UNIVERSIDAD POLITÉCNICA SALESIANA

Centros de Investigación

Perfil de Proyecto de Investigación Científica

22-feb-2018 3:42 AM

1. DATOS GENERALES DEL PROYECTO

Código:	CIMA-010213
Centro de Investigación:	CENTRO DE INVESTIGACION EN MODELAMIENTO AMBIENTAL
Programa:	Caracterización multivariada en la gestión de AP
Título del Proyecto:	Biodiversity, DNA Barcoding and molecular phylogeny of northwestern Ecuador
Grupo de Investigación:	Ecología y Gestión de Recursos Naturales
Area de Conocimiento:	Ciencias de la Vida
Línea de Investigación:	Ecología y Gestión de Recursos Naturales
Tipo de Investigación:	Básica
Campo :	Otro
Investigador Principal :	EDWIN FABIAN BERSOSA VACA
Proyectos Vinculados :	
Duración del Proyecto :	11 Meses
Localización del Proyecto :	the cloud forests of northwestern Ecuador: Los Cedros, Maquipucuna, Mindo and Pululahua reserves.
Fecha de ingreso :	30/09/2013 10:05

2. ANTECEDENTES

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3. BACKGROUND

The Neotropics harbor a paramount biodiversity and is currently acknowledged as the most species-rich region on Earth, being most of it represented by insects and plants (cited elsewhere). Globally, its relevance is even greater if we considered that many areas are highly threatened by human activities (Ojeda et al. 2003, Sarkar et al. 2008,Rieckmann et al. 2011) which is reflected in the six hotspots that have been indentified to date (Conservation International 2005, Mittermeier et al. 2011). The tropical forests in northwestern South America, known as the Tumbez-Chocó-Magdalena (Chocó for
short), and the Tropical Andean hotspots form an integral part of these endangered natural areas. In Ecuador, the rare but ecologically very important cloud forest ecosystems are part of these two biologically prominent regions. Despite their low representativeness in the country¿s natural reserves network (Sierra et al. 2001) these forests contain a very high biodiversity and endemism which are critically endangered today because of severe habitat loss (Gentry 1992, Bubb et al. 2004, Brehm et al.
2005). A tangible example is depicted throughout the northern Andean western slopes where ample forested areas have been converted into arable land (Sarmiento and Frolich 2002, Etter et al. 2006). Yet, many habitats in the Chocoan and tropical Andean regions have been poorly investigated (Toledo and Sossa1993, Moratelli and Wilson 2011) this is particularly true for the ubiquitous insects and their allies whose ecological role in ecosystem dynamics has been despised in our so-called developing countries.

However, according to Dr. Brian Brown in regards the slow progress in species
descriptions of Neotropical flies (Diptera), the problem are the ¿insufficient resources available to few available researchers¿ (Brown 2005, page 180).

Cloud forests located in the provinces of Esmeraldas, Imbabura and Pichincha, which have suffered intense human encroachment, are probably among the most
endangered areas in northwestern Ecuador (Young and Keating 2001, Sarmiento and
Frolich 2002). Few national and private reserves, namely Cotacachi-Cayapas,
Maquipucuna, Mindo, Los Cedros and Pululahua are the only refuges with pristine
forests hosting unique wild fauna and flora like for example, the Toucan Barbet
(Semnornis ramphastinus), the Garlepp¿s mouse (Galenomys garleppi), various orchids species (Orchidaceae) and many other plants and animals with narrow distributions,several of which are cited in the red list of the International Union for the Conservation of Nature - IUCN, and in the Convention on International Trade in Endangered Species of Wild Fauna and Flora ¿CITES (Rieckmann et al. 2011, Oleas et al. 2012).

Orchids are exceptionally diverse in Andean cloud forests (Gentry and Dodson 1987,Dodson 2003, Küperet al. 2004, Krömer et al. 2005, Suárez et al. 2006), it is estimated that 190 species occur at Los Cedros, a reserve with only 7000 ha, by comparison the entire North American continent has an estimated 200 species (NAOCC 2013). Many orchids depend on flies for their pollination, around 25% of all orchid species are fly pollinated (Van der Pijl and Dodson 1966, Christensen 1994). Recent findings in this reserve showed that, among other dipterans, several fruit fly species in the families Drosophilidae and Phoridae are attracted by the scents of the peculiar orchid Dracula
lafleurii (Endara et al. 2010). The flies pollinate the flowers and in exchange the insects feed on a sort of ¿liquid film¿ and use the sepals and petals as mating space and oviposition (Endara et al. 2010; Barbara Roy, Tobias Policha, Bryn Dentiger unpublished data; pers. obs.). Similar cases, between orchids and flies were observed repeatedly in Los Cedros, suggesting that orchid pollination by flies might be a 4 common and widespread ecological process in Andean cloud forests. Yet, the biodiversity of pollinating flies, their natural history, and the evolutionary ecology between these two rapidly diversifying groups (flies and flowering plants) remain mostly
unknown.

3. JUSTIFICACIÓN

We repeatedly hear in daily news the current threats to the conservation of natural ecosystems around the world, but how much have we done as scientists and nature conservationists in that regard? The immensely diverse natural biota hosted in the Neotropics, we should say mainly in the Tropical Andean corridor, faces a sad paradox:
there is no governmental support to scientific research in our countries where, in small protected areas coexist more animals and plants than in much bigger regions around the planet. If we further add to this issue the accelerated human encroachment on natural habitats, we will realize that urgent management and research initiatives are required. If we are to preserve our endangered tropical ecosystems, the first step should be inventorying its biodiversity.

In Ecuador, intensive work on vertebrates has yielded several monographs, to name a few: Mamíferos del Ecuador by Tirira (2007), Aves del Ecuador by Ridgely and
Greenfield (2007), Reptiles del Ecuador by Torres-Carvajal (2000-2008), Vertebrados del Ecuador by Albuja et al. (2012). In sharp contrast, the invertebrate diversity has been poorly investigated; insects in particular, require much attention from researchers.

Few studies have produced comprehensive national inventories of selected insect

groups like butterflies (Piñas et al. 1997-2006) or beetles (Pearson et al. 1999), as well as economically important groups (¿pests¿) (Rogg 2000). Whereas the majority of less charismatic, but by no means less ecologically remarkable insects, like flies, ants, true bugs and several other groups have remained in the shadows, which may explain their under-representativeness in conservation biology (Novotny et al. 2007). As the insects are the ¿most exuberant manifestation of Earth¿s many and varied life forms¿ (Tim New,
page xix) and create the biological foundation for all terrestrial ecosystems (Scudder 2009), then it should be worth study and inventory them.
Of the major insect groups the true flies (Diptera) and the ants (Hymenoptera) abound in tropical forests (Pinheiro et al. 2002, Davidson et al. 2003). In Andean cloud forests,our previous observations led us to suggest that dipterans are the dominant group (Figure 1). In addition to the great number of individuals the New World tropics harbor more Diptera species that any other place on Earth (Brown 2009b), similar to many other groups of organisms, i.e., birds (Welty and Baptista 1988); butterflies (Robbins and Opler 1997), mammals (Cole et al. 1994); orchids (Dodson 2003). Hammond (1992) places de number of flies in 1.6 million of species, this is roughly 10% of the
world¿s diversity of life (Brown 2005). This extraordinary species richness together with the large range of habitats the true flies occupy in many ecosystems around the Globe is tightly linked to their immense economical and ecological importance. For the former,it is widely known that flies are active vectors of many human diseases (Kato et al.2008) and cause crop damage (Hedstrom 1987). In regards the ecological importance,flies are efficient pollinators (Arroyo et al. 1985, Kearns 1990, Christensen 1994,Grimaldi et al. 2003, Goldblatt et al. 2004); are involved in decomposition (Pape 2009);and because many are parasites of other insects, they are sometimes used for biocontrol (Pape 2009). Thus, knowing the diversity of Diptera in a given region means within it.
It is estimated that flowering plants account for ca. one fourth of all described living organisms and close to 90% of these are pollinated by animals (Ollerton et al. 2011).
According to Evenhuis and his team (2008) close to half of the 150 Diptera families include species that feed at flowers as adults and around 550 species of flowering plants are regularly visited by flies (Larson et al. 2001). Flies are particularly important pollinators in regions that are cool and wet (Arroyo et al. 1985, Kearns 1990) such as the cloud forests. Orchids also thrive in these moist habitats, and many are fly pollinated, in fact it is has been estimated that up to 25% of all orchids are fly pollinated. (Van der Pijl and Dodson 1966, Christensen 1994). Considering that orchids
comprise a large proportion among tropical flowering plants (Gentry and Dodson 1987, Dodson 2003) and that Andean cloud forests are extremely rich in orchids (Gentry and Dodson 1987, Suárez et al. 2006) then, the diversity of ecological interactions between these organisms and their pollinating flies should be also considerably large, and by extension, critically important for plant-insect conservation in the Neotropics.
Our scarce knowledge on insect biodiversity and the evolutionary ecology of key
processes like pollination, together with the increasing threats to wild flora and fauna that are mainly illustrated by the accelerated expansion of agricultural frontiers in and around cloud forests, are factors that hamper our efforts to preserve ecosystems in South America. As forests in the Tropical Andes are critically endangered it is our responsibility to continue gathering information in biodiversity monitorings, this will expedite the understanding of nature¿s complex dynamics.

4. OBJETIVOS

4.1 Objetivo General

Examine the diversity and evolutionary relationships of orchid pollinating flies in the could forests of northwestern Ecuador

4.2 Objetivos Especificos

1 Determine the diversity of orchid pollinating flies (Diptera: Drosophilidae,

2 Develop DNA barcodes and examine the evolutionary relationships among

3 Georeference collection sites of all examined dipteran taxa.

4 Develop an illustrated guide to the orchid pollinating Diptera of northwestern

5. ESTADO DEL ARTE

Orchids are a major biodiversity component of the Neotropical flora (Foster and Hubbell 1990), close to one third of the world¿s described species can be found in the
western tropical Andes of Ecuador and Colombia (Cribb 2010).Their explosive diversification has been attributed to many factors, such as epiphytism or extreme seed production (Dodson 2003, Gravendeelet al. 2004), but undoubtedly another key factor
is the insect pollination specificity to many orchid species, which have led to reproductive isolation of both intervening parts (Dodson 2003).

There is an extensive literature related to several aspects about orchids worldwide, but current knowledge on the diversity, evolutionary biology and systematics of pollinating
Diptera in the Neotropics is still far from being seriously investigated, though, an exception is the recently published monumental Manual of Central American Diptera edited by Brown et al. in 2009. In sharp contrast, despite their paramount relevance in tropical ecology, the South American dipteran fauna is scarcely represented by few relevant studies where flies were included as focal study groups (Keiper et al. 2002,
Lavandeira 2005, Brown et al. 2009).
Within the orchids the Pleurothallidinae which are the most diverse orchid subtribe,comprising 5 ¿ 8% of the whole Neotropical flora (Jørgensen & León-Yánez, 1999), are
majorly pollinated by flies (Van der Pijl & Dodson 1966, Chase 1985, Dressler 1993,Christensen 1994, Borba & Semir 2001, Blanco & Barboza 2005, Albores-Ortiz & Sosa,
2006, Barbosa et al. 2009). Recently, in the cloud forests of the private reserve Los
Cedros, Troya et al. (unpubl shed data) observed that most of the insects that visit orchids are represented by flies, this was later supported by Malaise trap collections where it was found that a great proportion of the insect fauna belonged to the Diptera, most of them from the family Phoridae .
Furthermore, these researchers found that most of the Diptera potentially related to pollination of several orchid species in Los Cedros (e.g. those in the genus Dracula)belonged to the family Drosophilidae, particularly flies in the genus Zygothrica
(Dentinger and Roy 2010, Endara et al. 2010, Dentinger et al. unpublished data).
However, these observations do not exclude the possibility that other fly groups may take part in this system, for example, is likely that the hump-backed flies (Phoridae), that are known to pollinate many plants (Brues 1928, Young 1984, Brown 2009a) and
that were the most abundant insects collected in Los Cedros (Figure 1), are also involved in orchid pollination. Since many phorid species are attracted to mushrooms,and some Dracula orchids mimic agaric mushrooms (see details below), species in this family should also be visiting and possibly pollinating these flowers. Although the natural history of phorids has been comprehensively revised by Disney (1994), much of
their ways of life remains undiscovered (Disney 1994); this is especially true in the Neotropics where the Phoridae fauna is estimated to reach at least 10.000 species (Brown 2005).
Other Diptera groups that could potentially visit orchids are the crane flies (Tipulidae),fungus gnats (Mycetophilidae), black fungus gnats (Sciaridae) and gall midges (Cecidomyiidae). These fly groups were also collected in the reserve of Los Cedros
(Figure 1). Although these four families may be potentially linked to orchids, we will register only their frequencies at the family level in each collecting site due to the lack of taxonomic information that is required to identify their species.
So far, the observations suggest that species in Drosophilidae are probably more related to the pollination of several orchids, like i.e. Dracula lafleurii, D. felix at least in Los Cedros. Alluding to the observed cases where the orchid¿s pollinia was removed and afterwards adhered to the fly¿s dorsum after leaving the orchid¿s columnar chamber (where the reproducti e organs are located) of various D. Lafleurii and D. felix individuals (see details in Endara et al. 2010) , we hypothesize that in general
some drosophilid species could be exclusively pollinating a number of orchid species in the Andean cloud forests. Whether only drosophilids are directly linked to this process,
or additional factors contribute as well, e.g. other insects, autofertilization, etc., it is clear that flies are amongst the main actors.
As in many phorid species, most Zygothrica flies are mycophilous (Grimaldi 1987,1990a, 1990b), thus they are attracted by Dracula orchids whose labellum mimics the pileus of gilled mushrooms (like those in the family Marasmiaceae), and also expel
scents similar to those produced by the fungi (Kaiser 2006, Endara et al. 2010,
Dentinger and Roy 2010, Plicha et al. unpublished data). This case has been cataloged as a f orm of Batesian mimicry strategy and could be one of the principal
forces driving orchid diversity (Dentinger and Roy 2010), which in turn, may contribute to the diversity and speciation of the flies that visit these plants. Additionally, according to Endara et al. (2010) all observed Zygothrica species in Los Cedros carried pollinia after entering the orchid¿s columnar chamber unlike other smaller drosophilid visitors in the genera Cladochaeta and Hirtodrosophila, which suggests a certain level of specificity.

6. METODOLOGÍA

Area of Study
The observations and collections of specimens will be carried out in four preserves, two of which are private areas, dominated by tropical Andean cloud forest vegetation located northwest to the city of Quito, Ecuador. The altitude varies from 1400 m to
3800 m encompassing the provinces of Imbabura and Pichincha. The reserves are:
Los Cedros (0°19'38"N, 78°48'25"W) in the province of Imbabura; and Mindo (0°3'58"S,78°43'32"W), Pululahua (0°5'1"N, 78°31'55"W) and Maquipucuna (0°3'23"N,78°41'21"W) located in Pichincha (Figure 3). All of these are accessible via ground
local transport either from Quito or from other smaller cities as San Antonio de Pichincha, Ibarra or Cotacachi,

though in few cases as in Los Cedros it is also required to walk or use mules because the facilities are relatively isolated. In order to facilitate logistics we have already talked to several people working in each area.

Data collection Malaise trapping is a very effective method for obtaining large numbers of dipterans in short periods (Brown 2005, 2009) and is routinely used in species inventories and biomonitoring of the dipteran fauna around the world. In each selected area we will settle three Malaise traps located in opened places in the forest allowing for increasing chances of capturing flying insects from the surroundings. Additionally, in combination with Malaise trapping we will use pan trapping, another easy and efficient method for collecting flying insects. We will place two colored pan traps, yellow and blue at both sides of each Malaise trap so that we increase the likelihood of capturing the target dipterans. The use of both methods together maximizes the possibilities of capturing a broad array of species when performing biodiversity inventories (Campbell and Hanula 2007, Mazón and Bordera 2008). When applicable, we will install Malaise traps preferably in different habitats (hilled/plain forest) per area, each trap distanced approximately 500 meters. Insects attracted to Malaise traps will be collected in bottles containing 200 ml of either, 30% ethanol or a mix of water plus several drops of unscented detergent. We will proceed similarly as for the pan traps. The traps will remain in their positions for five days, checking their content daily and transferring death insects to other containers with 70% ethanol.

In addition to the above techniques we will set two Macphail aerial traps in 5 meters radius of each Malaise trap. We will hung Macphail traps on tree branches approximately 2-3 m above the ground for five days, making daily checks in each sample. We will use the same animal preservative (ETOH) in Mcphail trap containers as for Malaise and pan traps.

Cotacachi-Capayasr eserve

Every day in the field, we will classify to morphospecies the drosophilid and phorid specimens deposited in the samples, for this we will use standard 60X stereomicroscopes. Specimens for DNA and morphological analyses will be separated and depending upon the amount of available specimens per morphospecies we will select between two to five specimens for the molecular treatment which will be placed in 10 ml tubes with 95% ethanol in special cooling boxes so as to maintain the DNA in optimal conditions. The rest of specimens in each morphospecies will remain in 30 ml tubes with 70% ethanol for posterior morphological identification in the laboratory.

Molecular methods

Prior to DNA extraction, all insects collected in the field will be stored in a freezer at -60 °C. Ultra-freezing the insects facilitates the breakdown of their chitinized cuticules therefore enabling the escape of DNA into solution. In case ultra-freezers are not available we will use liquid nitrogen which yields even better results. Afterwards, each specimen is ground in 1.5 ml Eppendorf tubes using Proteinase K for chitin digestion.

Once extracted the DNA templates of all selected specimens, we will amplify through polymerase chain reaction (PCR) the subunit 1 of the mitochondrial gene Cytochrome oxidase (cox1) using standard primers described in Folmer et al. (1994). The short sequences (~ 600 base pairs) of this gene are currently used to support insect morphological identifications through the Barcode of Life Database (BOLD) with a relatively great success (Hebert et al. 2004, Smith et al. 2006, Smith et al. 2007, Fisher and Smith 2008). To examine evolutionary relationships among species in Phoridae we will use the ribosomal RNA (rRNA) genes 18S (small subunit) and 28 S (large subunit) which are widely used to examine insect molecular phylogenies.

DNA isolation and amplification will be performed in the genetics laboratory of the Zoological State Collection in Munich, Germany. The final product (DNA sequences)will be obtained at the Biocenter i the University of Munich. The genetic information will be analyzed at Instituto de Ciencias Biológicas, Escuela Politécnica Nacional (ICBEPN) and at UPS in Quito. We will use the program MEGA5 (Tamura et al. 2011) to make DNA alignments of all sequences. Phylogenetic trees will be constructed under maximum likelihood (ML) and Bayesian approaches. Once results are published, the sequences will be freely available in internet through GenBank (www.ncbi.nlm.nih.gov/genbank) and BOLD (www.barcodinglife.org) platforms; the latter will also contain photographs of each sequenced species together with notes on taxonomy and ecology.

Measuring of environmental variables We will count all orchid species growing in the surroundings of each sampling station (Malaise, Mcphail and Pan traps) in order to statistically relate the abundance of orchids to the amount of pollinating flies in the groups Drosophilidae, Phoridae,Tipulidae and Sciaroidea. In order to test whether the amount of vegetation influences the diversity of flies in each area we will count the number of trees > 10 cm DBH in 20 meters radius of each sampling station. We will also measure the following weather variables: elevation in each sampling station, daily temperature and rainfall per area.

Georeferencing species distributions At every collection site in each area we will record the coordinates using a standard GPS; afterwards, using the program ArcGIS (ESRI 2013), this information will be 12 visualized in a map showing the distribution of the species inventoried in the region.

This information will be stored in the database of the Department of Geography and Information at UPS under

the initiative of Mapping Species Distribution in Ecuador. The digitalization of the distributional maps will be performed at the mentioned Department at UPS.
Illustrated catalog
We will take high quality photographs of the flies, either in the field, as well as of collected specimens which will be mounted and deposited in the collections of the ICBEPN in Quito. The catalog will contain these photographs arranged by family, each of which will contain a short description of the number of genera and species found in the region; each species will contain a short taxonomic and ecological description. Finally we will build a taxonomic key to the orchid pollinating Diptera of the cloud forests in northwestern Ecuador. This illustrated work will serve as a basis for further scientific research on the biodiversity, evolutionary ecology and conservation of the insects the live in this megadiverse area of South America.

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8. RESULTADOS ESPERADOS

The proposed study will contribute to three main areas in science and society, in first place, our results will generate the first compendium of dipteran species associated to orchids, and in general to plants, pollination in the region; this will in turn boost the preservation of natural areas and its organisms through the study of insects and their ecological relationships with their plant hosts. Secondly, and as an extension of the latter, we will contribute to basic science in the fields of taxonomy and evolution by describing the fauna of dipterans; probably new species; and their evolutionary relationships in these poorly studied but highly endangered habitats of the Ecuadorian Andes. Finally, through the illustrative guide of pollinating dipterans, our investigation will provide a friendly and easy to read book which will capture the attention of either, the general public as well as the community of scientists who make research in this field, thus positively imprinting in the society a general aware of the wealth of animals and plants hosted in Ecuador.

We expect to produce the following concrete outputs,

1. The illustrated catalog to pollinating flies of the cloud forests in northwestern Ecuador, which will show information on the biodiversity, taxonomy, distribution,evolutionary ecology and conservation of these organisms in the proposed region of study.
2. At least two publications in national and/or international peer reviewed journals, for example, La Granja and Revista Politécnica, national journals published by UPS and EPN universities respectively, and Spixiana published by the ZSM in Germany. Possible related topics will include insect/animal biodiversity, molecular phylogenetics, and insect taxonomy and evolution. Both the catalog and publications in journals will require continuous scientific assistance and collaboration from two undergraduate students which will develop their theses in the framework of the present investigation. The three institutions participating in this project will share the credits for the proposed scientific publications

9. TRANSFERENCIA DE TECNOLOGÍA Y/O SOCIALIZACIÓN DE RESULTADOS DE INVESTIGACIÓN

The proposed field and lab work which will derive in publications and parallel presentations in congresses and symposia will also generate research opportunities through undergraduate theses for students at UPS, EPN and possibly at Universidad Central del Ecuador where Biology, Environmental Engineer and related careers are currently established. By means of the furtherance of the thesis work we, as researchers, will train and introduce in science to potential interested students.

Furthermore, both trainers and trainees will participate in national congresses for example, Jornadas Ecuatori

nas de Biología (Ecuadorian Symposia in Biology); and international meetings, as for example the Congreso Latinoamericano de Entomología (Latin American Congress in Entomology).
In addition, after obtaining preliminary results (see chronogram), we plan to give at least two scientific talks to students at UPS and EPN in the careers of Environmental
Engineer and Biotechnology in the framework of related topics within current academic programs. Although these talks will be primarily addressed to students at UPS and EPN we will encourage the assistance of the community therefore allowing for public
discussions, thus converting them in analytical forums and not just mere presentations of results.

On the other hand the proposed illustrated catalog, which will be in the majority of cases, provided by free to several universities, museums and research institutions in
Ecuador, will by itself render knowledge to society. We will arrange some public presentations of the catalog, either at the above institutions as well as in the communities living in the surroundings of the natural areas where this investigation will take place.

10. IMPACTOS DEL PROYECTO

1. The illustrated catalog and publications in national and/or international journals will generate cutting edge scientific information which will be available for the first time to the community, national and international researchers, people involved in conservation and ultimately to decision-making persons linked to institutions in the Government. These publications will form the scientific basis for further research in related topics.
2. During the execution of this study we will collaborate with several people from different institutions. By means of agreements between UPS and EPN Universities and with the Zoological State Collection of Munich, we will foster mutual collaboration leading ultimately to the advancement of science.

11. INFORMACIÓN DE COFINANCIADORES (en caso de que existieran)

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