which will be discussed in greater detail and along with the phonological results of the monitoring program will be discussed below.

Chondroscaphe embreei is a member of the Chondrorhyncha complex within the subtribe Zygopetalinae, as described by Garay (1969). *C. embreei* as used here is not yet a valid taxonomic name, but best describes the newest phylogenetic classification of the complex, which raises the subgenus *Chondroscaphe* Dressler to generic level, segregating it from the previous (and still currently valid) *Chondrorhyncha* Lindley (Dressler 2000). It should be noted that *Chondroscaphe* (Lindl.) Gerlach and Senghas has previously been proposed as a separate genus, but until DNA evidence was available, seemed superfluous and Dodson treated these differences at the subgeneric level (Dodson *et al*. 2003). The revived genus *Chondroscaphe* is separated from *Chondrorhyncha* based on DNA evidence from ITS and trnL-F chromosome regions, as well as several morphological features including: narrow grass-like leaves; exposed base of the inflorescence; 2-toothed callus; distinct stipe of the pollinaria; often with narrow pollinia; non-reflexed lateral sepals; and most notably, an often fimbriate lip (Dressler 2000). While DNA evidence from *C. embreei* specifically has not yet been analyzed, based on this morphological description, the previously named *Chondrorhyncha embreei* Dodson and Neudecker should be

reclassified as *Chondroscaphe embreei*. The most proper way currently to denote this species name, since it has not been formally described yet, would be *Chondrorhyncha* (=*Chondroscaphe* Dressler) *embreei* Dodson and Neudecker, however, thoughout this paper is has been shorten to *C. embreei* for the sake of brevity. Incidentally, it is quite possible that both of these genera may be reduced to subgeneric classifications of *Cochleanthes*, however, the current trend is towards segregating the subgenera based on morphological characteristics described above.

Orchid Pollination Monitoring Program: Methodology

Experimental subpopulations of *C. embreei* were selected based on initial surveys of its natural population distribution at the Maquipucuna Reserve (see site descriptions below) done in June-July 2002 and February 2003. A total of 24 plants in two subpopulations (16, Humedal trail and 8, Tranquilo trail) were marked in 2002 and an additional 36 plants in 2003, including a new subpopulation (4, Humedal, 12 Tranquilo, and 20, Cascadas trail). All results from the monitoring include at least 54 plants, with 60 total beginning June 6, 2004. The densities of two subpopulations (Humedal and Cascadas) were manipulated by the addition of 20 introduced plants (one-third of total plants). These plants had either been previously cultivated in a local orchid garden or collected from surrounding forest, and were tied with string to tree trunks and limbs among the existing plants, thereby mimicking a natural habit when possible. These plants were immobile and thus the density of each population was constant throughout the experiment. All three subpopulations were located approximately 1-3 km from each other, so they were treated as one population, due to the ability of euglossine pollinators to easily transverse this distance in a day. The forest immediately surrounding each subpopulation did not contain any individuals of *C. embreei*, though some individuals were found scattered throughout the Reserve. The species appears to cluster naturally in this manner, probably due to dispersal limitations and

local forest heterogeneity. The monitoring program was performed weekly (every 5-8 days) between February 26, 2003 and March 2, 2004, for a total of 12 months, with only three missed weeks (mid-April, early October, and late February). The monitoring consisted of observing each plant and recording whether it was reproductively active, and recording the number of buds, flowers, and fruits. Pollinator visitation was assessed by pollinaria removal (male pollination success) and seed set (female pollination success).

Additional environmental variables were recorded, such as nearest neighbor index (NNI), plant fitness index (PFI), host tree species DBH, and plant height off the ground. The NNI was the average of the three closest individuals in the subpopulation, measured in meters. The PFI is a composite index of several vegetative and reproductive measurements, including percent change of the longest leaf length (ΔL), percent change in number of vegetative shoots (ΔV), and the ratio of number of buds produced to number of flowers + ratio of capsules produced per flower (RS), represented by the following equation: $[\Delta L + \Delta V + RS]/3$. The NNI has been previously used in frequency dependent experiments (Meléndez-Ackerman and Ackerman 2001). The PFI index was created for this experiment as a composite of fitness characteristics, since other published methods were not immediately applicable to this species. Because its input factors are percentages and ratios, it has a range of -1.0 to 1.0, averaging the vegetative growth (negative or positive) and reproductive output of the individual during the year. The host tree DBH and plant height were only recorded once at the beginning of the monitoring. Three plants were disturbed by branchfall, including one which disappeared after a treefall, and which was replaced by another plant in approximately the same location. Environmental variables were correlated with pollination success and fruit set to determine the effects of resource and pollinator limitation, as well as intrapopulation density, on fruit set. Single factor ANOVA tests

were performed to determine inter-seasonal variation of bud, flower, and capsule production across and within the subpopulations. For statistical testing of seasonal effects, the rainy season was defined as mid-December through May and the dry season, June until mid-December.

Site Descriptions

Humedal subpopulation:

This subpopulation is located near the swamp along the Humedal trail at approximately 1250 msl (see Figure 4.2). The forest is a heterogenous mixture of mostly secondary succession forest, though the swamp represents a significant disturbance and light gap; most of the plants are directly located on the southern edge of the swamp. A total of twelve plants were naturally available and eight were introduced. The average neighborhood density (NNI) was 18.3 ± 16.6m, average host tree DBH was 7.9 cm $\pm$ 8.6 cm, and average plant height was 3.7 ± 2.5 m.

Cascadas subpopulation:

This subpopulation was located at approximately 1230 msl (see Figure 4.2) on the newest trail at the Reserve, which is only open for research. The surrounding forest was riparian old growth, since the site was within 100 m of the Rio Umachaca, however the local vegetation was predominately *Bactris* palms. Only eight of these plants were found naturally, though several individuals were later (March 2004) found above the subpopulation in the tree canopy, approximately 15 m off the ground. Due to the slope of the area and relative short distance upslope to reach this higher elevation, it is not surprising that a seed rain could have established plants at this height. Twelve plants were introduced to the area. The introduced plants were placed in the only flat area near the existing plants, and this arrangement made the neighborhood density much lower, an average NNI of 6.0 ± 3.5 m, but provided an intermediate density measure. The average host DBH was 5.7 ± 2.5 cm and the average plant height was 2.1 ± 0.9 m.
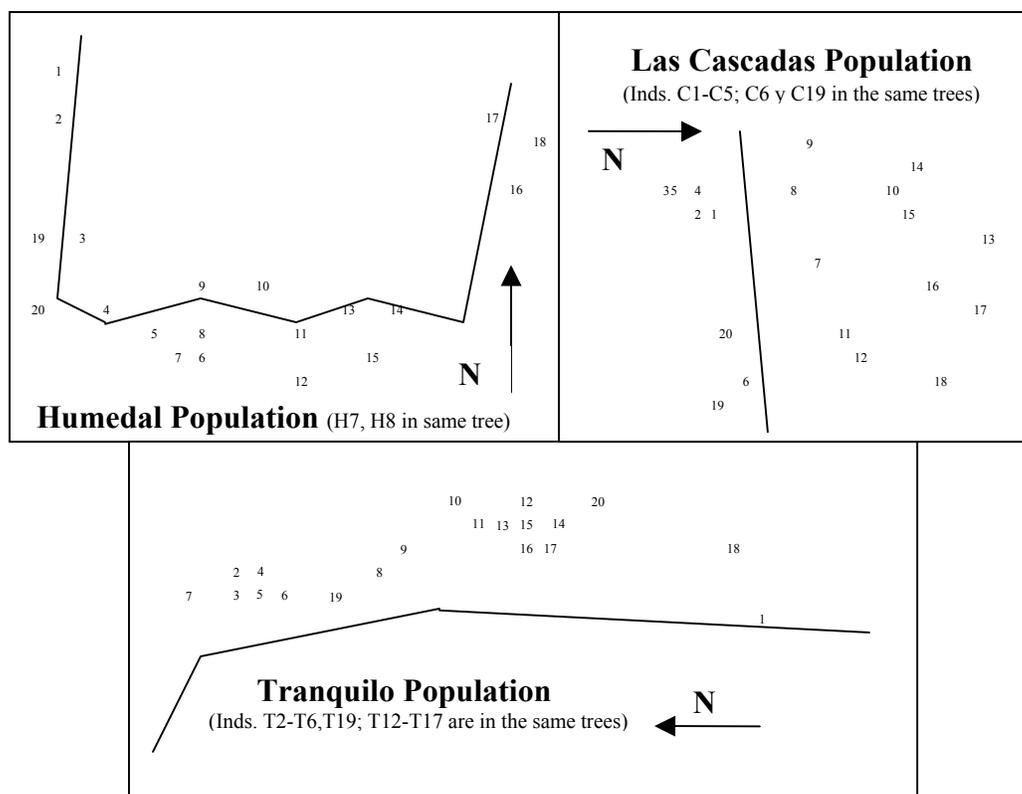
Figure 4.2. Maps of the three *C. embreei* subpopulations. The maps are not to scale, but provide a general outlay of the plants. The Humedal subpopulation is the least dense (NNI = 18.3 m) and the Tranquilo is the most dense (NNI = 5.3 m), despite the two outliers T1 and T18.

Tranquilo subpopulation:

This subpopulation was located the farthest from the other two, on the opposite facing slope of the Umachaca valley at approximately 1430 msl (see Figure 4.2). The surrounding vegetation was a mixture of regenerating pasture and secondary forest found between existing pastures of *Setaria sphacelata*, a common pasture grass in the region. This population was left intact and no plants were introduced. This population was the most dense, with an average NNI of 5.3 ± 10 m. The high variance is due to two plants isolated at the far southern end of the population; when these outliers are excluded, the adjusted NNI (N=18) becomes 2.4 ± 1.6 m, a

better reflection of the actual density of this subpopulation. The average host tree DBH was 5.8 ± 3.9 cm and the average height off the ground was 2.81 ± 2.5 m.

Results of Monitoring Program of *C. embreei*

A total of 55 surveys were carried out over the year-long monitoring period. The sixty plants produced a total of 318 buds, 192 flowers, and 2 fruits for a flowering success rate of 60.38% and a 1.04% fruit set (Table 4.1). Male pollination success rate was 7.29% with 14 pollinaria removed. Thirty buds were produced in the final weeks of the surveys and are not included in these results since their fate was not recorded. Due to insufficient pollination events, it was not possible to determine any density dependent effects between the subpopulations; however, results of the male pollination success are not highly correlated with the nearest neighbor index (NNI) density measurement (Pearson's correlation coefficient, $r^2 = 0.13$). Only the Humedal subpopulation showed some correlation to NNI with its three pollinaria removals ($r^2 = 0.48$), though the Cascadas subpopulation had the highest frequency of pollinaria removal and it was the least well correlated to neighborhood density ($r^2 = 0.09$). It should be noted that on several occasions, pollinaria were disturbed, but not removed, indicating potential visitation without male pollination; since these pollinaria were no longer in a position for effective removal they are not included in male pollination success.

Phenology was highly variable among the subpopulations with the majority of flowers produced by a few number of individuals. Half of all buds and flowers (49% and 52%, respectively) were produced by only 10 plants (16.7%), while only three individuals produced 18% of buds and two individuals, 15% of flowers (Figure 4.3). The five individuals with the most flowering also had some of the highest PFI values (0.68, 0.38, 0.38, 0.28, 0.34). The two individual that set fruit had high PFIs (0.68 and 0.40) as well. This provides weak support for

Table 4.1. Pollination success of *C. embreei*, 2003-2004. The species is characterized by extremely low pollinaria removal and fruit set. Fruit set (FS) for male and female flowers is a percentage of pollinaria removed per flower and capsules formed per flower, respectively.

Population	# of Buds	# of Flowers	# Capsules	Pollinaria	Male FS	Female FS
Humedal	137	85	1	3	3.5%	1.2%
Cascadas	98	56	0	7	12.5%	0%
Tranquilo	83	51	1	4	7.8%	2.0%
Total	318	192	2	14	7.29%	1.04%
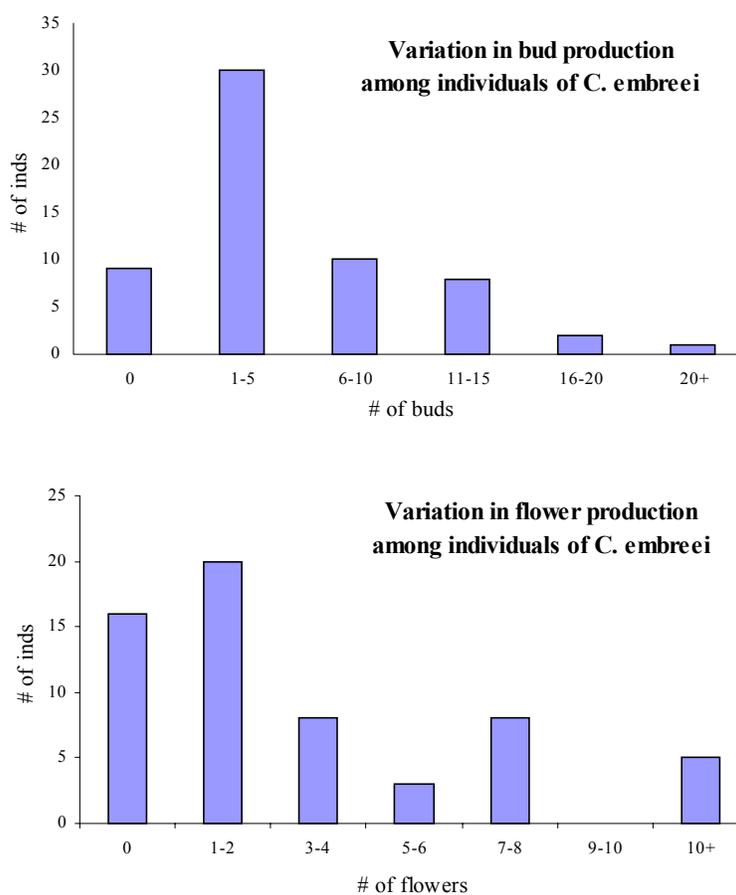
Figure 4.3. Variation in phenology among individuals of *Chondroscaphe embreei*. Nine plants did not produce buds and sixteen plants did not flower at all in a year. The majority of buds (49%) and flowers (52%) are produced by only ten individuals.
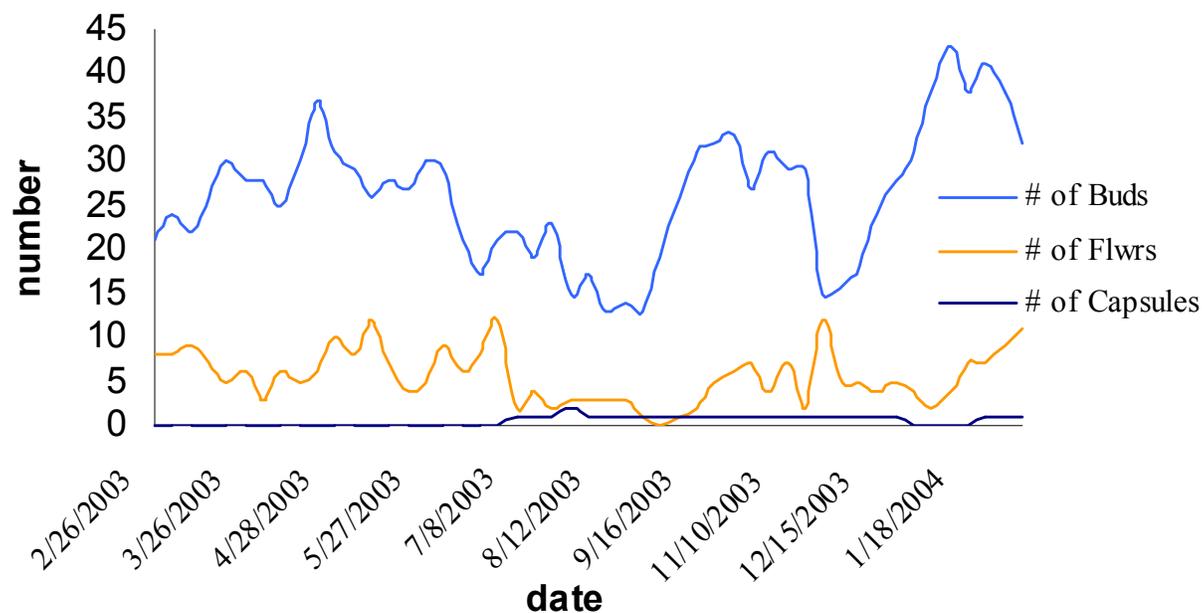
**Figure 4.4.** Seasonal variation in phenology of *Chondroscaphe embreei*. Note the low fruit set across all seasons. Bud production and flowering is highest in the early and mid-late rainy season (January, April-May) with a small spike at the onset of rainy season (November). Lowest flower production is in early dry season (August-September). Thirty buds formed at end of study were not included, accounting for the decline at the far right side of the curve.

the second hypothesis that phenology and fruit set is related to plant fitness. Seasonal effects were significant across the subpopulations. Bud and flower production were significantly higher in the rainy season (Single factor ANOVA – avg. buds: 30.1 ± 36.2 rainy and 22.1 ± 44.3 dry, $F=19.3$, $P<0.00007$; avg. flowers: 6.3 ± 5.6 rainy and 4.7 ± 10.5 dry, $F=3.94$, $P=0.05$; see Figure 4.4), but capsule production was higher in the dry season (single factor ANOVA – avg. capsules: 0.17 ± 0.15 rainy and 0.88 ± 0.20 dry, $F= 34.80$, $P<0.00005$). Flowering patterns between the subpopulations were asynchronous, but not significantly different in either season (single factor ANOVA - avg. rainy Humedal: 2.6 ± 2.0, Cascadas: 1.9 ± 3.6, Tranquilo: 1.8 ± 2.0, $F=1.85$,
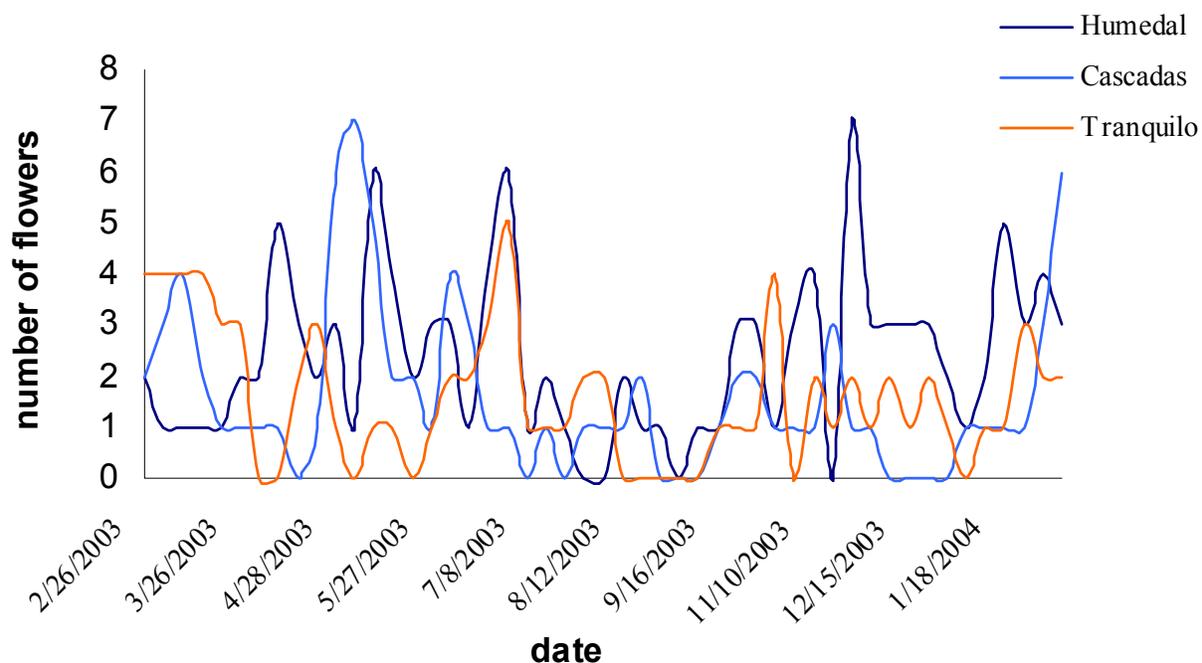
Figure 4.5. Seasonal asynchonicity of phenology among subpopulations. Most subpopulation flowering peaks are out of phase. Peak densities of flowering within each subpopulation are cyclic (large peak occurring ~4-6 weeks). Reduced phenology in dry season was found in all three subpopulations.

P=0.17; avg. dry Humedal: 2.1 ± 3.4, Cascadas: 1.2 ± 1.1, Tranquilo: 1.4 ± 1.6, F=2.89, P=0.06; see Figure 4.5), though the Humedal subpopulation did produce more flowers than the other two (113 total vs. 74 and 76 total). Despite this, the Tranquilo subpopulation had the highest average PFI (0.24 vs. 0.21, Humedal). Meanwhile, the Cascadas subpopulation, despite its high male population success, had an average PFI of only 0.086. No significant difference was found in plant fitness between reintroduced and naturally occurring plants (single factor ANOVA, avg. PFI reintroduced: 0.12 ± 0.05, natural 0.16 ± 0.11, F=0.20, P=0.66). Half of the pollinaria removed were from flowers of introduced plants, though none of these individuals set fruit.

Discussion: Deceit Pollination and Fruit Set

The fruit set and pollinaria removal rates of *C. embreei* are among the lowest recorded for any plant (Calvo 1993, Ackerman 1997, Neiland and Wilcock 1998). Its pollinator is *Euglossa trinotata*, a small euglossine bee, which was found several times at baiting stations carrying *C. embreei* pollinaria. This bee peaks in abundance during the late rainy season, which correlates to the peak flowering time of *C. embreei*. This may be an example of 'pollinator tracking,' a synchronicity between flower and pollinator abundance. As discussed in the previous chapter, euglossine abundance tends to peak in the early-mid rainy season due to resource availability, however, according to baiting data, *Euglossa trinotata* is the most abundant bee (measured by number of individuals observed at baits) at the Reserve in the late rainy season. This peak in abundance is probably due to emergence of young bees or possibly due to a seasonal competitive displacement. As a result, *C. embreei* is opportunistically pollinated by either naïve young bees or experienced bees that are switching resources between the rainy and dry seasons, and thus probe flowers which may be non-rewarding (Ackerman 1983b). The increase in capsule production during the dry season, whether statistically anomalous or not (low pollination frequency may affect this significance), indicates that *C. embreei* may flower year round in a 'steady state' strategy to take advantage of changing forest resources and euglossine bee recruits.

Food deceptive flowers will be avoided by their pollinators because of their lack of energetic return (Heinrich 1979). However, with such low frequency of floral visitation, density dependence effects on pollination success in this experiment cannot be accurately assessed. There are insufficient number of pollinaria removed and capsules produced for rigorous statistical comparison of the subpopulations. The average nearest neighbor measurements estimate population density, but also show subpopulation patchiness (Meléndez-Ackerman and

Ackerman 2001), which can affect pollination as well, since a bee visiting a non-rewarding flower usually leaves in a short period of time. Thus, high patchiness even within the subpopulation level may influence visitation of close neighbors or individuals with multiple open flowers. In the Cascadas subpopulation, the flowers visited most often had intermediate NNI (6.6m and 7.3m), while the ones visited in the Humedal subpopulation had high NNI (18.5m, 19.1m, and 69.7m), even relative to that subpopulation's neighborhood density, which was the largest of any of the three. The two fruits produced were on individuals with NNI of 18.5 and 2.7 m. Similarly, pollinaria were removed from flowers on plants as distant as 69.7 m from their nearest neighbor and as close as 2.7 m. Based on this, the negative frequency dependent threshold hypothesis remains inconclusive. It is uncertain whether a higher number of visitations could have resulted in a density dependent trend for fruit set, but even populations previously tested with much higher fruit sets remained inconclusive (Ackerman 1993). Moreover, if low fruit set led to the loss of nectar rewards in tropical orchids, with their dispersed, low density populations, it may be difficult to adequately separate the mechanisms for pollinator attraction in these deceit orchids.

The monitoring program of *C. embreei* is continuing to examine the relationship between pollinator and resource limitation in a nectarless orchid. Thus, two potential improvements to the methodology should be mentioned here. The NNI measurement that was used to determine density dependence included measurements of the three nearest neighbors. This index introduced the statistical error of pseudoreplication because some of the individuals overlapped their nearest neighbors. Due to the nature of the monitoring program, this was originally accepted as an unavoidable error. Secondly, the NNI measurement was static and used the three nearest plants as a density measure, however, it is more likely that nearest flower would be the appropriate unit

of density. Non-flowering individuals presumably have little affect on pollinator activity, at least in the context of density. The hypothesis followed in this experiment is that of lowered pollination in high-density patches due to high visibility of flowers and learned pollinator avoidance. The current density measurement does not accurately reflect floral display in a subpopulation, which must be corrected. The next phase of the monitoring program will incorporate this nearest flowering neighbor measurement as the density measurement. Incidentally, this measurement is less static and changes with nearest flowering neighbors every survey, also reducing the issue of pseudoreplication in the experiment. If fruit set is high enough to test for density dependence, at least the NNI measurement will accurately reflect this effect.

While it has been shown that it can be quite difficult to determine density dependence within subpopulations like these, this may be explained by two alternative hypotheses: 1) density dependence may occur between populations but not significantly within them (Ackerman 2001) or 2) density selection may occur on a very small scale, even between multiple flowers on a single plant (Folsom 1994).   The first hypothesis was proposed due to the current inability to experimentally determine intrapopulation frequency dependent selection in orchids. It is plausible, though no known studies are attempting pollination studies with orchids at such a large level. Considering the ability of euglossine bees for long-distance travel, this type of monitoring would have to occur in populations located a day or more by bee flight time from each other. The second hypothesis was proposed following pollination experiments with a weakly fragrant euglossine pollinated orchid that showed that pollen carryover is as often achieved between flowers on nearby plants as between distant plants. Thus intrapopulation gene flow occurs and maintains genetic diversity within the population. As discussed in the introduction, deceit pollination could have evolved as a self-incompatibility mechanism, in which case, the pollinator

constancy hypothesis, that is, learned avoidance of non-rewarding flowers, may not play such an important role in the maintenance of low densities in these populations. This point reveals another problem with the NNI measurement – it masks the effect of multiple flowers on a single individual. One plant in the Cascadas subpopulation had pollinaria removed from both flowers during the same survey. It is not known whether this was done by the same visitor or not, though one bee could have removed both without pollinating the second flower visited if the pollinarium was not yet in a position to contact the stigmatic surface. The NNI measurement however, does not record the multiple flower density on the same individual. If pollinations occur in higher flower densities according to the second hypothesis, then the nearest flowering neighbor index will better represent the floral display of an individual.

Pollination events occurred so infrequently in one year of monitoring *C. embreei*, that it is not even possible to assess the comparative effects of pollinator and resource limitation. The species is pollinator limited (see Chapter 2) and due to its reliance on food-deception as a pollinator strategy, even the relative abundance of *Euglossa trinotata* in the late rainy season does not result in successful pollination. Hand pollination trials in the orchid garden also showed that *C. embreei* is pollinator limited because fruit set in crossed plants was much higher (83%); however, not all of these hand-pollinated capsules matured, indicating some resource limitation as well. Resource limitation can also be inferred since many plants did not flower over the year. Future monitoring will indicate whether individuals that mature fruit continue to flower in following seasons. The life history traits of *C. embreei* – long-lived with steady-state flowering and no nectar investment – also lead to the conclusion that the low density, hyperdispersed populations of this species are likely due to pollinator limitation and pollination mechanism. As a consequence, many of these plants may only set fruit once or twice in a lifetime. This low

pollination success, however, is offset by high seed production, but still, limited dispersal distance of these seeds may influence the patchiness of the subpopulations. This subpopulation trait can be characterized by an analysis of the genetic structure of the population. If patches share maternity, then most of the individuals in a subpopulation would be siblings, which could have interesting effects for the conservation genetics of the population. Importantly, the third hypothesis tested, that reintroduced plants would not significantly differ in plant fitness was supported. All of these individuals survived, and their overall fitness was not significantly less than that of natural plants in the same subpopulations. In fact, they were responsible for half of the overall male pollination success. However, the overall strength of this as a strategy for conservation of endemic species is still not well-understood. The strategy of reintroducing plants to supplement native populations will be discussed in the concluding remarks following this discussion.

In conclusion, fruit set and pollinator visitation is too low in *C. embreei* for frequency dependent selection to act on the subpopulation level. Furthermore, a one-year monitoring program is simply not long enough to test for reproductive limitations, such as costs of fruit production, and their effects on limiting population density of an endemic orchid. It appears that *C. embreei* takes advantage of peak abundances of its euglossine pollinator in conjunction with the change in resource availability accompanying the end of the rainy season to achieve pollination through food deception. A large percentage of flowering occurs in just a few individuals, and these individuals have high overall plant fitness values, and one of them set fruit. This large floral display may attract bees to the local area, but the NNI measurement used in the study does not account for this density of multiple flowers on the same individual. Reintroduction of adults into existing populations is a viable means of improving effective

population size, because it appears these plants are incorporated into the pollination syndrome. Monitoring of *C. embreei* will continue to investigate the long-term comparative effects of resource vs. pollinator limitation and interseasonal phenologies of the orchid and its pollinator, in an effort to better understand how pollination mechanism, especially deception, can affect rarity of orchids in the Andes.

CHAPTER FIVE

CONCLUDING REMARKS: WHEN IS IT OK TO BE RARE?

<u>Conservation Strategies for Rare Orchids</u>

Whenever deciding on whether to implement pro-active conservation strategies with rare or endangered species, the causal mechanisms behind their condition must be analyzed carefully to avoid causing unforeseen negative consequences in the natural population (Schemske *et al*. 1994). For example, in the case of *C. embreei*, it is apparent that the species is pollinator limited and furthermore, due to its lack of reward, it is pollinated much less frequently than other euglossine pollinated orchids in the neighboring forest. It is classified as nearly threatened by the IUCN because while locally common at the Maquipucuna Reserve (I found 100+ in two years of surveys), it is limited to the western slopes of the Ecuadorian Andes. This research showed the population dynamics of *C. embreei*, like so many orchids in the Andes, are naturally mediated, even adapted to low density, dispersed populations through morphological adaptations.

So, why attempt to 'save' populations when there is no immediate threat to their populations? Pro-active conservation strategies may be necessary in certain cases, such as in areas of high deforestation, overcollection by locals, or pollinator declines through natural or human mediated causes, but it is still not known how severe these immediate threats are to a typical orchid population. Several of these strategies, specifically micropropagation, reintroduction, public education and outreach, and research, are currently being utilized in northwestern Ecuador and will be briefly discussing in the following concluding remarks.

Micropropagation and Responsible Markets

The Chocó-Andes Community Laboratory in Santa Marianita, a small community 4 km from the Scientific Station at the Maquipucuna Reserve, was established in 2001 with the goal of propagating economic and rare species. The lab produces 'test-tube' plants through either the tissue culture of meristems or the sowing of seed on agar gels *in vitro* and in a contaminant free area. This lab is the first of its kind *in situ*, that is, within such close proximity to a large forest reserve in Ecuador. It is currently propagating ten orchid species and has already successfully produced three previously unavailable species, including *C. embreei*.

Micropropagation takes advantage of the mass seed production by orchids. Even an orchid with fruit set as low as *C. embreei*, can have thousands of seedlings grown from a single seed because germination rates *in vitro* are not limited by mycorrhizal associations and microclimatic variation as in nature. Thus, enough plants to promote 'responsible markets,' that is, markets based on known artificial propagation, not wild collection, of a rare orchid species can be provided from small populations of plants in a non-intrusive manner. Commercialization, or inclusion in trade markets, of orchid species through propagation has already been an effective conservation tool in several species, such as *Epidendrum ilense*, *Phagmipedium bessae*, and *Paphiopedilum rothschildianum* (Koopowitz 2001). The sale of the plants also provides income for training local technicians who work in the lab, the self-sustainability of the lab itself, as well as continued research on orchids in northwestern Ecuador. In addition, seedlings produced by the lab are currently included in research conducted through the Atlanta Botanical Garden on the potential of reintroduction as a conservation strategy for rare orchids.

<u>Why Reintroduce Orchids?</u>

Reintroduction is a controversial topic in tropical orchid conservation (Koopowitz 2001). I propose three common scenarios for potential positive effects of reintroduction of rare orchid species: 1) local extirpation of a species in an area, but when reintroduced, serves as a metapopulation seed source in the region and/or attracts pollinators of the species that may have abandoned the area; 2) after habitat destruction, as part of a regeneration or restoration project where orchids and other epiphytes are known to be a keystone part of the original ecosystem; and 3) when populations have passed through a bottleneck or other scenario when genetic diversity in a population is so low as to affect survival of the species. This third scenario is not well-understood, as discussed in Chapter 1, and may even provide speciation mechanisms with local extinction a necessary result (Dodson and Gentry 1991). However, if the goal is preservation of the species, reintroduction could increase genetic heterogeneity of metapopulations and potentially prevent declines as predicted by models. The key aspect of all three scenarios is that known habitat destruction or degradation has occurred in orchid populations. Reintroduction of species without these events occurring prior is not strictly conservation oriented (i.e. display gardens or 'flowering' trails), though it could be considered an appropriate strategy in the context of environmental education and public outreach as discussed below.

This project included a subcategory of reintroduction, translocation, as a means of manipulating densities within the subpopulations of *C. embreei*. They are considered 'translocated' because all of them inhabited the same local forest area at some point in their life. This should not be confused with the idea of 'reintroduction' in the sense of

reestablishing an extirpated species population, which is more controversial (Berg 1996). This method provided an opportunity to examine the technique of reintroduction as a potential strategy to supplement existing populations. The translocated plants were taken from either the local orchid garden, fallen plants on trails, or moved between subpopulations. The plants that were found fallen on trails are considered 'rescues' because it has been shown that fallen epiphytes quickly die on the forest floor (93% mortality in 21 months, Matelson *et al*. 1993). There was a zero percent mortality rate among the translocated plants, not counting mortality due to disturbance (one individual disappeared after a treefall event). A recent study with *Lepanthes eltoroensis* also showed that post-disturbance translocation of plants led to high survival rates (Joubert *et al*. 2003). It should be remembered that translocating adult plants is very different from reintroducing seedlings. Translocation focuses on maintaining potential seed production in existing adults, while reintroducing seedlings usually aims to establish new populations or augment existing population numbers. Maintaining seed potential also can be achieved in some species by hand cross-pollinating flowers in the wild (Ackerman *et al*. 1996) or reintroducing 'sacrificial' adult plants, as reported in *Laelia crispa* in Brazil's Serra do Mar (Warren and Miller 1992, 1993). However, reintroducing adult plants may be also be important, especially in highly disturbed areas, such as stripped branches, where natural recolonization by epiphytes is very slow (>5 years), due to the necessity of microclimatic facilitation by bryophytes (Nadkarni 2000).

Is reintroduction a valuable strategy for conservation of species? Augmenting existing subpopulations of *C. embreei* through translocation did not affect the fruit set of over a year. It remains to be tested whether augmenting populations of a rewarding

orchid will increase pollinator visition and fruit set within a population, though it appears possible and even probable. However, if, in deceit pollinated flowers, such as *C. embreei*, future monitoring reveals negative density dependence for pollination success based on predicted pollinator avoidance, then increasing densities may negatively affect population dynamics. It is also possible that the added local flower display may attract pollinators to the area, as discussed in the previous chapter. The natural histories of most orchid species are simply too poorly understood to assume that simply adding more individuals will affect pollination success. For example, resource limitation may limit plant density through seedling mortality, which could be overcome by reintroducing adult plants, or flowering displays may be resource limited, in which case reintroduction have little effect. Alternatively, higher abundance of pollinators may affect pollination success, since competitively displaced naïve bees may be forced to probe non-rewarding flowers, or it could have no effect, since naïve bees should learn quickly to avoid non-rewarding flowers. These are important questions, and despite (or maybe because of) the lack of effect on pollination success, translocation is a useful tool for researching (sub)population dynamics. However, more research in individual case studies of reintroduction is recommended before using it as a primary tool for species conservation. For example, future projects will investigate the affect of multiple species reintroduction, since it has been shown that the presence of certain 'magnet' plants increase the pollination success of nearby deceit pollinated plants (Ackerman 1986). It has also been suggested that reintroduction strategies should follow a metapopulation model of population dynamics, with success being defined as incorporation into natural pollinator relationships and seedling recruitment mechanisms, not survival of individual plants (Primack 1996).

Research, Environmental Education and Public Outreach

The need for further research on orchid pollination ecology, population dynamics, and conservation opportunities cannot be more immediate. It is not possible to put all the pieces together when only half the pieces exist. One example will suffice for this discussion: how is it possible to recommend reintroducing an endemic species when the original range is listed as unknown? Currently, the most common sources of information on endemic species are 'red lists,' which list all known information on endemic plants or animals in a country. Ecuador's 'Red Book' (Pitman *et al*. 2000), as previously discussed, lists over 1,300 endemic orchid species for the country, based on restricted range, existing collection data, and future threats to survival. While this method is quite useful in prioritizing conservation activities, some criticize even this approach as subjective, erroneous and perpetually outdated (Ecuador will have a new 'Red Book' in 2005; Endara, *pers. comm*.). Indeed, long lists of endemic species are not useful for conservation planning if many of these species face no immediate threats to their survival; this approach can even be harmful to the credibility of the conservation groups promoting their usage. Ibisch *et al*. (2002) propose combining GIS data and extrapolation modeling to produce range and habitat conservation status maps. This method shows less species as vulnerable, concluding that the 20,000 km^2 range restriction used for determining vulnerability status is too large in Andean areas. Moreover, they argue that because developing nations are notoriously data-poor, usually with incomplete plant inventories, a more comprehensive approach that does not rely on just known collection data is imperative. As research continues, the 'endangered' nature of many range-

restricted orchids may be called into question. Conservation priorities should focus on larger landscape scale priorities before attempting species-specific conservation plans.

While further research is clearly needed, public education and outreach is even more important, because appropriate and effective environmental education can slow the immediate threats facing most of these orchid populations, such as habitat conversion, road construction, and deforestation. Near the Maquipucuna Reserve, local deforestation as a major source of income has already been surpassed by ecotourism dollars (Justicia, *pers. comm.*). Maquipucuna's Niño Naturalista program encourages Ecuadorian schoolchildren to value the cloud forest for both its inherent value and potential future values. Meanwhile, local orchid rescue gardens are becoming a new tool for community conservation, as they are converted to display and education gardens to promote orchid conservation and earn income for preservation of orchid habitat, conservation of species, and maintain a rural way of life. Future conservation priorities for orchids at Maquipucuna include specialized environmental education programs and integration with local school curricula to show the importance of orchid diversity and necessity of protecting local forests as orchid habitat. A new focus on higher level education will be instituted through a fund providing research opportunities to Ecuadorian university students, which will hopefully provide a stimulus for further orchid research in the region. Finally, a network of orchid gardens throughout northwestern Ecuador is planned to highlight orchid conservation as a priority for cloud forest conservation in the Tropical Andes. These display gardens will advertise the beauty and diversity of orchids, as well as provide an applied, *in-situ* tool for orchid conservation through the maintenance of living plant collections and propagation of individuals.

In conclusion, perhaps the diversity of orchids is only matched by the sheer diversity of responses they evoke in people. Whether they call themselves orchidologists, orchidophiles, or 'orchidiots', there are orchid lovers everywhere in this world who support their local orchid societies, attend orchid congresses, and travel across the world to see their favorite flowers bloom in nature. Orchid conservation should strive to include research, education, as well as the aesthetic appeal of orchids in an integrated appeal to protect these plants as well as the habitats where they are most threatened, including northwestern Ecuador and all of the Tropical Andes.

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APPENDIX:

2004 ORCHID LIST OF THE MAQUIPUCUNA RESERVE, ECUADOR

ORCHIDACEAE (80) Reviewed and augmented by Alexander Reynolds (* det. C. Dodson; ** det. J. Beckner; others as indicated.) Some taxa are vouchered by photos.

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[A large number of orchids have been collected by Dodson, Luer, and others along the road between Nono & Tandayapa, but most of these lie outside our boundaries; those within approximately 1' are listed in brackets.]

1. *Ackermania cornuta* (Garay) Dodson & R. Escobar
   Prim for., 1200-1600 m, Sendero Tranquilo, Río Umachaca; ep., lvs. grayish-green, fls. cream-yellow with red markings, lip apically pointed: AR21 (?)

2. *Ada elegantula* (Rchb. f.) N. H. Williams
   Prim. for., Nono-Nanegal, Tandayapa, 1600 m; fls. small, greenish with pink spots on lip: Hirtz 1287 (photo).

3. *Ada glumacea* (Lindl.) N. H. Williams
   Prim. for., Tandayapa, 1600 m; ep.: Dodson 18794.

4. *Ada ocanensis* (Lindl.) N. H. Williams
   Prim. for., Tandayapa, 2000 m; ep.: Dodson 1097; Luer 4711*. [32921, at 1300-1350 m, has the habit of *Ada ocanensis*, but the flower color (green with white lip) of *Ada andreettae* Dodson.]

   [*Altensteinia fimbriata* Kunth: Calacalí to Nanegalito, 2400 m, terr.: K 1015.]

5. *Altensteinia virescens* Lindl.
   Roadside embankment, Calacalí-Nanegalito, 2200 m; terr., fls. greenish yellow.

6. *Anguloa virginalis* Linden ex B. S. Williams
   Sec. for., Pahuma, 1800 m; fls. fleshy pink or white with maroon spots, strongly fragrant.

7. *Barbosella cucullata* (Lindl.) Schltr.
   Nono-Nanegal, km 17, 2160 m; ep.: Dodson 10783; El Pahuma, 2000 m; ep.: Suin 459.

   [*Barbosella* sp. 1: Río Alambi, 2250 m, Molau & Ohman 1391.]

8. *Brachionidium* sp.
   Upper mont. for., Cerro Montecristi, 2750 m; terr., perianth purplish & green: 30558*.

9. *Brachtia andina* Rchb. f.
   Upper mont. for., 1800-2470 m; ep., fls. yellow: F 1380, 1479; P 3578; Croat 72873 (det. E. A. Christenson); Dalström 1604.

10. *Brassia arcuigera* Rchb. f.
    Sec. for., 1350 m; ep., fls. pale greenish mottled with brown: 31025.

11. *Brassia* sp. 1
    Sec. for., 1250 m; ep., fls. pale yellow: 31008.

12. *Campylocentrum polystachyum* (Lindl.) Rolfe
    (500) 1000-2500 m; ep., infl. extending past lvs., fls. white.

13. *Chondrorhyncha embreei* Dodson & Neudecker
    Sec. for., 1200-2000 m; ep., fls. greenish-white with burgundy markings: 27648*, 27649*, 28842*, 31173; C 5933*.

14. *Chondrorhyncha thienii* (Dodson) Dodson
    Prim. for., 2000 m; ep., fls. pale yellow, column purple-spotted: 28723*.

15. *Cleistes* sp.
    Sec. for., 1725 m; terr., fls. orange: 31925.

16. *Comparettia falcata* Poepp. & Endl.
    Sec. for., 1250-1900 m; ep., fls. magenta: 31001; C 13045; Suin 501*.

17. *Cranichis antioquiensis* Schltr.
    Nono-Nanegal, orchid garden, 1250-2200 m, terr., flrs greenish-white, Sparre 16815.

18. *Cranichis ciliata* (Kunth) Kunth
    Prim. for., Tandayapa, 2320 m; terr.: Luer 7305.

19. *Cranichis fertilis* (F. Lehm. & Kraenzl.) Schltr.
    Banks in sec. for., 1250-1800 m; terr., fls. white: 27166*, 27636*; V 12265; Harling & Andersson 11623; Todzia & Grimes 2479.

20. *Cranichis* sp. 1
    Sec. for., 1200-1300 m; terr., fls. whitish: 27783, 28840.

21. *Cranichis* sp. 2
    El Pahuma, 1800 m; terr., fls. red.

22. *Crossoglossa caulescens* (Lindl.) Dodson
    Nanegal, 1500 m; terr.: 1855, Jameson s.n.

23. *Crossoglossa nanegalensis* Dodson
    Sec. for., Tandayapa, 1700 m; terr., fls. yellow-green: Hirtz 2210* [type collection].

24. *Crossoglossa tipuliodes* (Lindl.) Dodson
    Sec. for., Nanegal, 1200-1300 m; terr.: Harling & Anderson 11599*.

25. *Cryptocentrum latifolium* Schltr.
    Sendero Tranquilo, 1300 m; ep., lvs. 1.5 mm wide, fls. yellow-green: 31598, AR4.

26. *Cryptocentrum lehmannii* (Rchb. f.) Garay
    Sendero Tranquilo, 500-2000, 2500-3000 m; ep., lvs. <1 mm wide, fls. yellow-green: AR5.

27. *Cryptocentrum* sp. 1
    Sec. for., 1400 m; ep., lvs < 1 cm wide, fls. yellow-green, base of petals extend past base of sepals: AR13

28. *Cyclopogon inaequilaterus* (Poepp. & Endl.) Schltr.
    Sec. for., 1200-1350 m; terr., fls. yellowish-green: 27780*, 28109*, 28169*.

29. *Cyclopogon ovalifolium* C. Presl
    Sec. for., 1300 m; terr., fls. white: 28783*.

    [*Cyclopogon pelagallanus* Dodson, from its type locality at 2800 m near Loma Pelagallo, Hirtz 2618, is 1' E of the area boundary.]

30. *Cyclopogon* sp. 1
    Sec. for., 1300 m; terr., roots fleshy, fls. green with white lip: 27616.

31. *Cyclopogon* sp. 2
    Sec. for., 1300 m; terr., roots fleshy, fls. green with pink lip: 27617.

    [Two additional collections of *Cyclopogon*, 28725 and 29994, remain unidentified.]

32. *Cyrtochilum cimiciferum* (Rchb.f.) Dalström
    Sec. for., Pahuma, 1800-2400 m; ep., long infl., fls. yellow-brown: Suin 430; Calacalí-Niebli 2000 m: Hirtz 1482 (both det. S. Dalström).

33. *Cyrtochilum flexuosum* Kunth [= *Oncidium hartwegii* Lindl.]
Prim. for., 2050-2275 m; ep., fls. brown and yellow: 28934*.

34. *Cyrtochilum geniculatum* Königer
Sec. for., Nono-Nanegal, 2200-2400 m; terr., fls. red with pink edges: Hirtz 1041.

35. *Cyrtochilum macranthum* (Lindl.) Kraenzl.
Sec for., roadside embankments; terr., fls. large, orange, yellow and purple: 30591; El Pahuma, 2400 m; ep.: Suin 428 (det. S. Dalström).

36. *Cyrtochilum meirax* (Rchb. f.) Dalström
Prim. for., 1200-2100 m; ep., fls. dull yellow with brown spots: 27447 ex p.*, 27450*, 28132*, 28951*, 29308*, 30371*; T177*, Filskov 37131, Holm-Nielson 24504.

37. *Cyrtochilum ramosissimum* (Lindl.) Dalström
Nanegal; ep.: Sodiro 112.

38. *Cyrtochilum serratum* (Lindl.) Kraenzl.
Prim. & sec. for. & clearings, 1500-2225 m; common, ep. or terr., infl. to 4 m, twining or clambering, fls. yellow & brown: 27960*, 28058*, 28891*, 29402, 30100*, 30590; F 1192; Dodson & Thien 1102; Holmgren 851; Holm-Nielsen 24484; Suin 367 (det. S. Dalstrom), 441(?). "Margarita"

39. *Cyrtochilum williamsianum* (Dodson) Dalström
Sec. for., Sendero Tranquilo; 1500 m; ep., fls. pink with pink callus.

40. *Cyrtochilum* sp. 1
Roadside embankment, Utopia, 1800 m; ep./terr., fls. yellow.

41. *Dichaea longa* Schltr.
Sec. for., Tandayapa, 1500 m; ep.: Dodson 16506.

42. *Dichaea morrisii* Fawc. & Rendle
Sec. for., 1500-2200 m; ep.: 28020*, 28761*; Sparre 17045.

43. *Dichaea sodiroi* Schltr. (incl. *Dichaea pendula* (Aubl.) Cogn. & *Dichaea chasei* Dodson)
Prim. & sec. for., 1500-2100 m; ep., fls. yellow with purplish lip: 27453*, 28874*, 28900*, 33594; C 5903*; Hirtz 1496 (Calacalí-Niebli, 2200 m); 1979, Luer s.n.; Suin 391.

44. *Dichaea* sp. 1
Sec. for., 1300-1400 m; ep., fls. yellow: 29301.

45. *Dichaea* sp. 2
Prim. & sec. for., 1300-1775 m; ep.: 27579, 29273, 29579, 30391.

46. *Dracula dodsonii* (Luer) Luer
   Nono-Tandayapa, km 17; ep.: Dodson 10792, 16501.

47. *Dracula felix* (Luer) Luer
   Sendero Tranquilo, 1000-2500 m, ep., fls. white with purple.

48. *Dracula navarroörum* Luer & Hirtz
   Sec. for., Nanegalito, Tandayapa, 1600-1800 m; ep.: Dalström 1661; Hirtz 4854; Luer 15259.

49. *Dracula sodiroi* (Schltr.) Luer
   Sec. for., 1750-2400 m; ep.: F 1199*; Dodson 15972; Suin 427.

50. *Dracula vespertilio* (Rchb.f.) Luer
   Prim. for., Pahuma, 1800 m; ep., fls. yellow, spotted with purple, lip white with pink spots
(det. S. Dalström).

51. *Dracula wallisii* (Rchb.f.) Luer
   Prim. for., Pahuma, 1800 m; ep., fls. whitish, spotted brown or purple, lip white, mobile (det.
S. Dalström).

52. *Dressleria fragrans* Dodson
   500-1000 m; ep., fls. greenish, fleshy, fragrant.

53. *Dryadella simula* (Rchb. f.) Luer
   2000-3500 m, near Utopia; ep., fls. greenish with purple markings.

54. *Dryadella* sp. 1
   Prim. for., 2000 m; ep., fls. purplish: 31843.

55. *Elleanthus aristatus* Garay
   Cerro Negro, 1800 m; ep., fls. orange: 30449*.

56. *Elleanthus aurantiacus* (Lindl.) Rchb. f.
   Sec. for., 2000-2500 m; ep./terr.: K 1521; Suin 494*.

57. *Elleanthus capitatus* (Poepp. & Endl.) Rchb. f.
   Sec. for., 1300-2000 m; ep./terr., stems to 1 m: 27534*, 27611*, 31547; C 5905*; L 11327;
Bohlin 1100; Suin 401.

58. *Elleanthus discolor* (Rchb. f. & Warsz.) Rchb. f.
   Sec. for., 1300 m; ep./terr., bracts yellowish: 27708*.

59. *Elleanthus formosus* Garay
   Banks in sec. for., 1250 m; terr., fls. brick orange: 27301 (listed by Dodson *et al*. as a separate
species from *Elleanthus ampliflorus* but this collection # is *E. formosus*)

60. *Elleanthus fractiflexus* Schltr.
Sec. for., 1400-1550 m; terr., fls. white: C 39783; Dodson 6982; Sodiro 35*.

61. *Elleanthus gastroglottis* Schltr.
Sec. for., 1800-2500 m; ep./terr., perianth purple: C 5939*; Hurtado 1425*; Luer 2363; Suin 493.

62. *Elleanthus graminifolius* (Barb. Rodr.) Lojtnant
Sec. for., 1200-1250 m; ep./terr.: 27531*, 28966*.

63. *Elleanthus linifolius* C. Presl
Sec. for., 1200-1250 m; ep./terr.: 28967*.

[*Elleanthus myrosmatis* (Rchb.f) Rchb.f; at Yunguilla: Dodson et al. 16499.]

64. *Elleanthus oliganthus* (Poepp. & Endl.) Rchb. f.
(0)1000-3000 m; ep./terr., fls. orange-red.

65. *Elleanthus petrogeiton* Schltr.
Prim. for., Yunguilla-Sta. Lucia, 2500 m; terr., fls. white with purple.

66. *Elleanthus robustus* (Rchb. f.) Rchb. f.
Prim. for., 1650-1800 m; ep. or terr., stems to 1 m: 27447 ex p.*, 31565(?), 31917.

67. *Elleanthus smithii* Schltr.
Banks, 1200-2000 m; terr., fls. orange, foetid: 28161*; Dodson & Thien 1107, Dodson 16505; Harling & Andersson 11613.

68. *Elleanthus vernicosus* Garay
Sec. for., Tandayapa, 2000 m; terr.: Plowman & Davis 4446*.

69. *Elleanthus* sp. 1
Sec. for., banks, 1550-1600 m; fls. white or magenta: 31119, 31122.

70. *Elleanthus* sp. 2
Sec. for., Sendero del Río, 1300 m; ep., fls. white, solitary from terminal cone.

71. *Elleanthus* sp. 3
Sec for., orchid garden, 1250 m; terr., lvs. purplish below, fls. orange, in terminal cone.

72. *Epidendrum arachnoglossum* Rchb. f. ex André
Sec. scrub, above Río Pichan, 2050 m; terr., fls. white, lip with yellow spots: 30099*.

73. *Epidendrum* vel aff. *arbusculum* Lindl. (*Arbusculum* subgroup)
Prim. for., 1950-2000 m; ep., fls. green: 28715*.

74. *Epidendrum aristatum* Ackerman & Montalvo
  Prim. & sec. for., 1500-2250 m; ep. or terr.: 28004*, 28932*.

75. *Epidendrum blepharistes* Barker ex Lindl.
  Sec. for., carretera Nanegal-Marianitas, 1300 m; ep., white fls.

76. *Epidendrum brachyglossum* Lindl.
  Sec. for., 1800-2600 m; ep./terr.: Dodson 6990; Molau & Eriksen 30571.

77. *Epidendrum brachystele* Schltr.
  Sec. for., Tandayapa, 2160 m; ep., fls. green, non-resupinate: Dodson & Thien 1093, Dodson & Dodson 16502.

78. *Epidendrum calanthum* Rchb. f. & Warsz.
  Sec. for., 1250 m; ep., fls. yellow.: 28132*.

79. *Epidendrum caloglossum* Schltr.
  Sec. for., Nanegalito, 1600 m; ep.?: Dodson 6987.

80. *Epidendrum cochlidium* Lindl.
  Prim. & upper mont. scrub, 2000-2500 m; ep. or terr., fls. red or orange: 28085*, 28104*, 30260*, 30587*; F 1491; Asplund 17265; Dodson & Thien 1104.

81. *Epidendrum* cf. *cornanthera* Lehm. & Kraenzl.
  Sec. for., 1550 m; ep.?, viny: 31785.

82. *Epidendrum coryophorum* (Kunth) Rchb f.
  Loma Pahuamba, 2300 m; ep./terr., fls. white with purple spots: F 1446*.

83. *Epidendrum diothonaeoides* Schltr.
  Loma Pahuamba, 2300-2450 m; ep./terr.: F 1350, 1434.

84. *Epidendrum elleanthoides* Schltr.
  Prim. for., 21 km west of Calacalí, 2500 m: L 13681.

85. *Epidendrum embreei* Dodson
  Sec. for., 2000-2100 m; fls. reddish pink: P 3589; Dodson & Thien 1090.

  [*Epidendrum excisum* Lindl. Sec. for., Yunguilla, 2600 m; ep., large paniculate infl., fls. creamy yellow-white (det. E. Hágsater)]

86. *Epidendrum fimbriatum* Kunth
  Sec. for., 1800-2000 m; usually terr., fls. white with purple markings: L 14046, 14048; Bohlin 1119; Harling & Andersson 11611; Dodson & Thien 1095.

87. *Epidendrum gastropodium* Rchb. f.
    2200-2500 m; ep. or terr.: K 1564; Dodson 10764; Suin 495.

88. *Epidendrum geminiflorum* Kunth
    Sec. & upper mont. for., 2000-2700 m; ep./terr., fls. green, non-resupinate: F 1413; K 1468; Dodson & Thien 1100; Plowman & Davis 4436(?) ; Suin 473.

89. *Epidendrum goodspeedianum* A. D. Hawkes
    Prim. for., 1800-2100 m; ep., fls. green with pink lip: 28688*; Bohlin 1127; Dodson 10819; Suin 327.

90. *Epidendrum hymenodes* Lindl.
    Sec. for., Cerro Palo Seco, 1250-1300 m; ep., fls. green with white lip: 27510*.

91. *Epidendrum* cf. *incomptum* Rchb. f.
    Sec. for., 1550-1600 m; ep., fls. greenish- or pinkish-brown: 31120, 32962. [Not in CVPE.]

92. *Epidendrum jamiesonis* Rchb. f. (incl. *E. evectum* Hook f.)
    Banks in sec. for., 2000 m; ep./terr., to 1.5 m, fls. magenta: 31854; Dodson & Thien 1098.

93. *Epidendrum macroöphorum* Hágsater & Dodson
    Nanegal, 1000-1800 m; ep./terr., fls. greenish white with white lip, ovary up to 25 cm long: Wallis 3219; Banks, 1200-1650 m; terr., stems to 2 m, fls. greenish-white with creamy white lip: 28163*, 28389, 31922; Holm-Nielsen 24507*.

94. *Epidendrum mancum* Lindl.
    2500 m; ep., fls. reddish purple with white.

95. *Epidendrum marsupiale* F. Lehm. & Kraenzl.
    Banks in sec. for., 1250 m; ep./terr., fls. pale green: 31087**.

96. *Epidendrum microcarpum* Hágsater & Dodson
    Prim. for., 2400 m; ep./terr., fls. small, greenish-yellow (det. E. Hágsater).

97. *Epidendrum nanegalense* Hágsater & Dodson
    Nono-Nanegal, Río Alamb??, km 43-45, 2200-2500 m: Sparre 15960 (type); Nanegalito, Sendero Principal, 1700 m; ep., terminal infls., pink fls.: Sparre 14866.

98. *Epidendrum nanum* C. Schweinf.
    El Pahuma, 2400 m; ep. fls, purple and white: Suin 431.

    [*Epidendrum pallatangae* Schltr.: Nono-Nanegal, 2200 m, Harling 14866.]

99. *Epidendrum paniculatum* Ruiz & Pav.
    Sec. for., Nono-Nanegal, 1600-2000 m; terr.: Dodson & Thien 1094, Dodson 6986. [Endemic to Peru, needs to be revised.]

100. *Epidendrum parvilabre* Lindl.
     Upper mont. for., Cerro Montecristi, 2250 m; ep., fls. green with white lip: 28917*.

101. *Epidendrum peraltum* Schltr.
     Prim. for., 2000 m.; ep., fls. magenta: 28861*.

102. *Epidendrum porphyreum* Lindl. (incl. *Epidendrum spathatum* Schltr.)
     Banks, 1600-2450 m; ep. or terr., fls. pink to magenta: 31918; F 1334; Sec. for. & scrub, 1650-2400 m; ep. or terr., fls. orange: 30067*, 30098*, 31181**, 31792; F 1068, 1402; Croat 72862*; Hurtado 1437*, 1441*; Suin 361, 376, 429.

103. *Epidendrum quitensium* Rchb. f.
     2000-3500 m; ep./terr., fls. purple, non-resupinate: Dodson et al 3732; Gudiño 1409*.

104. *Epidendrum ramosum* Jacq.
     Sec. for., 1200-1500 m; ep.: 28968*, 32916; Dodson 16509.

105. *Epidendrum renilabium* Schltr.
     Sec. for., 1700-2000 m; ep./terr., fls. yellowish: 27951*, 31924.

     [*Epidendrum repens* Cogn.: Nono-Tandayapa, km 16, ep./terr., Dodson 10768.]

106. *Epidendrum rostratum* Garay & Dunst.
     1000-2000 m; ep., plant pendent, branched, fls. greenish.

107. *Epidendrum scharfii* Hágsater & Dodson
      Sec. for. and clearings, 1250 m; ep.; fls. greenish, lip reniform

108. *Epidendrum* cf. *secundum* Jacq.
     Sec. for. & banks, 1200-2000 m; ep. or terr., fls. orange: 27502*, 28051*, 28153, 31631, 33610; Suin 447. [Needs to be revised, does not occur in Ecuador.]

     [*Epidendrum sodiroi* Schltr., described from near Gualea, may occur within the western boundary of our area.]

109. *Epidendrum tandapianum* Dodson & Hágsater
     Prim. for., 2200 m; ep./terr., fls. green: 28931*.

110. *Epidendrum trachysepalum* Hágsater
     Nono-Nanegal, 1200 m: Luer 774.

111. *Epidendrum* sp. 1
     Sec. for., 1200 m; ep.: 28843*.

112. *Epidendrum* sp. 2
     Sec. for., 1300 m; ep., fls. pale green: 31111.

113. *Epidendrum* sp. 3
    Nanegalito, 1800 m; ep.: C 13064.

114. *Eriopsis rutidobulbon* Hook.
    1500-2000 m; ep., fls. yellow with red margins and yellow column.

115. *Erythrodes clavigera* (Rchb. f.) Ames [= *Erythrodes boliviensis* Cogn.]
    Prim. & sec. for., 1650-2000 m; terr., fls. pinkish: 28223*, 28858*, 30066*.

116. *Erythrodes erythrodoides* (Schltr.) Ames
    Sec. for., 1450 m; terr., fls. greenish: 28849.

117. *Erythrodes jamesonii* (Garay) Dodson
    Nanegal, 1300 m; terr.: Jameson s.n.

118. *Erythrodes* sp. 1
    1400 m; terr.

    [A number of additional collections of *Erythrodes* remain unidentified to species.]

119. *Eulophia alta* (L.) Fawc. & Rendle
    Sec. for., 1300-1400 m; terr., fls. whitish: 29275.

120. *Eurystyles cotyledon* Wawra
    Sec. for., 1450-1500 m; ep./terr.: 31905.

121. *Gomphichis adnata* (Ridl.) Schltr. [= *Gomphichis hetaerioides* Schltr.]
    Sec. for., Nanegal to Gualea, 1300 m; ep./terr.: 30336; Río Umachaca; Sodiro s.n.

122. *Govenia sodiroi* Schltr.
    Between Nono & Tandayapa, 2100 m; terr.: Harling 19976.

123. *Govenia tingens* Poepp. & Endl.
    Sec. for., 1300 m; terr. fls. pale yellow with reddish-purple markings.

124. *Habenaria dentifera* C. Schweinf.
    Sec. for., 1350-1400 m; terr., fls. greenish: 27593*, 32359.

125. *Habenaria floribunda* Lindl.
    Sec. for., Nanegal; terr.: Sodiro s.n.*.

126. *Habenaria monorrhiza* (Sw.) Rchb. f.
    Sec. for., 1200-1850 m; terr., fls. white or greenish: 27089*, 27781*, 27785*, 28038, 28055*,
28164*, 28264*; Dodson 10824; Harling & Andersson 11560; Todzia 2478.

127. *Isochilus linearis* (Jacq.) R. Br.
   Sec. for., 1300-1350 m; ep.: 32360.

128. *Kefersteinia ocellata* Garay
   500-1500 m; ep., fls. greenish white, striped and spotted purple.

129. *Kefersteinia sanguinolenta* Rchb. f.
   Sec. for., Tandayapa, 1600 m; ep.: Dodson 18799A.

130. *Kefersteinia taurina* Rchb. f.
   Pahuma, Sendero Principal, 1500-2500 m; ep., fls. whitish green with red markings.

131. *Kefersteinia* sp. 1
   Rip. for., 1900 m; ep.: 31592.

132. *Lepanthes acarina* Luer
   1000-3000 m; ep., plant miniscule, fls. white with reddish violet petals (det. L. Jost).

133. *Lepanthes ballatrix* Luer
   Prim. for., 2400 m; ep., fls. white with yellow and purple (det. L. Jost).

134. *Lepanthes biloba* Lindl.
   Sendero Principal, 2000-3500 m; ep., fls. yellow or brownish with red-purple petals.

   [*Lepanthes brachypogon* Luer: west of Tandayapa, 2320 m, ep., Luer 7301 (type collection).]

135. *Lepanthes cassidea* Rchb. f.
   Forests, Nanegal; ep.: 1854, Jameson.

136. *Lepanthes effusa* Schltr.
   Nono-Nanegal, 2000 m; ep.: Dodson & Thien 1106.

137. *Lepanthes gargantua* Rchb. f.
   Prim. for., 1550-1600 m; ep., lvs. purplish, fls. white: 31055.

138. *Lepanthes kuijtii* Luer & Hirtz
   Prim. for., Santa Lucia, 1600 m; ep., fls. red with yellow-green margins, sepals with spreading tails (det. L. Jost).

139. *Lepanthes magnifica* Luer
   1000-2500 m; ep., lvs. purplish, with pubescent ridges underneath, fls. white with red-violet margins on petals.

140. *Lepanthes mucronata* Lindl.
   Forests, Nanegal; ep.: 1854, Jameson.

141. *Lepanthes nanegalensis* Rchb. f.
   Nanegal, ep.: "1864" [1854?], Jameson.

142. *Lepanthes pecunialis* Luer
   Sec. for., 1250-1600 m; ep., fls. orange: 27613*, 28130*, 31564(?); T 595; Dalström 1609; Luer 4753.

143. *Lepanthes pelyx* Luer & Hirtz
   Prim. for., 1600-1800 m; ep., lvs. thinly coriaceous, fls. light brown, petals green with fuschia lip (det. L. Jost).

144. *Lepanthes pilosella* Rchb. f.
   Nono-Tandayapa, km 17; ep.: Dodson 10788.

   [*Lepanthes pteropogon* Rchb. f.: Yunguilla, 2000-3500 m; ep.]

145. *Lepanthes rhodophylla* Schltr.
   Nanegal Valley near Río Frio; ep.: 1902, Sodiro 1896.

146. *Lepanthes villosa* Lojtnant
   Prim. for., Sendero Tranquilo; 1600 m; ep., fls. cream and purple, striped dark purple.

147. *Lepanthes zygion* Luer
   Sec. for., Tandayapa, 2000 m: Hirtz s.n. [cited by Valencia et al., 2000: 311].

   [Luer, 1986, reports 2 additional *Lepanthes* species of from near La Liberia & Pellagallo, just east of our boundary: *L. columbar* Luer and *L. stupenda* Luer.]

148. *Lockhartia chocoensis* Kraenzl.
   Sendero Tranquilo, 500-2000 m; ep., fls. yellow with red markings.

149. *Lockhartia longifolia* (Lindl.) Schltr.
   Sec. for., 1250-1600 m; ep., fls. yellow: 27625*, 28964*, 30428*.

150. *Lycaste ciliata* (Ruiz & Pav.) Lindl. ex Rchb. f.
   1200-2000 m; ep., fls. white, lip fimbriate.

   [*Lycaste fimbriata* Poepp. & Endl.: Calacalí, Reserva Geobotanica Pululahua, Cerón 1495, 00.05N, 78.30W.]

151. *Lycaste gigantea* Lindl. [in CVPE as *Lycaste longipetala* (Ruiz & Pav.) Garay]
   1500-3500 m; ep./terr., fls. greenish-yellow with red lip: Dalström 1606.

152. *Lycaste* sp. 1
   Nanegalito,1800 m; terr.: C 13048.

153. *Lycomormium ecuadorense* Sweet
    Sec. for., Inca Trail, 1350 m; ep., buds whitish, fls. pink with purple spots: 31083.

154. *Macroclinium perryi* (Dodson) Dodson
    Sec. for., Nanegalito, 1400-1600 m; ep.?: Dodson 6995 [type collection]; Hirtz 993.

155. *Malaxis* cf. *andicola* (Ridl.) Kuntze
    Sendero Humedal, 1300 m; ep., fls. greenish.

156. Malaxis fastigiata (Rchb. f.) Kuntze
    El Pahuma, 2500 m; terr.: Suin 490.

157. *Masdevallia anachaeta* Rchb. f.
    Nono-Tandayapa, km 17, 2160 m; ep.: Dodson 10787.

158. *Masdevallia angulata* Rchb. f.
    Sec. for., 1500-2000 m; ep.: C 5938; Luer 4723*; Suin 359, 403.

159. *Masdevallia nidifica* Rchb. f.
    Sec. for., 1900-2000 m; ep., fls. cream & purple: F 1020; Luer 2371*; Suin 422.

160. *Masdevallia ophioglossa* Rchb. f.
    Sec. for., 1700 m; ep.: Luer 5211*.

161. *Masdevallia parvula* Schltr.
    Nono-Nanegal, 2000 m; ep.: Dodson 1096.

162. *Masdevallia* aff. *saltatrix* Rchb. f.
    El Pahuma, 2000 m; ep., fls. purple with yellow base: Suin 445.

163. *Masdevallia ventricularia* Rchb. f.
    Prim. for., 1675-2250 m; ep., fls. maroon with orange tips: 31136; Luer 1695.

164. *Masdevallia* sp. 1
    Sec. for., 2100-2200 m; ep., fls. yellow: 30088.

165. *Masdevallia* sp. 2
    Prim. for., 2000 m; ep., fls. greenish-white: 28860.

166. *Masdevallia* sp. 3
    Nanegal, 1200-1700 m; ep.: Tipaz & Quelal 163*.

167. *Masdevallia* sp. 4
    Prim. for., 2125 m; ep.: 28948*.

168. *Maxillaria acutifolia* Lindl.
    Sec. for., 1200-1250 m; ep., fls. orange with burgundy lip: 27512*, 27536*.

169. *Maxillaria aggregata* (Kunth) Lindl.
    Sec. for., roadsides, Utopia; ep., fls. pink, thickly bunched at base of lvs.: AR1.

170. *Maxillaria aurea* (Poepp. & Endl.) L. O. Williams
    Loma Pahuamba, 1900-1950 m; ep./terr.: F 1146.

171. *Maxillaria calantha* Schltr. (incl. *Maxillaria nigrescens* Lindl.)
    Nono-Nanegal, 2000 m; ep.: Dodson & Thien 1099; El Pahuma, 2400 m: Suin 422 (aff.).

172. *Maxillaria cryptobulbon* Carnevali & J. T. Atwood
    Sec. for., 1375-1500 m; ep., fls. white or yellowish: 30396*, 31872, 32924.

173. *Maxillaria ecuadorensis* Schltr.
    Sec. for., 1200-2100 m; ep. or terr., fls. yellow with reddish-brown lobes: F 1249, 1495; N 8655*; Hurtado 1420*.

174. *Maxillaria grandiflora* (Kunth) Lindl.
    Sec. & upper mont. for., 2000-3200 m; ep., fls. 6-8 cm across, perianth white, lip yellow within, purple-striped without: 32815.

175. *Maxillaria jamesonii* (Rchb. f.) Garay & C. Schweinf.
    Nono-Tandayapa, 2200 m: Harling & Andersson 11633.

176. *Maxillaria lehmannii* Rchb. f.
    700-2000 m; ep., fls. large, up to 13 cm across, white with yellow inside lip and red outside: Dodson 15749*.

177. *Maxillaria lepidota* Lindl.
    Prim. & sec. for., 1600-2400 m; ep., fls. yellow with purple spots: 30457*, 31080, 32839; C 13030; P 3579*; Dalström 1605; Dodson & Thien 1089; Suin 339, 416, 450, 457.

178. *Maxillaria nanegalensis* Rchb. f.
    Nanegal; ep.: Jameson s.n.

179. *Maxillaria pardalina* Garay
    Nono-Nanegal & Loma Pahuamba, 1850-2100 m; ep., fls. yellow with red spots: 30186*; F 1493; Dodson 1091; Suin 266, 465.

180. *Maxillaria parviflora* (Poepp. & Endl.) Garay
    Sec. for., 1100-1225 m; ep.: 31160; Dodson 17063.

181. *Maxillaria. polyphylla* Rchb. f.
    Nono-Nanegal, 2000 m: Dodson & Thien 1092.

182. *Maxillaria porrecta* Lindl.
   Sec. for., 1200-1300 m; ep., fls. yellowish: 27511*, 27532*, 27610*.

183. *Maxillaria pseudoreichenheimiana* Dodson
   500-1500 m; ep., lvs. spotted white, fls. orange with white lip: AR17.

184. *Maxillaria ramosa* Ruiz & Pav.
   Sec. for., 1250-1300 m; ep., viney, fls. green: 27615*.

185. *Maxillaria* sp. 1 (sp. nov.)
   Sec. for., 1200-1400 m; ep., fls. white, sepal tips pink.

186. *Maxillaria* sp. 2
   1250 m; ep., cane-stem habit, aerial roots, fls. axillary, yellow-white.

187. *Maxillaria* sp. 3
   1800 m; ep., creeping, fls yellow with purple.

188. *Maxillaria* sp. 4 (rufescens group)
   1250 m; ep., fls. pink.

   [Two species of *Maxillaria* collected from Nono to Nanegal at 2100-2500 m appear to be slightly outside our limits: *M. luteo-rubra* (Lindl.) Rchb. f., Luer 4710; Mites 469; Plowman & Davis 4443; and *M. squarrosa* (Schltr.) Dodson, Dodson 16504.]

189. *Myoxanthus* sp. 1
   Sec. for., 1600-1900 m; ep., fls. pale yellow: 27821, 27966, 28010.

190. *Myoxanthus* sp. 2
   Sec. for. & banks, 1250-1350 m; ep. or terr., fls. greenish: 31031, 31088.

191. *Myrosmodes* sp.
   Prim. for., 1900 m; ep.: 30458*.

192. *Odontoglossum armatum* Rchb.f
   Prim. & sec. for., Pahuma, 1800 m; ep., fls. yellow brown with red spots.

193. *Odontoglossum cirrhosum* Lindl.
   Scrub on banks, above Río Pichan, 2000 m; ep./terr., fls. white with red dots, lip yellow: 30589**; Luer 2373.

194. *Odontoglossum cristatum* Lindl.
   Sec. for., 1850 m; ep., fls. yellow & brown: 30382**; Dodson 16507.

195. *Odontoglossum* "*denticulatum*" F. Lehm. ex Dalström ined.
    Prim. & upper mont. for., 2000-2750 m; ep., fls. yellow & brown: 30556**; Andreetta 216; Lehmann 8549. [Not in CVPE; unpublished name.]

196. *Odontoglossum hallii* Lindl.
    Prim. for., 1800-2000 m; ep., fls. yellow-green spotted with brown: 27333*.

197. *Odontoglossum* sp. 1
    Prim. for., Montecristi, 2725 m; ep.: 29513.

198. *Oerstedella medinae* (Dodson) Hágsater
    Sendero Tranquilo, 1300 m; ep., fls. green with pink lip.

199. *Oncidium hapalotyle* Schltr.
    Sec. for., Tandayapa, 1900 m; ep.: Harling & Andersson 11615*.

200. *Oncidium heteranthum* Poepp. & Endl.
    Nono-Tandayapa, km 17, 2160 m; ep.: Dodson 10784.

201. *Oncidium klotzscheanum* Rchb. f. (incl. *O. obryzatum* Rchb. f.)
    Sec. for., 1300 m; ep., fls. yellow with brown spots on lateral tepals: 27619*, 32929.

202. *Oncidium orthotis* Rchb. f.
    Sec. for., 1300-1600 m; ep., fls. yellow with brown spots: 27937*; Dodson 16510.

203. *Oncidium pentadactylon* Lindl.
    Prim. & upper mont. for. & scrub, 1550-2500 m; ep. or terr., fls. yellow: 28093*; K 1487; Dodson 6991; Holm-Nielsen 24505. "Margarita".

204. *Oncidium* sp. 1
    Sec. for., Pahuma, 1800 m; ep., yellow-red with white lip.

205. *Otoglossum anixopterum* (Rchb.f) Garay & Dunst.
    Prim & sec. for., Sta. Lucia, 2000-3000 m; ep., fls. orange and yellow with purple lip.

206. *Pachyphyllum falcifolium* Rchb. f.
    Calacalí-Nanegal; ep.: K 1523.

207. *Pelexia ecuadorensis* Schltr.
    Nanegal, 1300 m; terr.: Jameson s.n.

208. *Peristeria* cf. *lindenii* Rolfe
    Sec. for., 1350 m; ep., buds white: 31024.

209. *Phragmipedium lindenii* (Lindl.) Dressler & N. H. Williams
  Sec. for., 1300-1700 m; terr., tepals yellow with greenish veins, lobes purplish: 27968*; Dodson 10821; Sodiro s.n.

210. *Phragmipedium longifolium* (Rchb. f. & Warsz.) Rolfe
  Sec. for., Nanegal; terr.: Hartweg s.n.*

211. *Platystele* sp. 1
  Prim. for., 1650-1700 m; ep., fls. greenish: 31143.

  [*Platystele alucitae* Luer, Dodson 7315; and *Platystele microscopica* Luer, Dodson 7311; both from Tandayapa-Mindo, 2100 m, ep.]

212. *Pleurothallis anceps* Luer
  Sec. for., Nanegalito, 1600 m; ep.: Dodson 6993; El Pahuma, 2000 m: Suin 468.

213. *Pleurothallis antennifera* Lindl.
  Prim. for., Guantopungo, 2400 m; ep., fls. greenish yellow.

214. *Pleurothallis bicruris* Lindl.
  Prim. for., Montecristi, 2400 m; ep., fls. white mottled with red.

215. *Pleurothallis bivalvis* Lindl.
  Prim. & sec. for., Sendero los Gallos, 1300 m; ep., lvs. cordate, fls. green and red.

216. *Pleurothallis brachyblephara* Schltr.
  El Pahuma, 2000 m; ep., fls. purple: Suin 389.

  [*Pleurothallis cassidis* Lindl.: at Hacienda Yunguilla, 2800 m; ep., Haught 3181.]

217. *Pleurothallis chloroleuca* Lindl.
  Sec. for., 1500-1700 m; ep., fls. yellow: Q 121 (det. G. Carnevali).

218. *Pleurothallis cordata* (Ruiz & Pav.) Lindl.
  Prim. & upper mont. for., 1700-2725 m; ep./terr., plants greenish or purplish: 27443, 28206*, 28253*, 29502, 30560, 30567, 31227; L 13678, 13685, 13688; Suin 358, 390 (both aff.).

219. *Pleurothallis cordifolia* Rchb. f. & Wagener
  500-2500 m; ep./terr., lvs. cordate, fls. yellow or green with red, brown, or purple lip.

220. *Pleurothallis crossota* Luer & Dalström
  1500-2000 m; ep./terr., lvs. cordate, fls. brown or purple, dorsal sepal five-veined (det. L. Endara).

221. *Pleurothallis crucifera* Luer & Hirtz
  Sendero Tranquilo, 1250 m; ep., fls. yellowish-brown (det. L. Endara).

222. *Pleurothallis deflexa* Luer
Pahuma, 1800 m; ep., fls. deep purple with brown lip (det. L. Endara).

223. *Pleurothallis dibolia* Luer
Senderos Tranquilo & Principal, 0-1500 m; ep., fls. dull yellow with brown lip.

224. *Pleurothallis dunstervillei* Foldats
El Pahuma, 2400 m; ep., fls. purple: Suin 433.

225. *Pleurothallis ensata* Luer
Sendero Tranquilo, 1300 m; ep., lvs. narrowly cordate, fls. pink with yellow lip.

226. *Pleurothallis epiglottis* Luer
Pahuma, 1800 m; ep., fls. non-resupinate, white with blue (det. L. Endara).

227. *Pleurothallis erythrium* Luer
500-2500 m; ep., fls. reddish-purple (det. L. Endara).

228. *Pleurothallis gelida* Lindl.
1250 m, orchid garden; ep., long, congested raceme with translucent white fls.

229. *Pleurothallis imperialis* Luer
Banks between El Carmen & Marianitas, 1200-1250 m; ep.: 28159*.

 [*Pleurothallis jupiter* Lindl., Yunguilla, 2600 m; ep., lvs. cordate, fls. large, yelow brown

230. *Pleurothallis lacera* Luer
Montecristi, 2400 m; ep., fls. dark wine red, lip lacerate.

231. *Pleurothallis linguifera* Lindl.
Nono-Nanegal, 1200 m; ep., fls. yellow-brown to reddish-brown: Luer 798.

232. *Pleurothallis macra* Lindl.
Upper mont. for., 2500-2750 m; ep., fls. yellow: K 31979; L 13675, 13682, 13683.

[*Pleurothallis oblonga* Luer & Hirtz, from La Liberia, at 2800 m, Hirtz 1416, is 2' E of the area boundary.]

233. *Pleurothallis ramulosa* Lindl.
Nono-Nanegal, 2000 m; ep.: Dodson & Thien 1103.

234. *Pleurothallis restrepioides* Lindl.
Pahuma, 1800-2500 m; ep., fls. white or yellow, heavily suffused with red, lip purplish: Suin 492.

235. *Pleurothallis ruberrima* Lindl.
     Prim. & sec. for., 500-2500 m; ep., fls. red with yellow.

236. *Pleurothallis ruscifolia* (Jacq.) R. Br.
     Prim. for., 1700-1800 m; ep., common, fls. yellow: 27811*, 28061*, 28062, 28069*, 28194*.

237. *Pleurothallis scabrilinguis* Lindl.
     Sec. for., 1800 m; ep.: Luer 13321.

238. *Pleurothallis sclerophylla* Lindl.
     Loma Pahuamba, 1900-2000 m; ep.: F 1141; Suin 448.

239. *Pleurothallis sicaria* Lindl.
     Sec. for., 1350-1400 m; ep., fls. & frs. dark green: 32918 (det. D. Kelch).

240. *Pleurothallis stevensonii* Luer
     Prim. for., 1750 m; ep., fls. white with red spots: 28846*.

241. *Pleurothallis tripterantha* Rchb. f.
     Sendero Humedal, 1300 m; ep., fls. cleistogamous.

     [*Pleurothallis truncata* Lindl.: Yunguilla, 2600 m, fls. orange-red.]

242. *Pleurothallis undulata* Poepp. & Endl.
     Sec. for., Tandayapa, 1200-1300 m; ep.: Hirtz 1892, 1893.

243. *Pleurothallis zephyrina* Rchb. f.
     Sec. for., 2150 m; ep.: Dodson 15750.

244. *Pleurothallis* sp. 1
     Sec. for.; Pahuma, 1800 m; long raceme, yellow frs. with red.

245. *Pleurothallis* sp. 2
     Sec. for., Sendero Tranquilo, 1250 m; lvs. cordate, red fls., cross shaped.

246. *Pleurothallis* sp. 3
     Sec. for.; 1250 m, orchid garden; long raceme, small white fls.

247. *Pleurothallis* sp. 4
     Prim. for., Montecristi; 2500 m; pink fls., pendent with 'cap' dorsal sepal.

248. *Pleurothallis* sp. 5
     Prim. for.; Montecristi; 2400 m; like sp. 4, but smaller, with yellow lines.

249. *Pleurothallis* sp. 6
     Sec. for., Pahuma, 1800 m; ep., lvs. cordate, fls. red-brown.

[*Pleurothallis* sp. 7: Yunguilla, 2600 m; ep., fls. red and yellow, pubescent.]

[A considerable number of collections of *Pleurothallis* remain unidentified to species.]

250. *Polystachya* cf. *foliosa* (Lindl.) Rchb. f.
Sec. for., 1250-1600 m; ep., fls. translucent yellow: 30423, 31567. [These specimens may be *Polystachya concreta* (Jacq.) Garay & H.R. Sweet].

251. *Polystachya concreta* (Jacq.) Garay & H.R. Sweet
Sec for. & clearings, 0-1500 m; ep., fls. yellow-white.

252. *Ponthieva disema* Schltr.
Sec. & upper mont. for., 2000-2600 m; ep./terr., fls. white: 32402; Haught 3162.

253. *Ponthieva pseudoracemosa* Garay
Sec. for., 1600-2500 m; terr.: Smith 1946; Sparre 16795.

254. *Porphyrostachys* sp.
Sec. for., 1300-1400 m; terr., fls. green: 32358. [Our plants differ from *Porphyrostachys pilifera* (Kunth) Rchb. f. in the distinctly green, rather than red, flowers.]

255. *Porroglossum amethystinum* (Rchb. f.) Garay
Prim. & sec. for., 1200-2000 m; ep., fls. purplish: 27963*, 29201*; T163*.

256. *Porroglossum muscosum* (Rchb. f.) Schltr.
Sec. for., 2000 m; ep.: C 5952.

257. *Porroglossum* sp. 1
Sec. for., 1475 m; ep. on mossy log, fls. pale maroon: 31898.

258. *Prescottia stachyodes* (Sw.) Lindl.
Sec. for., 1300-2000 m; terr., fls. greenish-white: 32384, 32455; Hirtz 1890.

259. *Prosthechea fragrans* (Sw.) W.E. Higgins
Sec. for., 1200-1250 m; ep., fls. white with red spots: 27535*.

260. *Prosthechea hartwegii* (Lindl.) W.E. Higgins
Sec. for., 1300-2000 m; ep., fls. greenish-yellow with red: V 12309; Hirtz 1891*.

261. *Prosthechea pamplonense* (Rchb. f.) W.E. Higgins
Tandayapa, 1800 m; ep., fls. green with black markings, fragrant: Dodson 6989.

262. *Prosthechea vespa* (Vell.) W.E. Higgins
Sec. for., 1700-2000 m; ep., fls. green and black or reddish-brown: 28037*, 31643.

263. *Pseudocentrum macrostachyum* Lindl.
Sec. for., 2200-2650 m; terr., fls. green: Dodson 15774; Molau 3037.

264. *Psygmorchis pumilio* (Rchb. f.) Dodson & Dressler
Sec. for., 1250 m; ep., fls. yellow: 28129*.

[*Pterichis triloba* (Lindl.) Schltr.: Yunguilla, 2600 m, ep., entire plant pubescent, fls. yellow-orange, non-resupinate.]

265. *Restrepiopsis tubulosa* (Lindl.) Luer (incl. *R. viridula* (Lindl.) Luer)
Nono-Tandayapa, km 17, 2160 m; ep.: Dodson 10786.

266. *Rodriguezia lehmannii* Rchb. f.
Sec. for., 1250-1700 m; ep., fls. white with purplish or reddish lines: 27447 ex p.*, 27845*, 27977*, 31109; T 597*. [29078, with yellowish-green fls., may represent another species.]

267. *Scaphosepalum ophidion* Luer
Prim. for., Sta. Lucia, 1500-2500 m; ep., fls. yellow-brown or purple with purple markings throughout.

268. *Scaphosepalum swertiifolium* (Rchb. f.) Rolfe
Prim. for., Sta. Lucia, 500-2500 m; ep., fls. white or yellow with brown or purple, extended sepaline tails.

269. *Scaphosepalum* sp. 1
Prim. & upper mont. for., 2250-2700 m; ep., fls. purple: 28940*; K 31983.

270. *Scaphyglottis prolifera* Cogn.
Sec. for. & clearings; 0-1500 m; ep., fls. white with violet colorations.

271. *Scelochilus chiribogae* Dodson
Sec. for., Nanegalito, 1400 m; ep.: Hirtz 989*.

272. *Scelochilus heterophyllus* Rchb. f.
Sec. for., Tandayapa, 1800 m; ep.: Hirtz 2179*.

273. *Scelochilus jamiesonii* Lindl.
Sec. for., Tandayapa, 2000 m; ep.: Dodson 16653.

274. *Schlimia stevensonii* Dodson
Prim. & sec. for., 1200 m, ep., fls. pink with red spots.

275. *Sertifera purpurea* Lindl. & Rchb. f.
Prim. for., 21 km west of Calacalí, 2500 m; terr.: L 13677.

276. *Sertifera* sp. 1
   Prim. for., 2250-2300 m; ep.: 28919*.

   [*Sigmatostalix adamsii* Dodson: Quito-Tandayapa, 2300 m, ep., Dodson 13093.]

277. *Sigmatostalix picta* Rchb. f.
   Sec. for. & clearings, Sendero Tranquilo, 0-2000 m; ep., fls. yellow and brown.

278. *Sigmatostalix* sp. 1
   Sec. for., 1300 m; ep., fls. dull yellow with brown spots: 27684.

279. *Sobralia atropubescens* Ames & C. Schweinf.
   0-2500 m; terr., stems 1 m, fls. orange-brown, lip pinkish suffused white and yellow.

280. *Sobralia crocea* (Poepp. & Endl.) Rchb. f.
   Sec. for., 1600 m; ep./terr.: Dodson 6992; Molau 2230.

281. *Sobralia ecuadorana* Dodson
   Sec. for., Tandayapa, 1850 m; ep./terr., fls. pink, lip white with yellow, strongly fragrant of vanilla: Dodson 10820.

282. *Sobralia gloriosa* Rchb. f.
   Sec. for., 1200-1500 m; terr., stems 1 m, fls. white with maroon lip: 31941*; V 12264*.

283. *Sobralia klotzscheana* Rchb. f.
   Sec. for., 1450-2100 m; terr., to 1.5 m, fls. pink or white with pink lip: 27967*, 30451, 30481(?), 30502, 32969. "Maygua"

284. *Sobralia lancea* Garay
   500-2000 m; orchid garden; terr., stems to 50 cm, fls. creamy white with a purple spot.

285. *Sobralia pulcherrima* Garay
   Banks, 1200-1800 m; terr., to 4 m, fls. pink, lip with purplish lines: 28302, 29979*, 31941; Harling & Andersson 11751 (between Nanegal & Nanegalito, [type collection]); Holm-Nielsen 24516*.

286. *Sobralia rosea* Poepp. & Endl.
   Steep banks in sec. for., 1200-1300 m; terr., canes to 3 m, fls. white, lip with purplish lines: 27079*; Asplund 1571; Sodiro 134 .

287. *Sobralia valida* Rolfe
   Sec. for., 1300 m; terr., canes to 1 m; fls. fragrant, white with yellow throat, lip violet-edged: 31342**.

288. *Stanhopea impressa* Rolfe
   Sec. for., Tandayapa, 1600 m; ep.: Dodson 18799.

289. *Stanhopea* sp. 1
    Sec. for., Hacienda El Carmen, 1250 m; ep.: Osbourn s.n.

    [*Stelis alba* Kunth: at Hacienda Yunguilla, 2800 m, ep., Haught 3187.]

290. *Stelis allenii* L.O. Williams
    1250 m, orchid garden; ep., fls. reddish-purple (det. L. Endara).

291. *Stelis argentata* Lindl.
    Prim. & sec. for. & clearings, 0-1000, 1500-2000 m; ep., pink or purple fls.

292. *Stelis calotricha* Schltr.
    Prim. & sec. for., 1250 m; ep., plant to 2 cm, fls. purple (det. L. Endara).

293. *Stelis columnaris* Lindl.
    Sec. for. & roadsides, 1800 m; ep., fls. purple, column extended past sepals (det. L. Endara).

294. *Stelis concinna* Lindl.
    Sec. for. & roadsides, 2000 m; ep., fls. purple, pubescent, on long, flexuous infl. (det. L. Endara).

295. *Stelis eublepharis* Rchb.
    Prim. & sec. for., Pahuma, 1800 m; ep., fls. pink, sepals pubescent.

296. *Stelis flacca* Rchb.f.
    Prim. for., Sendero Tranquilo, 1600 m; ep., fls. purple: AR18.

297. *Stelis hirtzii* Luer
    Prim. & sec. for.; 1200-1800 m; ep., fls. greenish-purple: AR6 (det. L. Endara).

298. *Stelis jamesonii* Lindl.
    Prim. for., Sendero Tranquilo, 1600 m; ep., fls. pink with yellow: AR22 (det. L. Endara).

299. *Stelis* cf. *lindenii* Lindl.
    Prim. for., Sendero Tranquilo, 1600 m; ep., fls. yellow-green with purple: AR19 (det. L. Endara).

300. *Stelis morganii* Dodson & Garay
    Sec. for., 1550-2000 m; ep., fls. cream, pubescent: 31784; T 596*; Suin 449 (aff.).

301. *Stelis* cf. *mucronata* Lindl.
    Sec. for. & roadsides; 1800-2000 m; ep., fls. purple (det. L. Endara).

302. *Stelis nanegalensis* Lindl. (incl. *S. vulcanica* Rchb. f.)
    Nono-Nanegal, 2000 m; fls. pink or yellow: Luer 6325A; AR16.

303. *Stelis pusilla* Kunth
   Prim. for., 1650-2500 m; ep., fls. pale yellow: 27964*; L 14045; Suin 491.

304. *Stelis striolata* Lindl.
   Prim. & sec. for. & roadsides; 1200-2000 m; ep., fls. yellow with purple.

305. *Stelis triseta* Lindl.
   Sec. for. & roadsides, 1800-2000 m; ep., fls. purple and green. (det. L. Endara).

[A number of additional collections of *Stelis* remain unidentified to species.]

306. *Stellilabium andinum* (L. O. Williams) Garay & Dunst.
   Sec. for., Nanegalito, 1400 m; ep.: Hirtz 991*.

307. *Stellilabium astroglossum* (Rchb. f.) Schltr. (incl. *S. tanii* Dodson)
   Sec. for., Tandayapa, Nanegalito 1500-1600 m; ep.: Dodson 6994, 6995, 16511, Palacios 12843.

308. *Stellilabium hirtzii* Dodson
   Sec. for., Nanegalito, 1400 m; ep.: Hirtz 992* [type collection].

[*Stenorrhynchos cernuus* Lindl., west of Nanegal, 1200 m, ep., Hartweg s.n. (type collection) is probably extralimital.]

309. *Stenorrhychos speciosum* (Jacq.) Rich. ex. Spreng.
   Prim for., Cerro Sosa, 2225 m; ep.: 29428*

310. *Symphyglossum sanguineum* (Rchb. f.) Schltr.
   Sec. for. & scrub, 1700-2000 m; ep., fls. red: 30155 (det. S. Dalstrom), 30588*; C 7175*; Dodson 6984.

[*Telipogon hagsateri* Dodson & R. Escobar, Yunguilla, 2600 m.]

311. *Telipogon steinii* Dodson & R. Escobar
   Prim. for., Pahuma, 1800-1850 m; ep., fls. yellow with red-brown veins, lip red with spines (det. Dodson); Stein 2863 [type collection].

312. *Trichopilia fragrans* Lindl.
   Prim. for., 1300-2000 m; ep. or terr., fls. white, fragrant: 32920; C 5926*.

313. *Trichopilia rostrata* Rchb. f.
   Prim. for., 1750 m; ep., fls. white, throat pink within: 28848*.

314. *Trichopilia steinii* Dodson
   1250 m, orchid garden; ep., fls. white with yellow throat, fleshy: #03-039 (fl in alcohol)

315. *Trichosalpinx dura* (Lindl.) Luer
   Prim. for., 1900-2000 m; ep., buds yellowish: 27751; Dodson & Thien 1105.

316. *Trichosalpinx memor* (Rchb.f.) Luer
   Prim. & sec. for.; 1250 m; ep., lvs. purple, fleshy, fls. red-purple with yellow.

317. *Trichosalpinx* aff. *microcharis* (Schltr.) Luer
   El Pahuma, 2000 m; ep., fls. cream: Suin 470.

318. *Trichosalpinx* sp. 1
   Sec. for., 1600 m; ep.: 31124.

319. *Trisetella vittata* (Luer) Luer
   Sec. for., Tandayapa; ep.: Luer 5209*.

320. *Xylobium elongatum* (Lindl. & Paxt.) Hemsl.
   Prim. & sec. for., 1250-1625 m; ep., fls. whitish, lip yellow: 28390, 31074.

321. *Xylobium foveatum* (Lindl.) G. Nicholson
   0-2000 m, orchid garden; ep., fls. white.

322. *Xylobium leontoglossum* (Rchb. f.) Rolfe
   Sec. for., 1200-2150 m; ep., fls. dull creamy white: 30368*, 31009, 31152(?); C 5927*; T
156*; Hurtado 1436*; Molau 3041; Suin 360, 440, 464.

323. *Xylobium pallidiflorum* (Hook.) G. Nicholson
   Sec. for., 1250-1600 m; ep., fls. yellow and white: 27933*, 28175*; T 594*.

324. *Xylobium* sp. 1
   1250 m; orchid garden; ep., pseudobulbs with 2-3 lvs, fls. pink, lip reddish-yellow.

325. *Zootrophion dayanum* (Rchb. f.) Luer
   Prim. & sec. for.; 1500-2500 m; ep., fls. red with yellow throughout.

326. *Zootrophion hirtzii* Luer
   Sec. for., 1600 m; ep., fls. yellow: Hirtz 2166.

327. *Zootrophion hypodiscus* (Rchb. f.) Luer
   Sec. for., Sendero del Río, 1000-2000 m; ep., fls. reddish-brown with yellow.