Chapter 2. Materials and Methods

Up to the age of 30 or beyond it, poetry of many kinds...gave me great pleasure, and even as a schoolboy I took intense delight in Shakespeare.... formerly pictures gave me considerable, and music very great, delight. But now for many years I cannot endure to read a line of poetry: I have tried to read Shakespeare, and found it so intolerably dull that it nauseated me. I have also almost lost any taste for pictures or music.... I retain some taste for scenery, but it does not cause me the exquisite delight which if formerly did.... My mind seems to have become a kind of machine for grinding general laws out of large collections of facts, but why this should have caused the atrophy of that part of the brain alone, on which the higher tastes depend, I cannot conceive.... The loss of these tastes is a loss of happiness, and may possibly be injurious to the intellect, and more probably to the moral character, by enfeebling the emotional part of our nature.

- Charles Darwin

The investigation of nature is an infinite pasture-ground, where all may graze, and where the more bite, the longer the grass grows, the sweeter is its flavor, and the more it nourishes.

- Thomas Henry Huxley (Cited in D. Boorstin, The Discoverers)

Study Site Selection

Potential wetland study sites were considered based upon a number of sources, including satellite images, vegetation maps, and topographic maps. Information was also obtained from other botanists, ecologists, ornithologists, and other researchers familiar with biological fieldwork in Bolivia. Additional input was sought from administrators of the Fundación Amigos de la Naturaleza (F.A.N.), the organization that manages a number of Bolivia's national parks, and from park guards and guiás del campo (field guides) in the National Park System. Potential study sites were also identified from site descriptions on herbarium specimens at the Museo Noel Kempff Mercado (USZ; Santa Cruz), the Herbario Nacional (LPB; La Paz), and the Herbario Forestal Martín Cárdenas (BOLV; Cochabamba). Study sites were chosen to create a reasonable representation of Bolivia's geographic, ecological, and elevational variability. At times, study site selections were weighted by necessity towards accessibility and the opportunity to coordinate with other ongoing biological investigations in the vicinity of the study sites.

Forty-six wetland systems were chosen as study sites (Table 2.1; Appendix A). Because meeting the objectives of this study necessitated establishing a large number of widespread study sites, it was critical to limit the types of wetland habitats included. Inundated riparian forest and seasonally inundated savanna can clearly be considered as wetlands (sensu Cowardin et al. 1979; Mitsch and Gosselink 1993), however, the inclusion of these types of habitats would have added enormously to the complexity of the project. It seemed that their exclusion would not detract significantly from attaining my study objectives, therefore, research focused on “traditional” (from a northern temperate viewpoint) aquatic habitats: those occupying basins or channels (i.e., lakes, ponds, rivers, and streams) and those inundated throughout the greatest part of the year (i.e., “marshes” and “swamps”). Nevertheless, although no inundated riparian forest or seasonally inundated savannas were included as study sites, general botanical reconnaissance and collecting were undertaken in these habitats and specimen data from this fieldwork were included in regional checklists (see Appendix B; Appendix D). Additionally, data from studies of a variety of inundated forest types (e.g., Foster et al. 1997; Junk 1989; Keel and Prance 1979; Klinge et al. 1990; Pires and Prance 1985; Worbes 1997) were utilized in the ascription of wetland species (see below).

The study sites encompassed a wide elevational range (Table 2.1) with the lowest site at about 90 m and the highest above 4400 m. Distribution of the study sites favored the lowlands. This distribution was partially due to the small number of wetlands in montane and dry valley habitats relative to the abundant lowland systems, and to the difficulties associated with locating wetlands in forested montane habitats. Moreover, as research progressed much more time was spent investigating the lowlands, as lowland systems generally proved to be richer and more interesting.

The study sites were also broadly distributed geographically throughout Bolivia (Figure 2-1), although there was a strong correlation between study site density and proximity to the city of Cochabamba, my primary place of residence during the course of field research. Nevertheless, at least one study site was established in all but one of Bolivia’s nine Departamentos (the principal political division, (Table 2.1; Figure 2-1), with Potosi the sole Departamento lacking a study site.

Table 2.1. Forty six study sites selected to represent Bolivian wetlands, with region, elevation, estimated area, Departamento (the 1st major political division), Provincia (the 2nd level political division), and major watershed. Key to Regions: AP, Andean Piedmont; CF, Cloud Forest; CH, Chapare; CQ, Chiquitanía; GC, Gran Chaco; HA, High Andean; LM, Lower Montane; T, Transition Zone; VS, Valles Secos; WW, Whitewater Floodplain.

Site	Study Site	Region	Elev. (m)	Area (ha.)	Departamento	Provincia	Watershed
P	Laguna Toro	HA	4420	2.5	Cochabamba	Ayopaya	Amazon
U	Huayalmarca Pond	HA	4300	0.1	Oruru	Cercado	Desaguadero
Q	Laguna Saythu Khocha	HA	4020	40	Cochabamba	Tiraque	Amazon
Q	Laguna Totora Khocha	HA	3620	120	Cochabamba	Tiraque	Amazon
P	Laguna Larati	HA	3540	124	Cochabamba	Chapare	Amazon
Q	Laguna Juntutuyo	HA	3360	244	Cochabamba	Arani	Amazon
O	Río Candelaria	HA	3165	1	Cochabamba	Chapare	Amazon
R	Laguna Chulichuncani	HA	3160	20	Cochabamba	Carrasco	Amazon
P	Laguna Alalay	VS	2550	170	Cochabamba	Cercado	Amazon
S	Río Mizque Wetland	VS	1970	0.5	Cochabamba	Mizque	Amazon
V	Río Guadalquivir Wetland	VS	1800	0.5	Tarija	Cercado	Paraná
P	Tiquipaya Irrigation Canal	VS	2620	0.02	Cochabamba	Cercado	Amazon
O	Chimpa Huata Bog	CF	2920	0.05	Cochabamba	Chapare	Amazon
O	Incachaca Pond	CF	2385	1	Cochabamba	Chapare	Amazon
O	Laguna Khonchu - East	CF	2620	0.07	Cochabamba	Chapare	Amazon
O	Laguna Khonchu - West	CF	2620	0.09	Cochabamba	Chapare	Amazon
O	Corani Pampa Marsh	CF	2470	0.02	Cochabamba	Chapare	Amazon
R	Siberia Marsh	CF	2800	0.75	Cochabamba	Carrasco	Amazon
M	Mariposa Wetland	CH	220	0.8	Cochabamba	Carrasco	Amazon
M	Ivirgarsama Marsh	CH	220	0.7	Cochabamba	Carrasco	Amazon
M	Senda F Wetland	CH	220	0.1	Cochabamba	Carrasco	Amazon
N	Villa Tunari Pond	CH	300	0.3	Cochabamba	Carrasco	Amazon
N	Sinahota Pond	CH	240	0.15	Cochabamba	Tiraque	Amazon
M	Valle de Sajta Curichi	CH	210	0.2	Cochabamba	Carrasco	Amazon
M	Puerto Villarroel Laguna	CH	190	30	Cochabamba	Carrasco	Amazon
A	Riberalta Ciénaga	WW	170	150	Beni	Vaca Díez	Amazon
A	Laguna Tumechuqua	WW	170	300	Beni	Vaca Díez	Amazon
B	Laguna Suarez	WW	160	600	Beni	Cercado	Amazon
K	Bermudez Curichi	AP	430	15	Santa Cruz	Andres Ibañez	Amazon
K	Viru Viru Wetland	AP	430	15	Santa Cruz	Andres Ibañez	Amazon
W	Laguna Yaguacua	CC	920	30	Chuquisaca	Luís Calvo	Paraná
G	Concepción Wetland	CQ	485	10	Santa Cruz	Ñ de Chavez	Amazon
E	Huanchaca Arroyo	CQ	760	0.04	Santa Cruz	Velasco	Amazon
F	La Toledo Curichi	CQ	220	6	Santa Cruz	Velasco	Amazon
F	Bahia Toledo	CQ	210	150	Santa Cruz	Velasco	Amazon
F	Río Paraguá	CQ	210	0.5	Santa Cruz	Velasco	Amazon
D	Cuatro Vientos Palm Swamp	CQ	205	690	Santa Cruz	Velasco	Amazon
C	Lago Caimán	CQ	200	575	Santa Cruz	Velasco	Amazon
H	Laguna Uberaba	P	85	30,000	Santa Cruz	Angel Sandoval	Paraná
H	Laguna La Gaiba	P	90	10,500	Santa Cruz	Angel Sandoval	Paraná
I	Laguna Mandioré	P	90	25,000	Santa Cruz	Angel Sandoval	Paraná
J	Laguna Cáceres	P	90	3,500	Santa Cruz	German Busch	Paraná
H	Puesto Gonzalo	P	90	2	Santa Cruz	Angel Sandoval	Paraná
L	Laguna Volcan	T	1150	3	Santa Cruz	Florida	Amazon
T	Yolosa Marsh	LM	1150	0.05	La Paz	Nor Yungas	Amazon
N	Cristalmayu Pond	LM	640	0.5	Santa Cruz	Andres Ibañez	Amazon

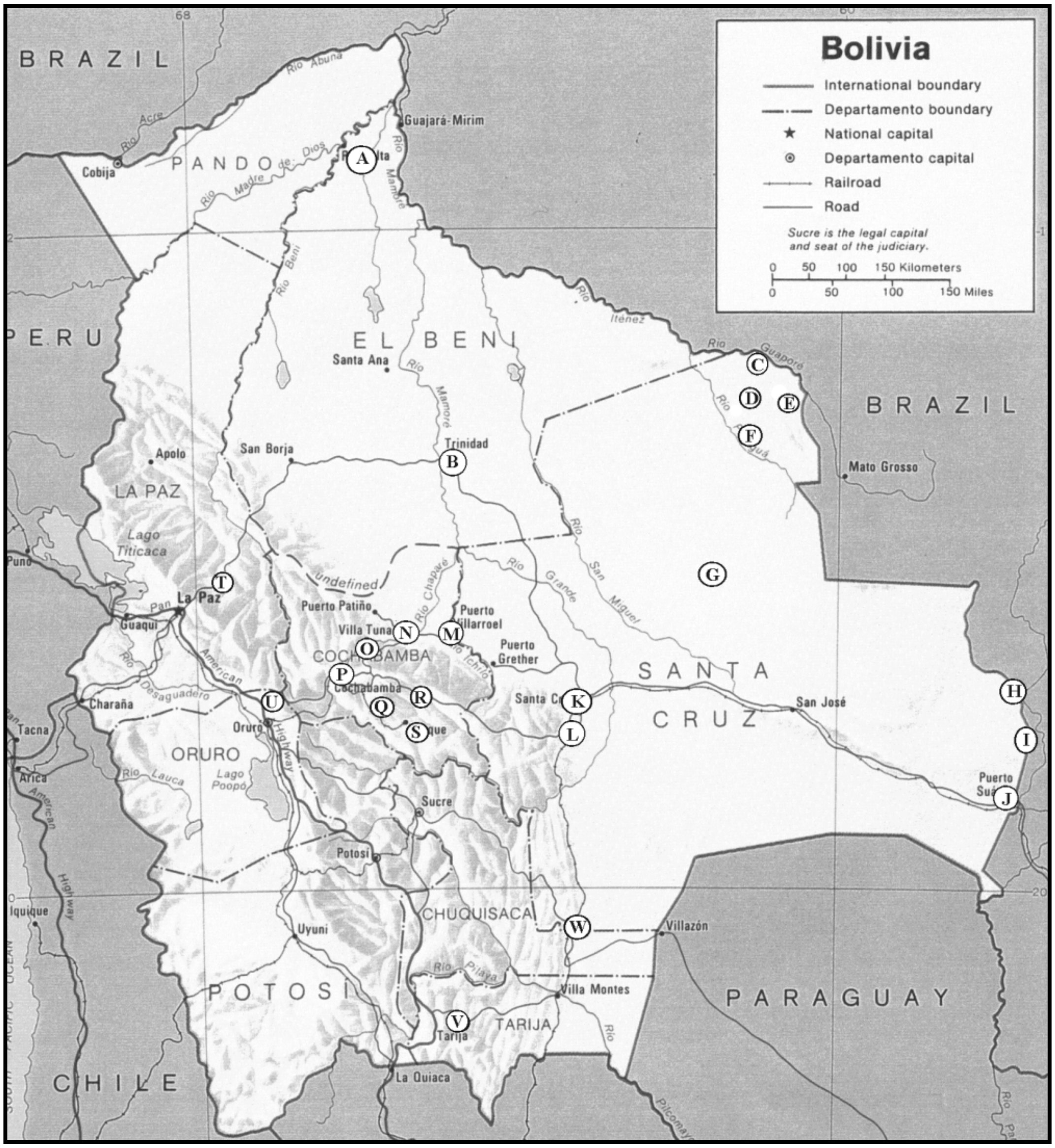


Figure 2-1. The forty-six Bolivian study sites. Letters in circles correspond to study sites as indicated in Table 2.1.

Vegetation Sampling

Initially, consideration was given to establishing a plot-based sampling methodology. In recent decades, researchers assessing phytodiversity in Neotropical terrestrial habitats have frequently employed standardized 0.1 hectare samples (e.g., Gentry 1988a, 1995). In this approach, plants with dbh >2.5 cm (trees, “treelets”, lianas and “even overgrown herbs” Gentry 1995) are censused from a series of 2 m X 50 m quadrats. Special problems are encountered in vegetation sampling in aquatic ecosystems; therefore, protocols developed for terrestrial habitats are often inadequate for aquatic research.

Some methodologies have been proposed specifically for floristic sampling in aquatic habitats. Crow (1993) cited the work of Gentry (1988a) and noted that no analogous approach had been developed for sampling in wetlands. Crow (1993, p. 253) suggested that 0.01 ha might serve as an appropriate sample size in wetlands, as “a relatively quick means of gathering data sets to make comparisons (of species richness) between various aquatic habitats.” Apparently, a single 0.01 ha quadrat was assumed to be sufficient for any size system. Jensen (1977, p. 107) proposed a methodology for sampling aquatic vegetation in lacustrine systems, with an aim toward developing “a rapid method to characterize and sample the macrophyte vegetation.” Sampling was limited to a series of nested relevés that were situated along a belt transect around the perimeter of the lake and along a series of profile transects. Relevé size was dependent upon the dominant life-form of the zone being sampled, while the number of profile transects were a function of shoreline length, system area, and fetch. Dubois et al. (1984) adopted the methods of Jensen and proposed some modifications for riverine systems.

Despite the demonstrated utility of some of these methods, applying any plot-based methodology to many of the Bolivian study sites would have been problematic. In some systems, the width of the vegetated zone from the marsh edge to open water approached 100 meters. This approach would have necessitated installing long, narrow quadrats barely a meter in width. The logistics of delineating such quadrats on the flexible and often weakly coalesced floating mats of vegetation that are characteristic of many lowland wetland habitats would have been daunting.

Furthermore, wetlands in the lowland Tropics are often extremely dynamic, with multiple "growing seasons" occurring in a single year due to seasonal changes in hydrology. Hence, an area dominated by a particular group of species during one set of hydrologic conditions may support an entirely different flora during a subsequent hydrologic period. In some systems, this process manifests as a seasonal change from hydrophytic to terrestrial vegetation. In other systems, however, different associations of hydrophilic vegetation may dominate the same area during different seasons. Clearly, in order to achieve a complete estimate of diversity a site should be sampled during as many different hydrologic stages as possible. Hence, additional difficulties would arise in any plot-based study because of the need to accurately re-locate quadrats during repeated sampling.

Although re-locating sampling areas might not present a significant problem in most temperate wetlands, floating mats in Neotropical wetlands frequently undergo physical changes, subsiding and rising in response to changes in water level, and foundering under certain conditions. Mats are also subject to other large-scale physical changes. For example, extensive sections of floating mats can break off and drift away and free-floating sections can merge with shore-bound mats. Therefore, in addition to the problems associated with delineating sampling areas on such a dynamic substratum, and in confidently re-locating quadrats, it is conceivable that a number of quadrats would be lost to migration or subsidence.

In addition to the need to devise a strategy to overcome the problems associated with sampling on floating mats and with seasonal variations in species composition, the question still would have remained as to whether plot-based sampling was the best approach for meeting the objectives of this study. Specifically, as the positive relationship between species richness and area has long been known (e.g., Brown 1988; Rosenzweig 1995), it is obvious that sampling from the maximum possible area (i.e., the entire system) would yield a more complete floristic account than sampling from only a portion of the system (i.e., quadrats). A plot-based methodology would have added significantly to the amount of time required at each study site and would not have replaced the need to conduct broad floristic surveys of the systems; therefore, quantitative sampling was eschewed and fieldwork focused on assembling comprehensive site floras.

Whenever possible, each study site received multiple visits. Fieldwork was widely spaced temporally so that the sites were observed under varying hydrologic conditions and in different seasons. Sampling commonly commenced at a convenient point of entry and entailed a systematic survey of localized areas until no new species were encountered, or until the time available at the site had elapsed. Smaller (i.e., < ca. 30 ha) systems were surveyed in their entirety; however, in many cases the sites were too large to allow this approach. Therefore, with large (i.e., > ca. 30 ha) systems, sampling was preceded by the identification of distinct communities and habitats. Subsequently, surveying focused on these areas. Criteria used in identifying these areas were as follows: 1) differences in dominant species; 2) discernible differences in hydrology; 3) microtopographic variations (i.e. differences in substratum or differences in the degree of sedimentation of floating mats); and, 4) obvious disturbance. Large study sites also received as complete a survey as possible; however, time and resource limitations minimized this activity at many systems.

Specimen Collection and Preparation

Plant specimens were taken preferentially from fertile individuals but sterile specimens were collected whenever fertile material was lacking. Despite the contention that there is little value in collecting sterile aquatic specimens (Haynes 1984), sterile specimens were collected to document the presence of a species. If, for a particular species, only sterile material was encountered during the initial visit to a site, a concerted effort was made to locate fertile material on following visits. In the same manner, if poor quality specimens of fertile material were collected during an initial visit to a site, an attempt was made to obtain more suitable specimens during a subsequent visit.

Many aquatic species possess flowers that are very delicate and that make substandard specimens when normal pressing and drying procedures are employed. In these cases, the flowers were dried separately in small (ca. 8 cm x 14 cm) presses, with only light pressure applied to the closures (Haynes 1984). At times, delicate flowers were also preserved in vials of 70 percent ethanol.

Submerged macrophytes with highly-dissected leaves (e.g., *Myriophyllum* spp., *Apalanthe* spp., and *Cabomba* spp.) often clump badly when pressed without special attention. Whenever possible, these species were “wet-mounted” (floated onto wet sheets of newsprint) to spread their leaf segments, and then dried using standard procedures (cf. Ceska and Ceska 1986; Haynes 1984; Taylor 1977). Additionally, for species with highly dissected leaves, cross-sections of stem nodes were also pressed to better present the leaves and leaf-arrangement (Haynes 1984).

Specimens were preferably dried in the field using a portable dryer with a propane stove as a heat source. In addition to yielding high quality herbarium specimens, this method preserves plant pigments, which can contain useful information (Ceska and Ceska 1986), and also allows for DNA samples to be taken from the herbarium specimens. If no portable drier were available, and if time and climatic conditions permitted, specimens were “sun-dried”, with the plant presses placed in direct sun and the newspapers and blotters changed frequently. Given appropriate weather conditions, specimens produced by this method are equal in quality to those produced by gas drying, although this method was quite laborious and, hence, was utilized only when necessary. At certain times, such as on protracted expeditions to remote areas, field drying was not feasible. In these instances, specimens were pressed overnight and then placed in polyethylene bags containing a 70/30 mixture of alcohol and water (cf. Liesner 1990). Specimens remained in bags for the duration of the fieldwork, after which time they were removed and dried in a specimen dryer.

Although every attempt was made to collect voucher specimens of all species present at a site, in rare instances when the number of specimens exceeded the capacity of the portable field dryer and weather conditions precluded sun-drying, specimens of a few of the most common and easily identifiable species were discarded. Whenever possible, photo vouchers were taken to compensate for the absence of dried specimens.

Specimen Identification

Provisional identifications of specimens were made in the field. Frequently, there was some uncertainty as to the number of taxa present at the site (e.g., as occurred with similar species in families with highly reduced and superficially similar flowers, such as the Poaceae and Cyperaceae). In these instances, specimens were taken from a number of different areas in the system and were given separate collection numbers. Further identifications and/or confirmations of specimens were carried out at the three primary herbaria in Bolivia (BOLV, LPB, USZ) and in the United States at the Hodgdon Herbarium (NHA), the Gray Herbarium at Harvard University (GH), and the Missouri Botanical Garden (MO). Specimens were also borrowed from other institutions when additional material was required. Voucher specimens were deposited at NHA and, depending on the region from which the collections were taken, at either USZ, LPB, or BOLV. When available, duplicates were also deposited at MO.

Whenever possible, all species noted at the study sites were included in the species richness estimates. When only sterile individuals of a particular species were encountered, and if clearly distinguishable from all other taxa at the site, that species was given a morphospecies name (e.g., *Cyperus* #1) and was incorporated in the estimate of site diversity. Morphospecies names were also assigned to any fertile species that I was unable to confidently determine to the level of species. Frequently, it was not possible to ascertain whether or not a morphospecies corresponded to a morphospecies from another site (e.g., it was not possible to differentiate between Poaceae #1 from site “A” and Poaceae #1 from site “B”). Hence, although these species were counted in the estimates of site diversity, they were excluded from floristic comparisons.

Wetland Species Database

To undertake floristic comparisons, a checklist of species associated with Neotropical wetlands was compiled and stored in a relational database (Ritter 2000). In assembling the checklist it was necessary to identify whether or not a particular species could be considered as a “wetland species”. Ideally, these were both the truly aquatic species (those that spend nearly their entire life cycle in contact with water), as well as semiaquatic species (those that require that the greatest part of their life cycle be spent in soils that are at least saturated, and which cannot survive extended periods of drought). This distinction was made to exclude those ruderal species that are tolerant of some inundation, but which are more characteristic of disturbed, terrestrial sites. It was often difficult to state with certainty whether or not a particular species met these criteria. In such cases, the species was designated as “possibly wetland” or “probably wetland”, and further information regarding the species’ typical habitat was sought. When information regarding the life history of a particular species was lacking, the habitat in which it was most often encountered was considered as an indicator of wetland affinity. Frequently, the delineation of wetland species in regions outside of Bolivia was based on habitat descriptions given by a single author. If it was unclear whether or not a habitat could be considered as a wetland (i.e., if there was insufficient information given on inundation regime), the dominant vegetation was used as an indicator of wetland status. Hence, as a result of these uncertainties, a “wetland species” referred to a species that was strongly associated with inundated habitats.

A species that was recognized as a wetland species in one region was considered to be a wetland species throughout its range. Hence, it was possible to undertake comparisons of wetland floras at the regional and country level by obtaining or compiling species lists for these areas and querying the wetland species database as to the “wetland” status of each species.

Species lists from numerous published floristic studies of Neotropical wetlands were incorporated into the database (Figure 2-2, Figure 2-3). Whenever possible, species lists were entered in their entirety, in order to accommodate changes in the ascription of wetland species. Thus, although a species might not be recognized as a “wetland species” at the time of the incorporation of data from a particular study, its presence in the region was still registered. Species lists from the following publications were entered in their entirety: Aristeguieta (1968), Armitage and Fassett (1971), Beck (1984), Bonilla-Barbosa and Novello R. (1995), Brandão et al. (1989), Bravo-Velásquez and Balslev (1985), Briones et al. (1997), Bumby (1982), Burkart (1957), Cabrera and Fabrís (1948), Cano et al. (1993), Conceiçâo and de Paula (1986), Crow and Rivera (1986), Crow et al. (1987),

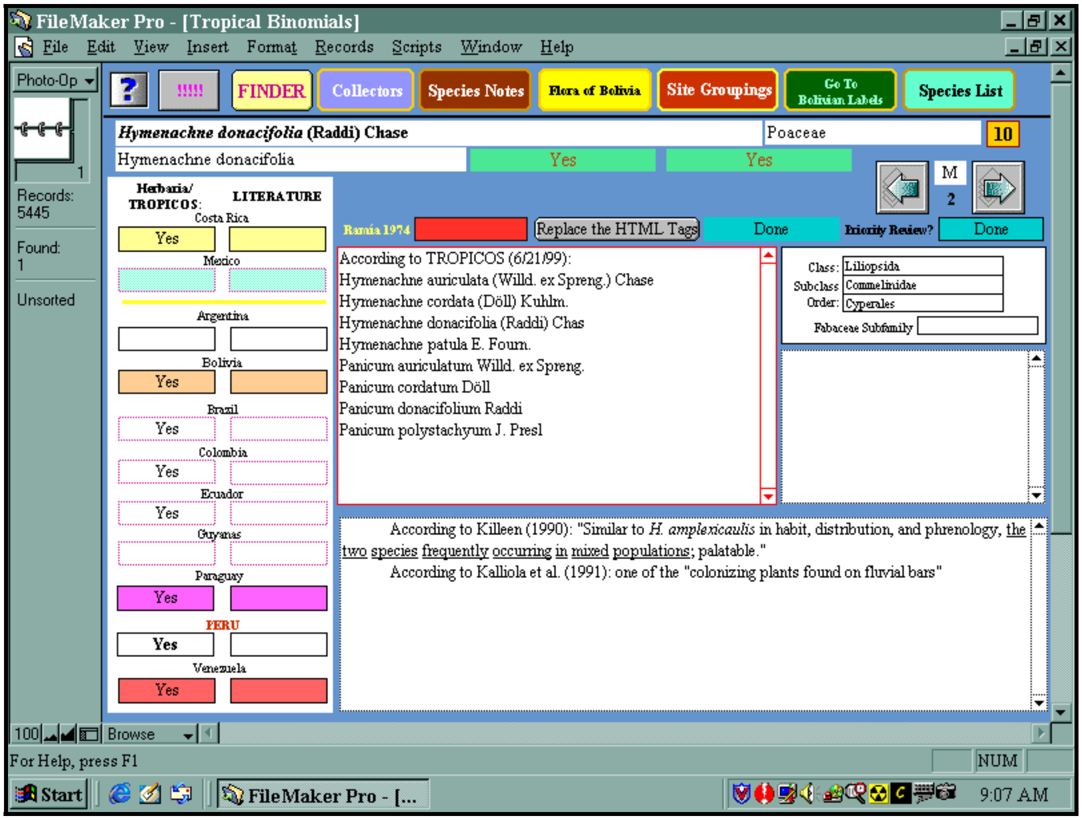


Figure 2-2. A record from the Wetland Species database. Fields along the left hand edge indicate whether references to the species were encountered in the TROPICOS database, in herbarium specimens, or in the literature. The large field in the center of the image stores information on synonymy and other taxonomic notes. The large field in the lower right hand corner contains information on species' habitats.

Estenssoro C. (1991), Franken (1991), Frey (1995), Galán de Mera (1989), Galán de Mera and Navarro (1992), Haase (1989, 1990), Haase & Beck (1989), Heckman (1998), Howard-Williams and Junk (1977) Junk (1983, 1986, 1989), Junk and Piedade (1997), Kalliola et al. (1991), Killeen (1990), Killeen and Nee (1991), Keel and Prance (1979), Klinge (1990), Lara R. & Cazas (1996), León et al. (1995), León and Young (1996), Loetschert (1954), López-Hernández (1993), Lot and Novelo R. (1988), Lot et al. (1986; 1999), Menalled and Adámoli (1995), Mereles et al. (1992), Navarro (1993), Neiff (1986), Pires and Prance (1985), Por (1995), A. Pott and V. Pott (1997), V. Pott and A. Pott (1997), Pott et al. (1986, 1989, 1992), Prado et al. (1994), Ramía (1974), Ramírez-García and Novelo R. (1984), Rangel & Aguirre (1983), Raynal-Roques (1991), Rojas and Novelo R. (1995), Sanabria and de Wilde (1998), Schulz (1961), Schmidt-Mumm (2000), Schulz (1961), Siebert (1994), Siebert and Menhofer (1992), Velásquez (1994), Wolf (1990), and Worbes (1997).

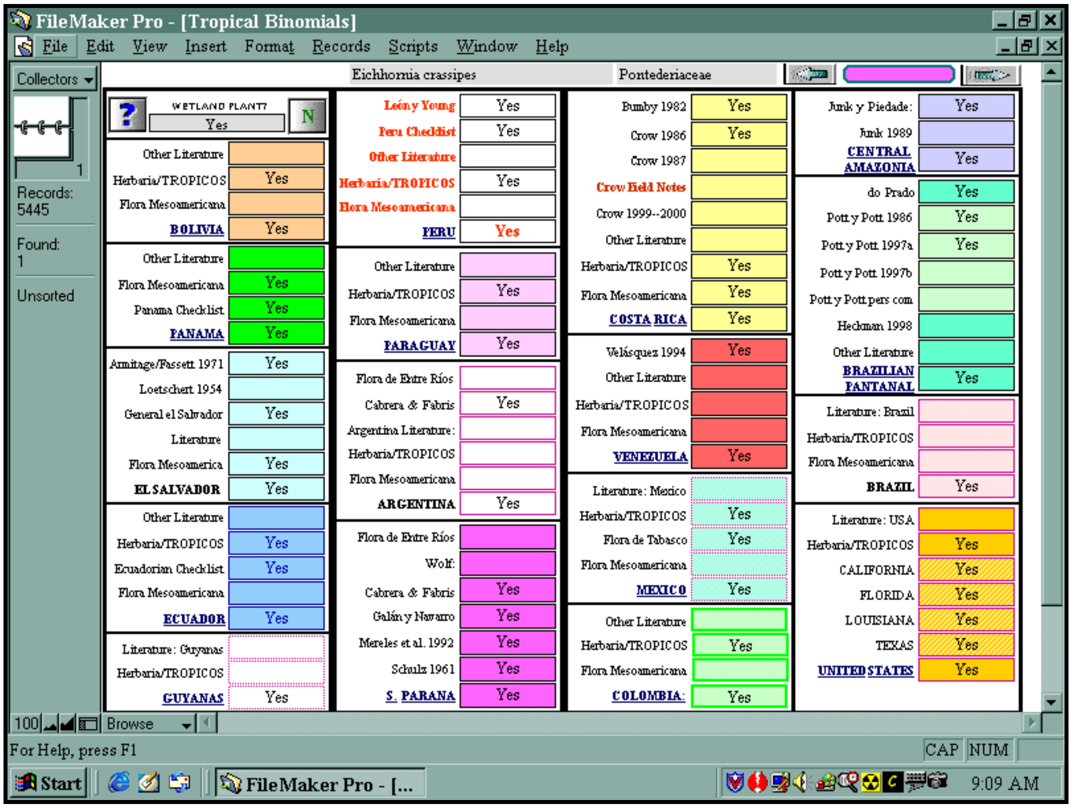


Figure 2-3. The same record from the Wetland Species database as in Figure 2.3. In this layout, the presence of the species in various countries and regions is displayed. Fields indicate the source of the species' references (e.g., various published accounts, the TROPICOS and Flora Mesoamericana databases, herbarium specimens, etc.). Note: Only a small subset of the sources used in compiling species list for each OGU are presented in this layout.

Information from the following regional floristic studies was also utilized in distinguishing wetland species: Burkart (1957, 1978), Davidse et al. (1994, 1995), Gómez (1984), Kahn (1993), Renvoize (1998), and Troncoso de B. et al. (1987). Although only a portion of the species lists from these sources were entered into the database, habitat descriptions were frequently used to identify wetland species and to resolve uncertainties regarding typical habitats of particular species. Likewise, habitat information from the many taxonomic treatments used during the identification of specimens was incorporated in the ascription of wetland species. Habitat data from herbarium specimens at BOLV, GH, LPB, MO, and USZ were also utilized in adjudging wetland species.

Additional input on species' typical habitats came from discussions and written communications with other researchers working in tropical wetlands and from a query (for species associated with wetlands) of the Biological Diversity of the Guianas Database (the Biological Diversity of the Guianas project, National Museum of Natural History, Washington, DC.; see Appendix B). Information was incorporated from Reed's (1996) checklist of species associated with wetlands in the United States and its extra-continental protectorates and associated territories. The inclusion of material from this checklist was intended to help identify wetland species from those regions (i.e., northern Mexico) which possess temperate and northern subtropical floristic elements. Based on the preceding sources, 2060 species in 149 families and 666 genera were identified as being associated with the OGS (i.e., Mesoamerica and tropical and subtropical South America).

Phytogeographic Analysis

Floristic comparisons were made at three scales of "Operational Geographical Unit" or "OGU" (cf. Crovello 1981): 1) between study sites; 2) between regions within Bolivia ("mesoregional scale" sensu McLaughlin 1994); and, 3) between regions and countries in tropical and subtropical South and Mesoamerica ("macroregional scale" sensu McLaughlin 1994).

In his overview of quantitative biogeography, Crovello (1981) listed the potential purposes of quantitative biogeographical studies as follows: 1) to elucidate observed patterns among OGUs; 2) to account for the factors that produce and maintain these patterns; and, 3) to predict the effects of different conditions and events on future patterns. In this thesis, phytogeographical analyses are intended solely to address the first of these purposes, the elucidation of patterns among the OGU floras.

Regions Within Bolivia

Bolivia was divided into ten regions (Figure 2-4). Sufficient study sites were present in eight of these to allow their inclusion in regional comparisons: three montane (High Andean, Valles Secos, and Cloud Forest) and five essentially lowland (Chapare, Andean Piedmont of Santa Cruz, White-water Floodplain, Chiquitanía, and Gran Pantanal).

Regions were delineated based on geographical features and predominant abiotic factors, principally precipitation. Descriptions of the regions are presented in the appropriate chapters. The Bolivian OGUs, their estimated area, range of elevation of the study sites within each OGU, and the watersheds present in each OGU are presented in Table 2.2.

In addition to data from the fieldwork, information on species' distributions were obtained from various other sources and incorporated into the regional wetland floras. Principal among these were: 1) published accounts of research in other Bolivian wetlands; 2) data from Bolivian specimens listed in the Missouri Botanical Garden database TROPICOS.

In addition to data from the fieldwork, information on species' distributions were obtained from various other sources and incorporated into the regional wetland floras. Principal among these were: 1) published accounts of research in other Bolivian wetlands; and, 2) data from Bolivian specimens listed in the Missouri Botanical Garden database TROPICOS. Specimen data from TROPICOS was obtained by querying the database for exsiccatae for each of the country's Departamentos. The selected records (ca. 61,000 records) were subsequently reviewed for locality errors (see Appendix C) and were apportioned to the proper region. At times, locality data from herbarium specimens and from floristic treatments were also incorporated into the regional checklists. A complete account of the sources used for compiling the Bolivian regional wetland floras is given in Appendix B. A checklist of the species associated with Bolivian wetlands (1026 species, in 126 families and 450 genera) plus regional presence/absence data is presented in Appendix D.

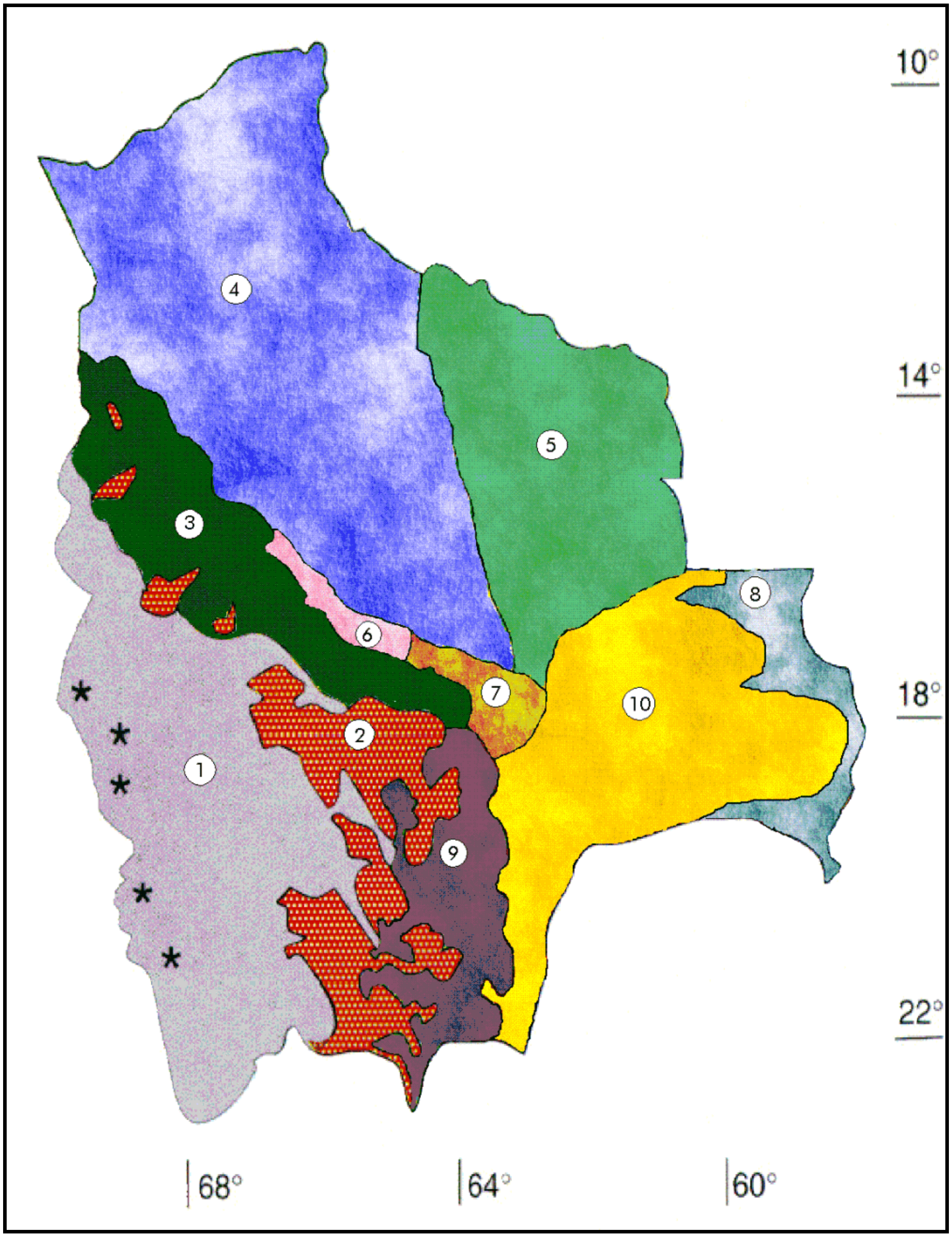


Figure 2-4. Bolivian Regions (modified from Killeen et al. 1993): 1) High Andean; 2) Valles Secos; 3) Cloud Forest; 4) White-water Floodplain; 5) Chiquitanía; 6) Chapare; 7) Andean Piedmont of Santa Cruz; 8) Gran Pantanal; 9) Bosque Tucumano-Boliviano; 10) Gran Chaco.

Table 2.2. The Bolivian regions, with estimated area, elevation range of the study sites, and major watersheds present in each region.

Region	Approximate Area (km2)	Elevational Range[A] (m.a.s.l.)	Watershed(s)
High Andean	210,000	3100-4500	Desaguadero, Amazon, Paraná
Valles Secos	83,000	1800-2550	Amazon, Paraná
Cloud Forest	33,000	2400-2920	Amazon
Chapare	4000	200-230	Amazon
Andean Piedmont	5000	400-430	Amazon
Whitewater Floodplain	325,000	200-220	Amazon
Chiquitanía	190,000	200-750	Amazon
Gran Pantanal	14,000	90-100	Paraná
A. Range of elevations of the study sites in the region. Regional checklists likely contain species which occur outside of this range.			

Extra-Bolivian Regions

Utilizing a diversity of sources, species checklists were either obtained or compiled for the following countries: Brazil, Colombia, Costa Rica, Ecuador, the Guianas (French Guiana, Guyana, and Suriname: treated here as a single OGU), Mexico, Panama, Paraguay, Peru, the United States, and Venezuela. The OGUs, their estimated area, total species, and total wetland species are presented in Table 2.2.

Preferably, complete species lists were obtained (in electronic format) for each country and incorporated into the database. When comprehensive checklists were unavailable for a country or region, representative floras were compiled from floristic studies, augmented by data from herbarium specimens, monographs, and other literature as previously described. Checklists were also compiled for three South American regions: the Gran Pantanal de Mato Grosso, the Central Amazonian (Brazil) region, and the Río Paraná Delta region. A checklist for a fourth region, Lowland Amazonian Peru, was compiled by querying the Peruvian checklist (see Appendix B) for all species not restricted to coastal habitats whose lower distributional limit was 0 m (e.g., 0-1000 m). A complete account of the sources used to compile the country and regional floras is presented in Appendix B.

Table 2.3. Countries and extra-Bolivian regions utilized in biodiversity and floristic comparisons, with estimated area, total species and total wetland species noted for each OGU. Sources used in compiling the Bolivian flora are given in Appendix B. Sources used in compiling the floras of extra-Bolivian OGUs are given in Appendix C.

OGU	Approximate Area [A] (km^2)	All Species	Wetland Species
Central America			
"Mid-Central America" [B]	394,474	1527	696
Costa Rica	51,160	9265 [D]	708
Mexico	1,972,550	9942	778
Panama	78,200	7576 [D]	607
South America			
Río Paraná Delta (Argentina)	23,700	297	297
Bolivia	1,098,580	9539	1026
Brazil	8,511,965	,634	1007
Central Amazonia (Brazil)	4000	411	255
Gran Pantanal de Mato Grosso (Brazil)	140,000	1193	425
Colombia	1,138,910	1301	870
Ecuador	283,560	15,812 [D]	756
The Guianas [C]	378,331	14,088 [D]	845
Peru	1,285,220	18,687 [D]	903
Lowland Amazonian Peru	533,100	6014	429
Venezuela	912,050	1384	887
North America			
United States and Associated Territories	9,629,000	25,267	3284

A: World Factbook (Central Intelligence Agency 2000).
B: Belize, El Salvador, Guatemala, Honduras, and Nicaragua.
C: Guyana, French Guiana, and Suriname.
D: Presumed to represent relatively complete national floras.

Additional information regarding species' distributions was obtained from the Flora Mesoamericana checklist (Davidse et al. 1999) that was downloaded from the Missouri Botanical Garden website and converted to database format. In this form, the checklist contained only species and family names, however, it was possible to obtain distribution data for individual species by querying the Missouri Botanical Garden's online database for the Flora Mesoamericana (http://www.mobot.org). To this end, a program was written to direct the computer to automatically submit queries to the website and transfer results to the wetland species database. In this manner, the website was systematically queried for each species, thereby compiling distributions (in the form of presence/absence for each country in Meso- and South America) for the approximately 12,000 species in the Flora Mesoamericana checklist.

Complete locality data from the following floristic treatments were entered into the database: Balslev (1996), Cialdella (1989), Galán de Mera and Navarro (1989), Haynes and Holm-Nielsen (1994), Wiersema (1987), Van Royen (1951, 1953, 1954), and Zardini and Raven (1991). Additionally, distribution data for all species listed for the Neotropics by Taylor (1989) were entered.

The wetland flora of the United States was also incorporated in floristic comparisons. The 1996 National List of Vascular Plant Species That Occur in Wetlands (Reed 1996) was downloaded as a text file and converted to a database (see Appendix B for an elaboration). Of the 7000+ species included in the checklist, 2034 were characterized as "Obligate Wetland Species" (OBL), i.e., those that under natural conditions almost always occur in wetlands (Reed 1996). Species that were categorized as OBL in any region were treated as associated with wetlands throughout their range.

Analytical Methods

Similarity Indices

Degrees of similarity were analyzed using Sørensen's Index (Magurran 1988):

$$S = 2j / (a + b);$$

where a is the total number of species noted for OGU 1, b is the total number of species noted for OGU 2, and j is the number of species common to both OGUs.

During the initial stages of statistical analysis, data from selected regions were also analyzed using Ochiai's index (McLaughlin 1994), with the resulting similarity matrix compared to that produced by Sørensen's index (cf. Hubálek 1982). Although some small differences were noted between the matrices generated by the two indices, ultimately, Sørensen's index was selected because it was one of the more commonly used indices (McLaughlin 1994). Moreover, as this index has been used in various other wetland studies it allowed comparisons between these studies and the Bolivian data.

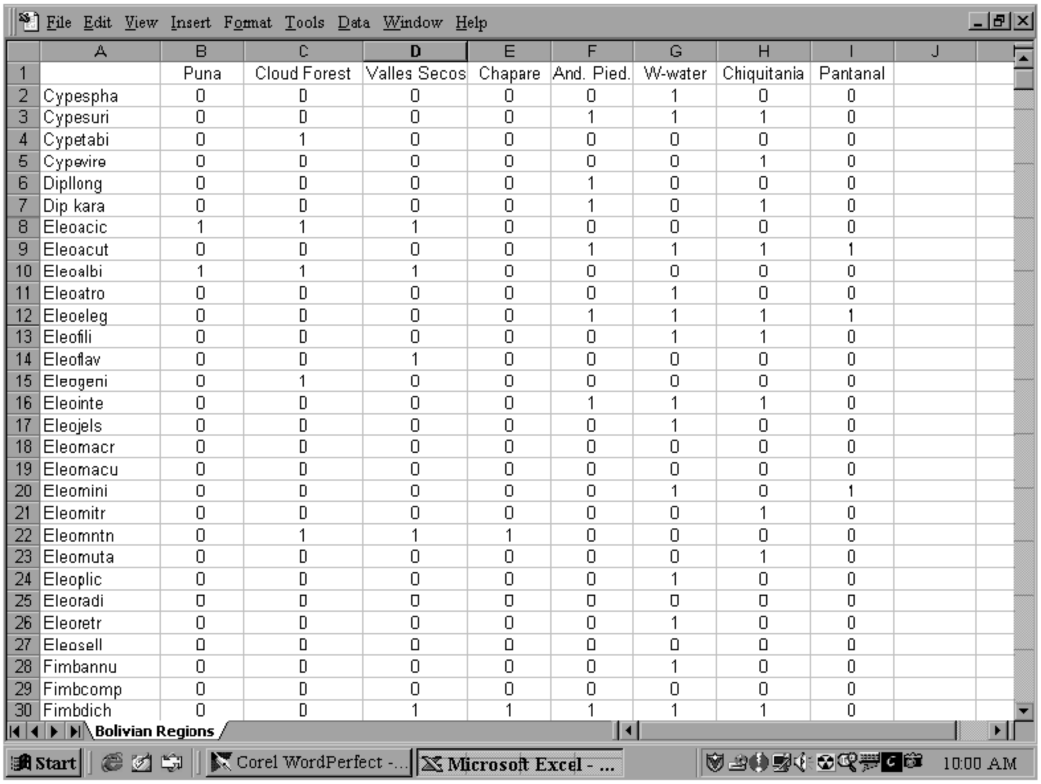
Ordination

In order to express floristic relationships among all OGUs simultaneously, data were organized into a binary matrix (see Figure 2-5) of OGUs versus species (recorded as presence/absence values) and ordinated using Detrended Correspondence Analysis (DCA, Hill and Gauch 1980). Ordinations were conducted using the software package, PC-ORD (McCune and Mefford 1997).

Initially, two approaches were used to test the validity of the ordinations. First, random draws from the original data were performed, thereby creating data sets in which each OGU possessed half as many species as in the actual flora. Ordinations were then performed on these sets. Next, for each species, presence/absence data were replaced by randomly generated numbers. These randomly generated data sets were subsequently sorted, reconverted to presence/absence data, and ordinated. The number of species present in each OGU was maintained, i.e., for each OGU the number of species after randomization equaled the number of species in the original data set. Each of these methods was repeated three times, and the resulting ordinations were compared to the ordination of the actual data.

Attempts at appraising the stability of the ordination were inconclusive. Ordinations of randomly drawn data sets (half-sized sets of the actual data) were often consistent with the complete data set. In these instances, the OGUs maintained their same relative configuration with the only differences limited to small-scale migrations of the data points. At other times, ordinations of the randomly drawn data were not faithful with that of the full data set. Moreover, ordinations of the randomly generated data sets were extremely problematic. In these tests, the three OGUs with the fewest species were strongly associated with axial endpoints (i.e., in most iterations the least species-rich OGUs formed the axial endpoints).

Clearly, the orientation of the OGUs in ordination space was influenced to a significant degree by sample size. Therefore, in order to establish a frame of reference for interpreting the ordinations of the actual data a null data set was created and classified by DCA. In this data set one half of the species in each OGU were shared with the other OGUs and the other half of the species were restricted to a single OGU. Floristic affinities were then interpreted by comparing the position of the actual data to the ordered data set.



	Puna	Cloud Forest	Valles Secos	Chapare	And. Pied.	W-water	Chiquitania	Pantanal
Cypespha	0	0	0	0	0	1	0	0
Cypesuri	0	0	0	0	1	1	1	0
Cypetabi	0	1	0	0	0	0	0	0
Cypevire	0	0	0	0	0	0	1	0
Dipllong	0	0	0	0	1	0	0	0
Dip kara	0	0	0	0	1	0	1	0
Eleoacic	1	1	1	0	0	0	0	0
Eleoacut	0	0	0	0	1	1	1	1
Eleoalbi	1	1	1	0	0	0	0	0
Eleoatro	0	0	0	0	0	1	0	0
Eleoeleg	0	0	0	0	1	1	1	1
Eleofili	0	0	0	0	0	1	1	0
Eleoflav	0	0	1	0	0	0	0	0
Eleogeni	0	1	0	0	0	0	0	0
Eleointe	0	0	0	0	1	1	1	0
Eleojels	0	0	0	0	0	1	0	0
Eleomacr	0	0	0	0	0	0	0	0
Eleomacu	0	0	0	0	0	0	0	0
Eleomini	0	0	0	0	0	1	0	1
Eleomitr	0	0	0	0	0	0	1	0
Eleomntn	0	1	1	1	0	0	0	0
Eleomuta	0	0	0	0	0	0	1	0
Eleoplic	0	0	0	0	0	1	0	0
Eleoradi	0	0	0	0	0	0	0	0
Eleoretr	0	0	0	0	0	1	0	0
Eleosell	0	0	0	0	0	0	0	0
Fimbannu	0	0	0	0	0	1	0	0
Fimbcomp	0	0	0	0	0	0	0	0
Fimbdich	0	0	1	1	1	1	1	0

Figure 2-5. Sample of the binary matrix of species versus OGUs. The top row indicates OGUs. The left-hand column lists the species, with species names abbreviated to eight characters in or to accommodate the restrictions of the analysis program (PC-Ord). The remaining columns contain presence/absence data for each species.

Cluster Analyses

Initially, OGU floras were also classified by cluster analysis using PC-ORD (McCune and Mefford 1997) with Sørensen's Index distance and nearest neighbor linkage. Stability of the clustering was tested using sets of randomly generated presence/absence data as per the preceding analysis. In this manner, it was determined that cluster analysis of the data was particularly sensitive to flora size. The OGUs with the smallest floras always occupied the outermost branches of the dendrograms generated both from actual data and from the three iterations of randomly generated data. Although cluster analysis is a common tool of biogeographical research (McLaughlin 1994), it was clear that differences in flora size had too large an effect for the results to be interpreted with confidence in this study.

Frequency Analyses

Although similarity indices are regularly used in phytogeographical analysis (McLaughlin 1994, Simberloff and Connor 1979), it should be recognized that these are ad hoc constructs (Simberloff and Connor 1979), i.e., they are not derived from any hypothesis regarding the factors that determine species' distributions. In most of these indices floristic similarities are calculated from the entire flora; however, the presence of shared rare species in a pair of OGUs can also serve as the criterion for adjudging similarity (Simberloff and Connor 1979). Therefore, a method was developed for graphically representing the relative contribution of species classes (e.g., species present in all OGUs, species restricted to 2 OGUs, etc.) to overall similarity (see Figure 2-6 for an illustration of this method).

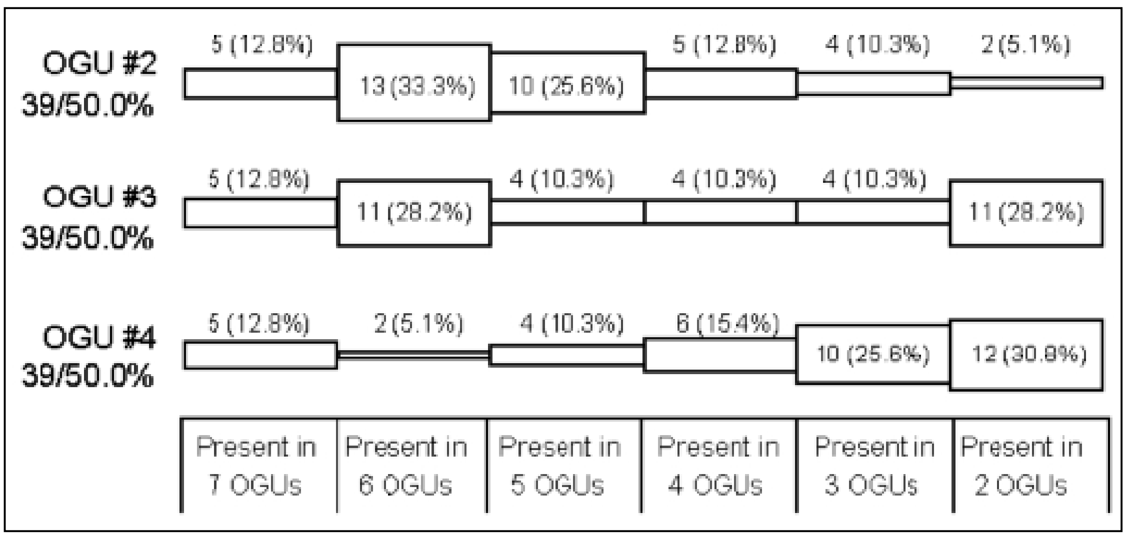


Figure 2-6. Frequency of species shared between a subset of hypothetical OGUs. Figures below the OGU names indicate the number of species shared between that OGU and OGU #1, followed by percent floristic similarity (Sørensen's Index) of the two floras. The boxes correspond to species classes (i.e., number of OGUs in which the species was present), as indicated by the key along the bottom edge of the figure. The vertical dimension of each box is proportional to the number of species that it represents. Figures associated with the boxes indicate the number of species that occurred in both the OGU and OGU #1, followed by the percentage that this portion of the flora contributed to the total species shared between the OGU and OGU #1. For example, considering the relationship between OGU #1 OGU #2, the initial (lefthand-most) box represents the 5 species that were present in all seven OGUs. These accounted for 12.8% of the species shared between these two OGUs. Continuing from left to right, the second box represents the 13 species that were present in both OGU #1 and OGU #2 and that occurred in exactly 6 OGUs. These accounted for 33.3 % of the species shared between these two OGUs. Note: due to rounding off, the percentages may not add up to exactly 100%.

In the following three chapters, descriptions and analyses are presented for three Bolivian regions selected for comprehensive analysis (the Cloud Forest, Chapter 3; the Chapare, Chapter 4; and, the Gran Pantanal, Chapter 5). Subsequently (Chapter 6), site-level diversity for all 46 Bolivian study sites is examined. In the final chapter, comparisons of macroregional-scale diversity are made among Bolivian regions, among the countries of the Neotropics, and between the Neotropical region and the New World temperate regions.