SPECIES LIMITS AND PHYLOGENETIC SYSTEMATICS OF THE DIURNAL GECKOS OF THE GENUS *GONATODES* (SQUAMATA: SPHAERODATYLIDAE)

by

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ABSTRACT

SPECIES LIMITS AND PHYLOGENETIC SYSTEMATICS OF THE DIURNALGECKOS OF THE GENUS *GONATODES* (SQUAMATA: SPHAERODATYLIDAE)

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The genus *Gonatodes* (family Sphaerodactylidae) is a diverse of group of about 20 species currently recognized of mostly diurnal geckos that are collectively distributed from southern Mexico to Brazil and Bolivia, and also including most of the Caribbean Islands. The systematics of this group remains poorly studied with published contributions limited mostly to the original species descriptions, natural history notes, and a recent, yet largely incomplete, molecular phylogeny. In this work, several contributions to the systematics of this genus are made.

The first chapter is a general introduction to the group. Chapter 2 is a phylogeographic study of the *G. vittatus* complex in northern Venezuela. The most

significant results from this chapter suggest mitochondrial introgression between *G. petersi* and *G. vittatus* at a novel contact zone. In addition to this, a new species is this complex is discovered and supported by both morphological and molecular data. Chapter 3 is a systematic study of the genus *Gonatodes* in the Guayana Shield. Two new species in this region are discovered and described, and molecular data supports a monophyletic radiation of the genus in the region. In Chapter 4 a novel approach to delimit species boundaries is proposed and tested in a group of species of *Gonatodes* found in montane habitats of Venezuela. This new approach uses a combination of different data including morphological characters, mitochondrial markers, and GIS-based ecological niche modeling to infer ecological barriers. Chapter 5 is a discussion on the phylogenetic relationships of the genus *Gonatodes* focusing in the major clades and the position of *G. antillensis*, which is the only known nocturnal species of *Gonatodes*. The molecular data supports inclusion of this species in *Gonatodes* and the notion that it has re-evolved character states associated with nocturnal life (elliptical pupil and use of vocalizations).

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CHAPTER 1

GENERAL INTRODUCTION

In light of the current biodiversity crisis, documenting the world's largely unknown biological diversity is arguably one of the most important tasks a biologist can undertake (Wilson, 1992). Rapid habitat destruction is a worldwide phenomenon driving many species to extinction. This is especially problematic in the tropics as countries located within this region harbor the greatest biological diversity and are generally the most lacking on biodiversity surveys and also suffering the most rapid rate of habitat destruction. Decades of work (if not centuries) are still ahead of us before we near completion of documenting the total diversity of species in our planet (debate exist on whether we will ever completely finish this task), as even in the best-studied groups, such as mammals, new species are found every year.

For the last decade I have been engaged in multiple studies of the fascinating diversity of amphibians and reptiles of Venezuela. During this time my collaborators and I have discovered several species new to science, including frogs, salamanders, lizards and snakes, many of which still remain to be formally described. This has been the result of many days of work in the field in multiple localities throughout most of the country. Venezuela, being a Neotropical country with many different bioregions (e.g. Andes, Coastal, Guiana Shield, Llanos) and habitats (e.g. cloud forest, paramo, rain forest, grasslands) determined by the topographic, geologic and climatic complexity

inherent to this country (Huber, 1997); contains one of the highest diversities of amphibians (298 species in most recent account; see Barrio, 2004) and reptiles (344 species in most recent account; see Rivas et al., in prep) in the world for a country of relatively small size. Furthermore, when the rate of discovery of new species in these two groups in Venezuela is examined through time there is no indication yet of a decrease in this rate (Schargel, unpublished); thus many more new taxa are expected to be discovered in upcoming years. Although I have worked on several different groups in different families of amphibians and reptiles, in the last four years I have focused my efforts almost completely on a single genus: *Gonatodes*. It is my goal in this dissertation to show my results so far on this genus, which is a member of one of the most fascinating and diverse clades of lizards, namely the Gekkota.

The Gekkota comprise one of three major clades of lizards (Vitt et al., 2003) and includes what we commonly know as geckos and pygopods (The latter Australia and New Guinea's legless lizards). With approximately 1100 species currently recognized (Kluge, 2001) this clade accounts for more than 25% of the total species diversity of lizards (Kluge, 1987). Perhaps the most distinctive traits of gekkotans are that the great majority of species in this lineage are nocturnal and also have highly complex adhesive toepads that allow them to climb smooth vertical surfaces with ease (Russell, 1972). In addition, many species of geckos are unique among lizards in being able to produce complex calls (e.g. multiple chirps) that are used in social interactions (Marcellini, 1977, 1978). Interestingly, the evolution of calls in geckos seems to be a compensation for the limitations of using visual displays at night (Marcellini, 1977). Within Gekkota

nocturnal activity is presumably the ancestral state (Vitt et al., 2003) with a few secondarily derived diurnal species allocated in some Old World genera of the Gekkonidae (e.g. *Cnemaspis*, *Lygodactylus*, and *Phelsuma*) and, with some exceptions, most members of the family Sphaerodactylidae (sensu Gamble et al., 2008).

The neotropical tribe Sphaerodactylini is a member of the family Sphaerodactylidae and is the only major group of mostly diurnal species of geckos and with about 145 species this group accounts for about 13% of the total species of gekkotans (Kluge, 1995). The phylogenetic position of sphaerodactyls within Gekkota remains somewhat controversial. Kluge (1987) based on morphology proposed that this group is nested within the subfamily Gekkoninae, whereas recent molecular work (Gamble et al., 2008; Han et al., 2004) and Kluge's (1967) own earlier morphological analysis indicate that sphaerodactylines are not nested within but sister to gekkonines. Whichever the case, the most parsimonious scenario is that diurnal activity has been secondarily evolved in sphaerodactyls. The species in this tribe are contained in the following five genera (number of species in parenthesis): *Coleodactylus* (5), *Gonatodes* (20), *Lepidoblepharis* (18), *Pseudogonatodes* (7) and *Sphaerodactylus* (95), with a collective distribution from Mexico to Brazil and Bolivia in mainland America, and also throughout most of the Antilles.

My interest in *Gonatodes* begun in the summer of 2002 when I first collected a member of what I now call the "montane group"; a group of large species restricted to the Andes and Coastal mountain range of Venezuela and highlands of Trinidad and Tobago. The species I collected was *G. ceciliae,* which I found while conducting

fieldwork in the humid forests of the mountainous regions of the Peninsula de Paria in extreme northeastern Venezuela. The first thing that struck me about this species was the great degree of sexual dimorphism in coloration. Males were by far the most attractive lizards I had ever seen, with a dark body beautifully ornamented with bright red, yellow and green markings, whereas females were cryptically colored much like the nocturnal species with which I had previous field experience (e.g. *Hemidactylus*, *Phyllodactylus*). Also interesting was the observation that males and females were territorial and directed visual displays towards conspecific intruders. Furthermore, males were able to change the intensity of their coloration depending on social interactions as was noted for a few individuals I kept in captivity. Males that became dominant in captivity increased the intensity of their bright colors, whereas submissive males did the opposite.

These incidental observations on *G. ceciliae* prompted me to search for literature on all aspects of the biology of the genus. Regrettably, little has been published about *Gonatodes* and the most important work on the genus remains the 1979 unpublished Ph.D. Dissertation of my Venezuelan colleague (and now collaborator and friend) Dr. Carlos Rivero-Blanco. Rivero-Blanco completed his doctorate at Texas A&M University in College Station, TX, working under renowned herpetologist Dr. James A. Dixon. His dissertation was a monographic work focusing mostly on the taxonomy of *Gonatodes*, but also presenting a valuable discussion about the genus on diverse topics such as evolutionary relationships, biogeography, natural history, coloration function, etc. Because published taxonomic work on *Gonatodes* is limited mostly to the original

species descriptions, many of which are old and not detailed, Rivero-Blanco's dissertation has become an important reference for the few people who have worked on the genus during the last two decades (e.g. Esqueda, 2004; Cole and Kok, 2006; Avila-Pires, 1995). Some of the most important results from Rivero-Blaco's dissertation are the discovery of three new taxa from Venezuela and one from Trinidad, and also the observation that, unlike most other lizards, many members of *Gonatodes* exhibit an extreme, male-limited, discrete polymorphism in color pattern. This polymorphism is so extreme that in some cases different color-morphs (= discrete variants of color pattern within a species), have been interpreted as different species living in sympatry (e.g. Donoso-Barros, 1966; see Rivero-Blanco, 1968). For those species of *Gonatodes* that exhibit this type of polymorphism there generally are two different color morphs, one in which males have reticulated solid black markings, and one in which males are devoid of these markings. However, in at least one undescribed species from the Coastal Mountain Range of Venezuela there are up to four different color morphs living in sympatry in some localities (Rivero-Blanco, 1979).

Another important piece of unpublished information on *Gonatodes* is the Ph.D. dissertation of Janna M. Ellingson. Ellingson (1994) studied sexual selection in *G. albogularis* and demonstrated that females preferred to mate with males that have a yellow head as opposed to males that have a reddish orange head. This result is quite significant, as most studies on sexual selection in lizards have failed to demonstrate female mate choice and the general trend is that sexual selection in lizards is driven by male-male competition (Andersson, 1994; Tokarz, 1995).

In addition to the interesting findings of Rivero-Blanco (1979) and Ellingson (1994), my own unpublished observations on different species of *Gonatodes* kept in captivity indicate that, much like some of the better studied diurnal families and unlike nocturnal geckos (see Marcellini, 1977), they have a diverse repertoire of stereotyped visual displays, such as push-ups, arm waving, tail flicking, head bobbing, etc. that are used in social interactions. I have found parallels in display function between *Gonatodes* and the well-studied, distantly related diurnal iguanids and agamids. For example, males used mostly push-ups in combination with other modifiers (e.g. arm waving, tail raising) in territorial encounters, whereas head movement is used mostly for courtship. Because nocturnal geckoes seem to have a limited visual display repertoire, there is a potential fascinating case of re-evolution of visual display signals in *Gonatodes* that are in some ways convergent with that of some diurnal families.

With this in mind, *Gonatodes* is an excellent candidate to be a model system for studying sexual selection and especially evolution of visual signals. I will summarize here the already mentioned aspects of why I think this proposition is valid: 1) *Gonatodes* is a diverse group with more than 20 species that vary mostly in aspects of sexually selected coloration; 2) there is extreme sexual dimorphism in coloration in the genus, 3) discrete male-limited polymorphism in coloration occurs in many species, 4) a diverse repertoire of displays in social interactions has been observed in those species studied, 5) female mate choice is present, and finally 6) they adapt well to captivity and it is easy to elicit territorial or courtship displays from individuals even when they have been kept captive only for a few days. As Ellingson (1994) stated, *Gonatodes* are

perhaps the lizards most similar to birds in terms of sexual dimorphism and sexual selection.

It is my goal to continue working on *Gonatodes* beyond this dissertation and to focus almost exclusively on studying the function and evolution of sexually dimorphic coloration and displays. For this to be possible, the first step is to have a sound taxonomy of the genus, one that reflects as accurate as possible the limits between species, and also to have a robust hypothesis of phylogenetic relationships that will allow reconstruction of character evolution. In this dissertation I have addressed this first step as thoroughly as possible given sampling limitations, using both morphological and molecular data.

Systematic studies of neotropical taxa generally represent difficult tasks, especially when relying at least partially on molecular data. Although in some cases there are sufficient museum specimens to allow a fair understanding of morphological variation in a group, this generally is not the case for tissue samples suitable for molecular work. Regrettably, it is only recently that researchers based in the Neotropics have begun to regularly preserve tissues that can be used for molecular analyses, especially for DNA sequencing. Because of this limitation, I had to obtain most of the tissue samples myself from specimens collected directly in the field. Fortunately, 13 of the 20 currently recognized species of *Gonatodes* occur in Venezuela, country in which, as mentioned above, I have focused my fieldwork for more than a decade now. I have collected multiple samples from different localities for all the 13 species that occur in Venezuela, and, through collaborators working in other countries, I have obtained tissue

samples of five of the seven species that do not occur in Venezuela. Thus, I only lack samples of two of the currently recognized species of *Gonatodes*, both of which are endemic to Brazil and have restricted distributions. However, for a relatively large and widespread genus such as *Gonatodes*, obtaining 90% of the taxa is fairly good as most studies of tropical genera of comparable size and distribution are generally able to sample only a much smaller fraction of species.

Limitations in obtaining samples for molecular work set aside, there are still other problems that have caused our knowledge of systematics of neotropical amphibians to be far from optimal. The fact that most systematics studies in the Neotropics have relied only on morphological data is not a great problem per se, as this type of data has an epistemological justification as good as any other type of data for inferring species boundaries or evolutionary relationships. The main problem is that most of the taxonomic work has been done under a traditional and obsolete framework dating back two centuries. In this traditional framework a typological species concept is implicitly used and species limits decisions are made inductively in an arbitrary and ad hoc way. There generally is no ontological consideration for what a species is, and little epistemological justification for the discovery operations used to delimit species boundaries. Traditional taxonomy basically translates into defining a species as a group of individuals in a somewhat defined geographic area that posses an arbitrary amount of differences when compared to other such species. As a consequence current taxonomy might not closely reflect the limits between the theoretical entities (e.g. evolutionary species concept) that we now equate the concept of species to (de Queiroz 1998).

Despite the limitations of traditional taxonomic methods it is necessary to point out that its current practice still remains vital to rapidly catalog the planet's biological diversity, and to set the grounds for more scientifically rigorous and sophisticated ways to test species boundaries.

For *Gonatodes*, the dissertation of Rivero-Blanco (1979) provides an excellent starting point on the taxonomy of the genus. In the first three chapters of this dissertation I attempt to delimit species boundaries and revise the taxonomy of three different monophyletic groups of *Gonatodes*, namely: 1) the *G. vittatus* group, which contains two closely related species, characterized by a white middorsal stripe, which are found in the lowlands and piedmonts of northern Venezuela and a few islands off the coast of this country; 2) the "Guyanan clade" a monophyletic radiation of species almost exclusively restricted to the Guyana Shield; and 3) the "montane group", a diverse radiation (current analysis are inconclusive about the monophyly of this group) of species that inhabit the humid montane forest of the Merida and Coastal mountain ranges of Venezuela, as well as the mountainous areas of Trinidad and Tobago. In delimiting species boundaries in *Gonatodes* I try to use a more scientifically rigorous approach than in traditional taxonomic studies. Following Frost and Kluge (1994) and Mayden (1997), I consider the evolutionary species concept (*sensu* Wiley, 1978), which in general defines species as the "largest integrating lineages," as the only purely theoretical species concept that is also consistent with ontological claims of individuality (for a discussion of species as individuals see Ghiselin, 1974; Hull, 1978; Mishler and Brandon, 1987). Thus, evolutionary species are the real entities in nature

that I try to delimit by using a priori defined criteria in analyses of morphology, genetics and presence of geographic barriers.

The final chapter of this dissertation is a general discussion of the phylogenetic relationship of *Gonatodes* focusing on the major clades recovered from an analysis using the 12s mitochondrial gene and the C-mos nuclear gene. I also focus in this chapter on the phylogenetic position of *G. antillensis*. This is the only nocturnal species of *Gonatodes* and the evolutionary implications of its phylogenetic position are discussed.

CHAPTER 2

MITOCHONDRIAL INTROGRESSION BETWEEN TWO ECOLOGICALLY DIVERGENT GECKOS: PHYLOGEOGRAPHY OF *GONATODES VITTATUS* AND *G. PETERSI* (SPHAERODACTYLIDAE)

2.1 Introduction

The genus *Gonatodes* is a diverse group of about 20 species currently recognized (Cole and Kok, 2006) of mostly diurnal geckos that are collectively distributed from southern Mexico to Brazil and Bolivia, and also including most of the Caribbean Islands (Rivero-Blanco 1979; Powell and Henderson 2005). The greatest diversity of *Gonatodes* is centered in northern South America, especially in Venezuela, country in which 12 species are known to occur (Esqueda, 2004), but also where several additional species remain to be described (Rivero-Blanco, 1979; Chapters 3 and 4). Two closely related species of *Gonatodes* that occur in Venezuela, namely *G. petersi* Donoso-Barros, and *G. vittatus* (Lichtenstein), are distinctive in having a conspicuous, white, middorsal stripe, a character shared with only two other species in the genus: *G. atricucullaris* Noble, from Peru and *G. eladioi* Nascimento, Cunha and Avila-Pires, from Brazil. *Gonatodes petersi* is endemic to the eastern versant of the Sierra de Perija in northwestern Venezuela (Rivero-Blanco, 1979; Rojas-Runjaic and Rivas, 2006), whereas *G. vittatus* is a widespread species along the coast, on the northern portion of the country. Both species have diverged ecologically as they occur in contrasting habitats. *Gonatodes petersi* inhabits submontane humid forests and semideciduous

riparian forests (Rivero-Blanco, 1979; Rojas-Runjaic and Rivas, 2006), whereas *G. vittatus* is found naturally associated with xerophytic vegetation in arid and semiarid regions, yet it can invade more humid regions in association with anthropogenic impact (Rivero-Blanco, 1979; La Marca and Soriano, 2004). These two species also differ is aspects of morphology including size, coloration (mostly in males), and subdigital scale counts. The characters that differ among *G. petersi*, *G. vittatus* and a related population of uncertain taxonomic status (see below) are specified in Table 2.1. Interestingly, *G. petersi* and *G. vittatus* come into contact in at least one transitional habitat locality in the northern portion of the Sierra de Perijá (this work). Contact zones between closely related and morphologically similar species are important windows into understanding the evolution of reproductive isolation mechanisms and ultimately the origin of species (Howard et al., 2003). Even when closely related species that have not developed genetic incompatibilities come into contact and hybridize, gene introgression can be limited if the species have diverged ecologically in habitat preferences (e.g. *G. petersi* and *G. vittatus*) and hybrids are selected against by the environment (Anderson, 1948).

In addition to this contact zone between *G. petersi* and *G. vittatus* there is a distinctive population (here forth referred as "SMLB population" following Rivas et al. 2006) occurring along the western versant of the Mérida mountain range and lowlands south of the Maracaibo Lake that, although morphologically distinguishable from both *G. petersi* and *G. vittatus*, in some aspects seems intermediate between these two species (see Figure 2.1). As a consequence the SMLB population has been referred in the literature to both of these species by different authors. Rivero-Blanco (1979)

Table 2.1. Morphological characters used to distinguish among species in the *G. vittatus* complex

Species/Population	Size (SVL)	Dorsal ferruginous coloration in males	White lateral spots in males	Dorsal background coloration in males	Infraproximal lamellae under fourth toe.
petersi	>35 mm	Absent	Present	Brown	Generally 6, rarely 5 or 7.
vittatus	<35 mm	Present, extending from head to body	Absent	Grey	Generally 7 or 8, rarely 6 or 9.
SMLB	>35 mm	Present, only on head	Present	Grey	Generally 6, rarely 5 or 7.

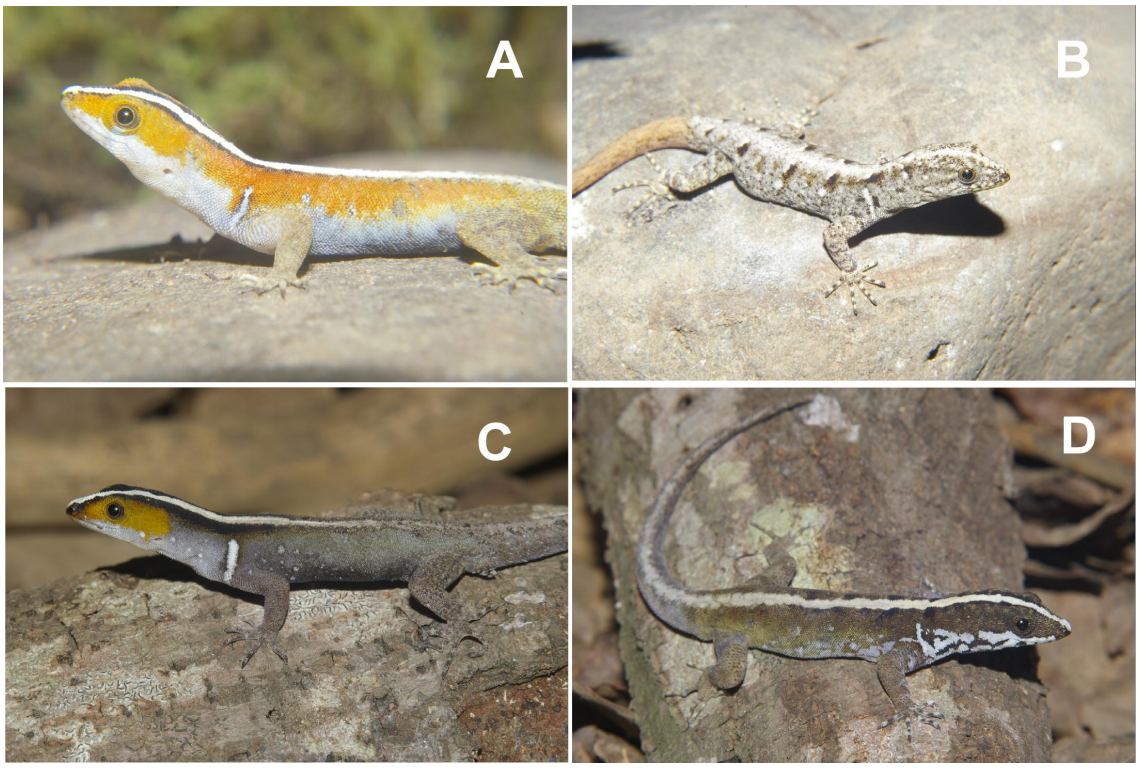


Figure 2.1 Specimens in life of a male *G. vittatus* (A), a female (B) and a male (C) of the SMLB population, and a male of *G. petersi* (D)

assigned this population to *G. vittatus* without further comment. Esqueda (2004) in a description of a new species of *Gonatodes* listed in the material examined section specimens of *G. petersi* that are referred to the SMLB population (Esqueda pers. comm.). Rivas et al. (2006) noticed the distinctiveness of the SMLB population, which they regarded as variation in *G. vittatus*. However, the SMLB population is actually more similar ecologically to *G. petersi* than *G. vittatus* as it inhabits similarly humid forests. Because there are still sampling gaps located between specimens referred to the SMLB population and the known distribution of *G. petersi*, there is a possibility that these are conspecific and that they represent morphological extremes in clinal variation. Contrary to what has been proposed in the literature, the SMLB population might

represent an independent lineage (i.e., a different species) that deserves taxonomic recognition.

Mitochondrial DNA (mtDNA) markers have become one of the most important data sources for examining phylogenetic relationships, species limits, and population history, in animal species (Avise, 2000). However, the use of mtDNA in these types of studies has been criticized because processes such as introgressive hybridization and incomplete lineage sorting can cause gene trees to deviate from reflecting phylogenetic descent. In light of this, studies of closely related species using mtDNA are of utmost importance to determine the ubiquity and the circumstances under which these processes occur. Herein I use data from two mitochondrial gene regions to examine phylogenetic and phylogeographic structure in *G. petersi*, *G. vittatus* and the SMLB population, a group which I will referred collectively as the "*G. vittatus* Complex", to answer the following questions: 1) what is the taxonomic status of the SMLB population? 2) are species in this complex reciprocally monophyletic for their mitochondrial lineages? 3) if they are not reciprocally monophyletic, is it due to incomplete lineage sorting, heterospecific mitochondrial introgression or both? 4) what other aspects of population history/structure can be inferred?

2.2 Materials and methods

2.2.1 Taxon sampling and outgroup selection

For the molecular analyses I obtained 10 samples of *G. petersi* from three localities, 38 of *G. vittatus* from 27 localities, and 16 samples referred to the SMLB population from 7 localities. One of these localities is the only known contact zone

between *G. petersi* and *G. vittatus*. Species identification was based on the morphological characters listed on Table 2.1. Males were always obtained in the different localities studied and because there were no specimens or populations intermediate in the distribution of the character states used to distinguish among species/SMLB population, identification was unambiguous. I also included in the analyses a few samples of *G. albogularis*, which is closely related (Chapter 5; see also Gamble et al., 2008) and partially sympatric with the group under study, to examine whether there was evidence of mitochondrial introgression with this species. Following the results of Gamble et al. (2008) I selected *G. daudini* from the Lesser Antilles as the outgroup in phylogenetic analyses, as this species is shown to be sister to the clade containing *G. albogularis* and the *G. vittatus* Complex. All samples used in this study with the associated locality data and field or museum numbers are listed in Appendix A.

2.2.2 Lab protocols and molecular analyses

Tissues (liver or muscle) were obtained from freshly killed specimens in the field and were preserved in 95-99% ethanol, and permanently stored at –70°C. Total genomic DNA was isolated using the DNAeasy Blood and Tissue Kit (Qiagen) following the Animal Tissue protocol provided by the manufacturer. DNA Sequences were amplified from the mitochondrial ribosomal small unit (12S primers: 12a: 5'-CTG GGA TTA GAT ACC CCA CTA-3'; 12b: 5'-TGA GGA GGG TGA CGG GCG GT-3') and from the cytochrome oxidase I (Cox1; COI primers: CO1F: 5'-CCT GCA GGA GGA GGA GAY CC-3'; CO1R 5'-AGT ATA AGC GTC TGG GTA GTC-3') gene regions using standard polymerase chain reaction (PCR) protocols. The PCR products

were purified using the ExoSAP-IT^{tm} kit (United States Biochemical) and used as templates in sequencing reactions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were cleaned-up using ETOH/Sodium acetate precipitations and were read on an ABI PRISM 3100xl Genetic analyzer. Contigs were assembled and edited in Sequencher 4.1 (Genes Code Corps., Inc).

Sequences were aligned online in T-COFFEE 5.56 (Notredame et al., 2000) using the "regular option" (default parameters). This service is provided through a web server (http://www.tcoffee.org) of the Swiss Institute of Bioinformatics (see Poirot et al., 2003). A Maximum Parsimony (MP) analysis of the concatenate dataset was performed on TNT 1.2 (Goloboff et al., 2000) using the Tree Branch Reconnection (TBR) algorithm with 100 sequence additions and retaining 10 trees per replication. Nodal support of the MP analysis was estimated using the standard (with replacement) Bootstrap option of TNT with 1000 replications. I also obtained a tree using the Neighbor Joining (NJ) method with uncorrected distances to visualize the relative sequence divergence between haplotypes and clades. The NJ analysis was conducted in MacVector. The geneology of the concatenated dataset was also examined by estimating a haplotype network using the statistical parsimony approach proposed by Templeton et al. (1992) implemented in the program TCS 1.21 (Clement et al., 2000). Network methods are often more appropriate for intraspecific data than traditional phylogenetic methods because they allow for different phenomena that are common at the population level, such as multifurcations, extant ancestral haplotypes, and reticulations (Posada and Crandall, 2001).

2.3 Results

2.3.1 Sequence characteristics

The 12s and Cox1 alignments were 409 and 612 bp long, respectively, for a concatenate dataset of 1021 bp. The 12s alignment contains several regions with gaps resulting from indel events. These gaps were small not exceeding 3 bp in length. The Cox1 alignment did not contain any gaps.

2.3.2 Phylogenetic analysis

The MP analysis recovered 17 equally parsimonious trees with tree length of 605. A strict consensus of all 17 trees is shown in Figure 2.2, with bootstrap values on most nodes. The NJ tree (Figure 2.3) with uncorrected distances is essentially the same as the consensus MP tree, recovering the same basal relationships and with only minor differences at the tips. Both the MP consensus tree and the NJ tree show that neither *G. petersi*, *G. vittatus* nor the SMLB population, are monophyletic for the mitochondrial haplotypes examined. The basal-most split in the haplotype tree of the *G. vittatus* complex is between samples of *G. petersi* from Finca El Deseo (163G, 193-195G) and Río Las Lajas (156G), and a large clade containing all remaining samples. However, samples of *G. petersi* from Río Mache (the contact zone between *G. petersi* and *G. vittatus*) fall sister and have little divergence (<1%) relative to samples of *G. vittatus* from the same locality and from the nearby locality of Hacienda Grano de Oro. Samples referred to SMLB fall in two different clades forming two monophyletic groups, respectively. The first of these two groups of haplotypes is sister to a large clade containing all the haplotypes found in *G. vittatus* as well as those found in *G. petersi*

from Río Mache and those on the second group of SMLB haplotypes, which is nested within *G. vittatus*. Haplotypes of *G. vittatus* fall in three clades. The first is sister to the other two clades and contains haplotypes only of from the State of Lara (samples 38G, 213G, 215G) in Venezuela. The other two clades are sister to each other, one of which contains samples of *G. vittatus* from northwestern and northcentral Venezuela and also the haplotypes of *G. petersi* from Río Mache as well as the second clade of SMLB haplotypes; the other contains only samples of *G. vittatus* form northcentral and northeastern Venezuela.

The statistical parsimony approach generated mostly small networks (not shown here) connecting few haplotypes, generally with several single nucleotide substitutions events inferred between them. Only one large network was recovered corresponding to *G. vittatus* samples mostly from northcentral and northeastern Venezuela. This network shows possible multifurcations as well as retained ancestral haplotypes.

2.4 Discussion

The morphologically cohesive nature of the SMLB population across its distribution, together with the high level of genetic divergence relative to both *G. petersi* and *G. vittatus*, as well as a lack of any evidence for recent mitochondrial introgression with these two species suggest that the SMLB population represents an independent evolutionary lineage and, as such, should be recognized as a different species for which a new name needs to be proposed. However, this SMLB species is not

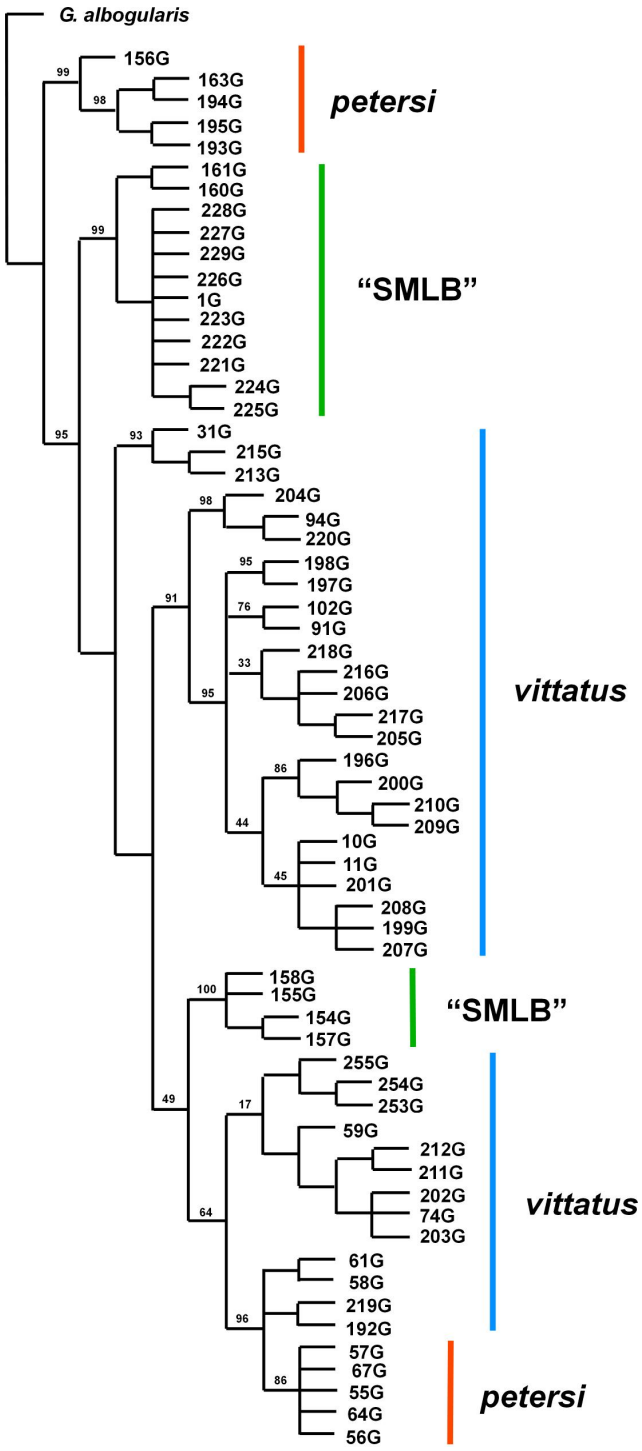


Figure 2.2 Strict consensus of 17 equally parsimonious trees in the *G. vittatus* Complex

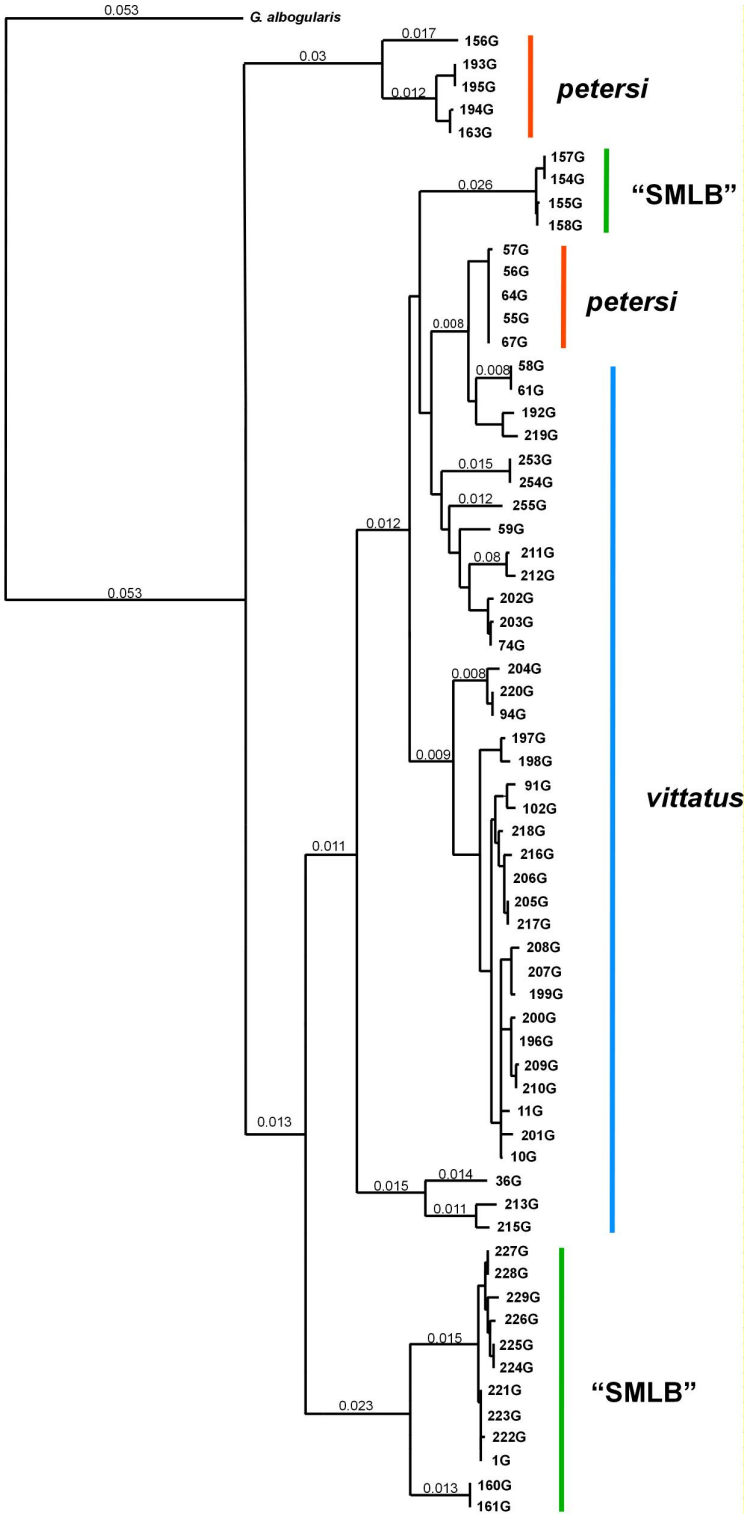


Figure 2.3 Neighbor Joining tree with uncorrected distances of the *G. vittatus* Complex

monophyletic for the mitochondrial markers examined, as it contains two divergent mitochondrial lineages. Whether this is the result of persistent ancestral polymorphism or past hybridization cannot be determined based on the available data. Future studies focusing on the potential contact zones between SMLB and *G. vittatus*, in the northern limit of distribution of the former, as well as between SMLB and *G. petersi*, in the southern range of distribution of both species, are needed to examine potential hybridization between these species. It is also important to note that the two haplotype clades of SMLB correspond with the geographic distribution of sampling localities. The first, basalmost clade contains samples from intermediate localities (Mérida State) as well as the northern extreme of distribution (samples 160 and 161G, both from Trujillo Sate). The second clade contains only samples from the southern extreme of distribution of the SMLB species (Táchira State). In the absence of any geographic barriers separating these two clades as well as an apparent lack of morphological differences, it seems unlikely that the two clades represent two distinct species.

With respect to *G. petersi*, the results seem to clearly indicate that the polyphyletic nature of the mitochondrial haplotypes of this species is the consequence of interspecific mitochondrial introgression rather than incomplete lineage sorting. This hypothesis is supported by the two following facts: (1) the haplotypes of *G. petersi* and *G. vittatus* that cluster together occur at the contact zone between these two species, (2) divergence observed between the haplotypes of these two species at the contact zone is minimal (less than 1%) suggesting that it postdates the point of divergence between these ecologically and morphologically differentiated species. The original ancestral

haplotypes of *G. petersi* are perhaps those found in the localities of Finca el Deseo and Rio Las Lajas, which are far removed from any potential contact zone with *G. vittatus*. These haplotypes are basal within the *G. vittatus* Complex and on average diverge by about 10% (12s + Cox1) from the rest. Interestingly, mitochondrial introgression seems to be unidirectional, having occurred only from *G. vittatus* into *G. petersi*. Without further study it is not possible to infer the evolutionary processes that account for this observation but the following has been suggested in the literature (see Ballard and Whitlock, 2004; McGuire et al., 2007) for similar cases of unidirectional mitochondrial introgression: 1) incompatibility of one mitochondrial genome in a hybrid genetic background (in which case it would be the *G. petersi* mitochondrial genome the incompatible one), 2) higher fitness effect of one mitochondrial genome, leading to a selective sweep (in which case the *G. vittatus* mitochondrial genome confers higher fitness), and 3) an asymmetry in reproductive isolation/sexual selection between species. It is noteworthy to mention that the size differences in *G. petersi* (larger) and *G. vittatus* (smaller) and the direction of introgression (from *G. vittatus* into *G. petersi*) are consistent with an asymmetry in reproductive isolation/sexual selection. Because larger male size is generally advantageous in both male-male competition and mate choice in lizards (Anderson, 1994; Tokarz, 1995), it is expected that females of *G. vittatus* would mate with the larger males of *G. petersi*, whereas it is unlikely that the large females of *G. petersi* would mate with the much smaller males of *G. vittatus*. However, the fact that there is some divergence between the haplotypes of *G. petersi* and *G. vittatus* at the contact zone, and also the fact that there is no evidence of morphological intergradation

in the area, suggest that hybridization might be rare or currently absent, and was possibly limited to the initial secondary contact between these species. Because *G. petersi* and *G. vittatus* seem to have diverged ecologically into occupying contrasting habitat, it is possible that the environment selects against hybrids and reinforcement of reproductive barriers (especially species recognition) has occurred between these two species.

Interestingly the few cases in which studies have found evidence of heterospecific mitochondrial introgression in lizards have shown that this is frequently unidirectional and concomitant with selective sweeps (see Morando et al., 2004; McGuire et al., 2007). I suspect that unidirectional mitochondrial introgression is a more common evolutionary phenomenon than currently understood (see also McGuire et al., 2007), yet more phylogeographic studies targeting multiple closely related species are needed to confirm this notion and to uncover possible ecological correlates.

Of the three species in the *G. vittatus* Complex only *G. vittatus* is not confined in to the Lake Maracaibo Basin, as it also extends in distribution to the east, mostly along the Coast of Venezuela and also on a few nearby Islands. Most of the genetic variation in *G. vittatus* occurs in northwestern (including the Lake Maracaibo Basin) and northcentral Venezuela, a region in which haplotypes from different localities can have maximum uncorrected divergences of over 5% (less than 5 % if the basal-most haplotypes from Lara are excluded). In contrast, there seems to be relatively lower genetic diversity in northeastern Venezuela, as maximum haplotype divergence in this region is around 1%, even across comparable geographic distances. It is also in this

region were a significant haplotype network structure is recovered by statistical parsimony, with haplotypes from nearby localities generally separated by few single nucleotide substitution events. This geographic pattern of genetic diversity is consistent with a recent expansion of *G. vittatus* into northeastern Venezuela. This scenario is also supported by the fact that the inferred ancestral area of origin of the *G. vittatus* complex using the progression rule principle (Humphries, 1992) is the Lake Maracaibo Basin. Notice that the basal-most split in the *G. vittatus* complex is between *G. petersi* and *G. vittatus* plus the SMLB species, and also that *G. albogularis* (putative sister species to the *G. vittatus* complex) also occurs in the Lake Maracaibo Basin but does not extends in distribution east from there.

CHAPTER 3

PHYLOGENETIC SYSTEMATICS AND SPECIES LIMITS OF THE GENUS *GONATODES* (SPHAERODACTYLIDAE) IN THE VENEZUELAN GUAYANA, WITH DESCRIPTION OF TWO NEW SPECIES

3.1 Introduction

The Guayana region in northern South America is arguably one of the regions of the world with the most fascinating biological diversity. This region, as delimited by Hoogmoed (1979), encompasses the whole of French Guiana, Guyana, Surinam, most of Southeastern Venezuela and adjacent areas of extreme northern Brazil, and represents one of the oldest geological formations in the world, dating back to the Precambrian. Topographically, the Guayana region is complex, but has been roughly divided in uplands (500-1500 m), highlands (>1500 m) and peripheral lowands (<500 m; McDiarmid and Donnelly, 2005). From a biogical perspective, the highlands, also known as "Pantepui", represent the most interesting areas, characterized by a large number of endemics, many of which are restricted to the summit of the peculiar table-top mountains or "tepuis" of Guyana and Venezuela. These highlands have fascinated naturalists for many decades and even captivated the imagination of Sir Arthur Conan Doyle, serving as the geographic setting for *The Lost World*, a novel he wrote about a fictitious expedition to the summit of a tepui thriving with prehistoric life.

Among the major groups of animals that occur in the Guayana region, it is perhaps the amphibians and reptiles that have received the most scientific attention in

recent decades. Several expeditions to the region, together with revisionary studies, mostly by researchers at the American Museum of Natural History, New York, and the La Salle Musem of Natural History, Caracas, have resulted in the description of a plethora of new species and even new genera of amphibians and reptiles, mainly from Venezuela (e.g. Myers and Donnelly, 1996; 1997; 2001; Gorzula and Señaris, 1998; Señaris et al., 1994; 1996). Understandably, their efforts have ben focused mostly on the highlands likely due to the high levels of local endemicity in these areas (Hoogmoed, 1979; McDiarmid and Donnelly, 2005). More recently, however, studies have started to draw attention to the complex, yet overlooked, patterns of diversification within low/mid- elevation groups of the Guayana region (e.g. Noonan and Gaucher, 2005; 2006; Hawkings et al., 2007). These recent studies have challenged the commonly held view that this region represented a stable refuge during the climatic fluctuations of the late Tertiary and Quaternary, acting as a biotic reservoir. Additionally, recent fieldwork has also resulted in several endemic new species of amphibians and reptiles from the lower elevations of this region (e.g. Cole and Kok, 2006; Kok, 2005; 2006a, 2006b; Smith and Noonan, 2001) suggesting that the high levels of endemicity are not necessarily restritcted to highland taxa.

The genus *Gonatodes* represents a diverse clade of neotropical diurnal geckos (lizards in Gekkota) with about 20 species currently recognized, and with a center of diversity located in northcentral South America (Rivero-Blanco, 1979). In the Guayana region three species of *Gonatodes* have been reported, two of which, *G. annularis* and *G. alexandermendesi*, are endemic to the region yet they occur at relatively low

elevations (<800 m), whereas *G. humeralis* is a widespread species in lowland rainforests of South America. Recent field work in the Venezuelan Guayana by the staff of the La Salle Musem in Caracas discovered several populations of *Gonatodes* that could not be assigned to any of the species known to Venezuela. I herein use morphological and molecular data to determine the taxonomic status of these populations. Our study reveals the existence of two sympatric undescribed species of *Gonatodes* from the peripheral lowlands of the Guayana region in the Puerto Ayacucho area. We also present a preliminary phylogenetic analysis of the genus focusing on the position of the Guayana endemics to examine if these species result from multiple colonization events by different lineages or if they are the result of within region diversification from a common ancestor. This study further support recent findings that suggest a higher level of diversification and endemism within the lower elevations of the Guayan region.

3.2 Materials and methods

3.2.1 Morphology and species descriptions

Museum specimens examined are deposited at Museo de Historia Natural La Salle, Caracas (MHNLS), and the Amphibian and Reptile Diversity Research Center, The Univerisity of Texas at Arlington (UTA). Individual measurements used in the descriptions were taken with a digital caliper to the nearest 0.1 mm and are listed below with the corresponding abbreviations. The species descriptions generally follow, with minor modifications and additions, the format and terminology used by Avila-Pires (1995) for Amazonian species of *Gonatodes*. Rivero-Blanco (1979) divided the

subdigital scale count of the third finger and fourth toe into infraproximals and infradistals, corresponding to the subdigtal scales on the two most proximal and two most distal phalanges, respectively. The point of division between infraproximals and infradistals also corresponds with the subdigital scale (included in the infraproximal count following Rivero-Blanco, 1979) located at the level where the digits breaks in an angle and where they bend when pressure is applied; this scale is also notably larger than those subdigital scales in the same area. Based on this point of division I extended the infradistal/infraproximal count to all digits.

3.2.2 Statistical analysis

Revell et al. (2007) recently demonstrated that occupation of rocky habitats by lizard lineages involves evolutionary change towards decreased head depth and longer limb elements. Because one of the undescribed species discovered in this work was found to be restricted to inselbergs (large, dome-like, rock outcrops) I used a Principal Components Analysis (PCA) to examine if this species differs in the morphometric variables associated with rock-dwelling adaptations from the two other species of *Gonatodes* that are endemic to the Guayana Shield (*G. alexandermendesi* and *G. annularis*), as well as from *G. ceciliae* from northeastern Venezuela (all three species are found mostly on trees). The measurements included in the statistical analysis (abbreviations and definitions in parentheses) are femoral length (FEM; distance on the hindlimb from knee to point of limb insertion on the body), tibial length (TIB; distance on the hindlimb from base of foot to knee), fourth toe length (FTL; distance on the fourth toe from distal point of claw to point of insertion on the foot), humeral length

(HUM; distance on the forelimb from elbow to point of limb insertion in the body), ulnar length (ULN; distance on forelimb from base of hand to elbow), fourth finger length (FFL; distance on the fourth finger from distal point of claw to point of insertion on the hand), and head depth (HDP). In order to remove the effects of size from each one of these variables prior to the PCA I used the residuals obtained from least-squared linear regressions using SVL as the independent variable. Other measurements used in the species description but not used in the statistical analyses are head length (HL; form tip of snout to anterior margin on tympanum), head width (HW; taken at widest point of the head), eye-nostril distance (EYN; distance from nostril to anterior margin of eye), and axilla-groin distance (AXG). All measurements were taken with a digital caliper to the nearest 0.1 of a millimeter. Results of the PCA were visualized on bivariate scatterplots of the principal components retained with eigenvalues higher than one. All statistical analyses were performed on SYSTAT 11.

3.2.3 Taxon sampling and outgroup choice

I included multiple samples of the three currently recognized species of *Gonatodes* that occur in the Guayana Shield plus samples of four putative new species (morphologically diagnosable groups of individuals) found only in this region. The putative new species include *G.* sp. 1, collected from forests near Puerto Ayacucho, Venezuela; *G.* sp. 2, collected from Inselbergs near Puerto Ayacucho, Venezuela; *G.* sp. 3., from Sierra La Paragua, Venezuela; and *G.* sp. 4, from Guiana. We also included a diverse sample of non-Guayanan species of *Gonatodes* in order to examine the phylogenetic position of the Guayanan species within the genus and examine the major

clades in *Gonatodes*. I chose *Lepidoblepharis* as the outgroup for the phylogenetic analyses based on the recent molecular results of Gamble at al. (2008), which show with high support that this genus is the sister group of *Gonatodes*. All samples used in the molecular work are listed in Appendix B with the associated locality data and field numbers.

3.2.4 Lab protocols and molecular analyses

Tissues (liver or muscle) were obtained from freshly killed specimens in the field and were preserved in 95-99% ethanol, and permanently stored at –70°C. Total genomic DNA was isolated using the DNAeasy Blood and Tissue Kit (Qiagen) following the manufacturers protocol. DNA Sequences were amplified from the mitochondrial ribosomal small unit (12S; primers: 12a: 5'-CTG GGA TTA GAT ACC CCA CTA-3'; 12b: 5'-TGA GGA GGG TGA CGG GCG GT-3') and from the nuclear (C-mos; primers: FU-F: 5'-TTT GGT TCK GTC TAC AAG GCT AC-3'; FU-R: 5'-AGG GAA CAT CCA AAG TCT CCA AT-3') gene regions using standard polymerase chain reaction (PCR) protocols. The PCR products were purified using the ExoSAP-IT^{tm} kit (United States Biochemical) and used as templates in sequencing reactions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were cleaned-up using ETOH/Sodium acetate precipitations and were read on a ABI PRISM 3100xl Genetic analyzer. Contigs were assembled and edited in Sequencher 4.1 (Genes Code Corps., Inc).

Sequences were aligned in CLUSTAL X 1.83 with default parameters. The 12s alignment was refined visually and regions that were considered to be ambiguously

aligned (those with large and/or multiple gaps) were excluded from the analysis. A heuristic Maximum Parsimony (MP) analysis of the concatenate dataset was performed on TNT 1.2 (Goloboff et al., 2000) using the Tree Branch Reconnection algorithm, with 100 replications (random addition sequences) and keeping 5 trees per replication. Node support of the MP analysis was estimated using the standard (with replacement) Bootstrap option of TNT with 1000 replications.

3.2.5 Species criteria

Following the work of many philosophers of the species problem (e.g. Frost and Kluge, 1990; Ghiselin, 1974; Hull, 1978), I consider species to be real entities (i.e. individuals in the philosophical sense) that exist in nature whether we can detect them or not with our current discovery operations (Frost and Kluge, 1994). These entities have been equated by some authors (e.g. Frost and Kluge, 1994; Mayden, 1997) to the Evolutionary Species Concept (ESC) of Wiley (1978). According to Mayden (1997), the ESC is the only purely theoretical species concept and, therefore, does not prescribe any particular discovery operation for delimiting boundaries between species. I advocate the notion that species delimitation should be subject to multiple complimentary discovery operations (Grant, 2002), preferably if they examine different contingent properties (e.g. reproductive isolation, morphological diagnosability, genetic divergence) of species as evolutionary lineages. I herein use a two-step approach for delimiting species. First, I follow the traditional approach of finding putative diagnostic (=fixed) morphological characters to generate hypotheses of species limits. In the second step I attempt to refute these hypothetical species with the molecular data. For

mitochondrial sequences two main criteria have been suggested for recognizing species. The first is to set a threshold value for sequence divergence that when exceeded different species are recognized. The second criterion is to recognize different species if their mitochondrial haplotypes form reciprocally exclusive groups (sensu Baum, 1992; =monophyletic for some authors) when a gene tree is examined. Both criteria have shortcomings and, thus, have both advocates and detractors. The threshold divergence approach has been criticized as being too arbitrary and also depends on having a good understanding of intraspecific sequence variation of the group in question (Moritz and Cicero, 2004). The criterion of reciprocal exclusivity (CRE) has been criticized as having a systematic bias against nested units of diversity (Kizirian and Donnelly, 2004), plus the fact that in using tree-based methods for delimiting species we depict relationships as hierarchical when the terminals (e.g. semaphoronts) are not themselves related hierarchically (Goldstein and De Salle, 2000). However, the natural tendency for lineages that are isolated to become reciprocally exclusive is an intuitive and widely accepted theoretical model; thus this represents an uncontroversial contingent property of species that is most parsimoniously explained by effective isolation. Some authors (e.g. Wiens and Penkrot, 2002) have proposed to delimit different species if there is no evidence of gene flow between basal lineages of putative species even when the CRE is not met. However, in the absence of any other independent line of evidence, especially knowledge of well supported geographic barriers between putative species, recognizing species with non-exclusive genetic data is problematic because species delimitation is partly based on assumptions of demographic exchangeability and if sampling is not

intense we risk recognizing a paraphyletic group of lineages as a species. The more conservative approach of limiting species recognition to those units that are reciprocally exclusive, although it might underestimate species diversity, at least our delimited species will likely correspond to historical groups (if not real species a monophyletic group of weakly divergent species) that can be further examined in the future with more intensive sampling and more markers. That said, for the purpose of this work I consider a hypothetical species defined by morphology to be refuted if they do not meet the CRE for the gene trees obtained; otherwise the hypothetical species are corroborated.

3.3 Results

3.3.1 Sequence characteristics

The 12s and C-mos alignments were 315 and 389 bp long, respectively, for a concatenate dataset of 704 bp long. The 12s alignment contains several regions with gaps resulting from indel events. The initial Clustal alignment contained more regions with gaps that were dropped because they were deemed to be ambiguously aligned. The C-mos alignment was not modified from Clustal as only two Indel events were present. The first is a 12 bp gap shared by all the Guayanan endemic species together with *G. hasemani* (see discussion). The second is a 3 bp gap found only in one montane species of *Gonatodes*.

3.3.2 Phylogenetic analyses and species limits

The Maximum Parsimony analysis resulted in 21 equally parsimonious trees, with tree length of 471. A partial (for the full tree see Chapter 5) strict consensus of these 21 trees is shown in Figure 3.1 with bootstrap values shown on the major nodes.

The phylogenetic analysis strongly supports (bootstrap value 93) a monophyletic group containing all the Guayana Shield endemics together with *G. hasemani* from the southern Amazon Basin. All of the putative new species, except *G.* sp .3 from La Paragua, Venezuela, met the CRE and are corroborated as valid species that require separate taxonomic recognition. The single individual referred to *Gonatodes* sp. 3 falls nested within the samples of *G. alexandermendesi*. Because this individual was separated from G. *alexandermendersi* solely on the basis of coloration, I consider this individual to be a new color morph this species. Bootstrap values for all clades representing species were high (>80).

3.4 Species accounts

Gonatodes alexandermendesi Cole and Kok

Gonatodes alexandermendesi Cole and Kok, 2006

Gonatodes sp. Gamble et al., 2008

Remarks.—A new color morph of *G. alexandermendesi* (See Figure 3.2) has been discovered herein, which was initially thought to represent an undescribed species (*G.* sp. 3). The new color morph has a darker body coloration with small ocelli on the sides and a yellow hood covering the head and extending onto the neck and the forelimbs. The presence of *G. alexandermendesi* is also confirmed in Venezuela for several localities including Sierra La Paragua, Jaua Tepui, and Sierra de Lema. The sample referred to *G.* sp. by Gamble et al. (2008) is herein shown to fall nested within *G. alexandermendesi* (49G) and is therefore considered to be from this species.

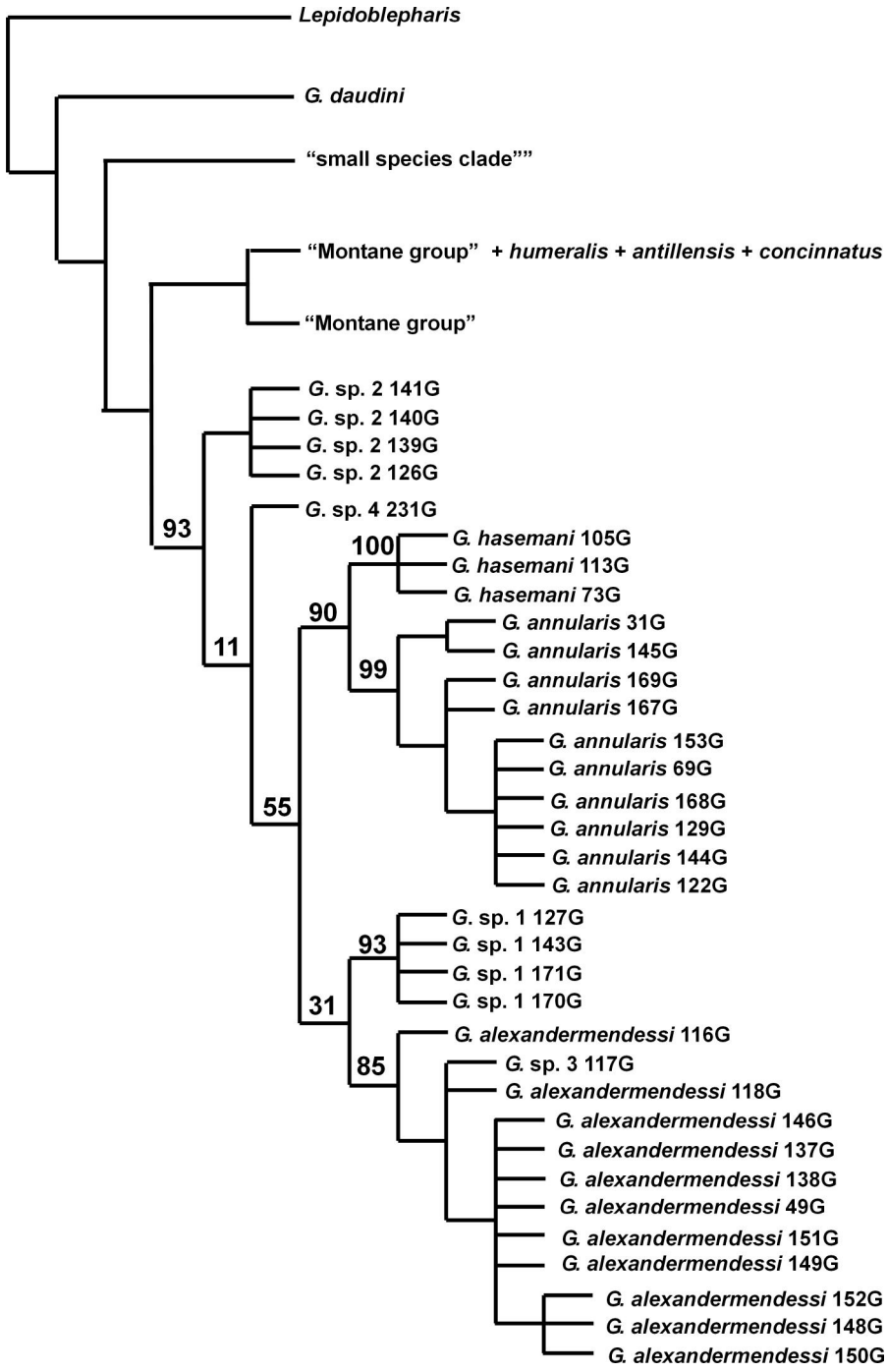


Figure 3.1 Partial view of a strict consensus tree of 21 equally parsimonious trees for the genus *Gonatodes*

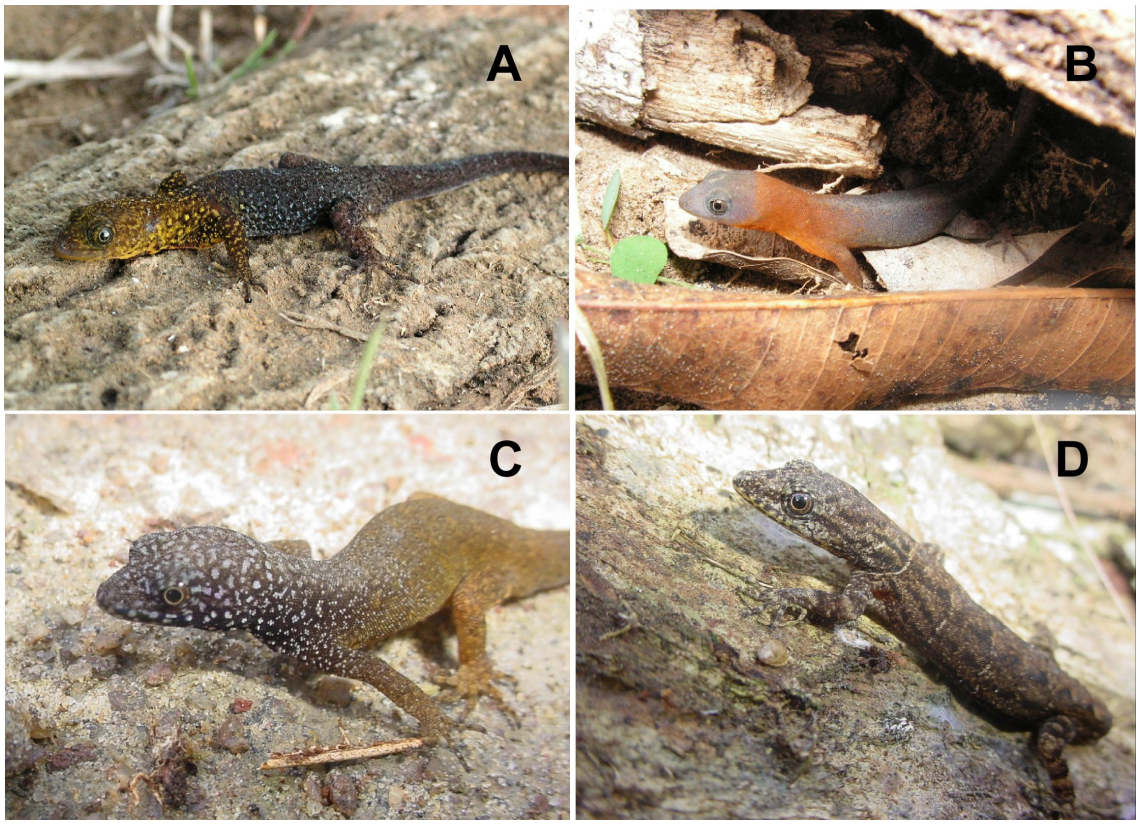


Figure 3.2 Specimens in life of the two color morphs of *G. alexandermendesi* (A and B), and a male (C) and female (D) of *G.* sp. 1

Gonatodes sp. 1, new species

Holotype: MHNLS 18417 (field number CJF4680), an adult male, collected on Sector El Infierno, road Puerto Ayacucho-Gavilan, Estado Amazonas, Venezuela, one of three specimens collected on 15 March 2007 by Gilson Rivas.

Paratypes: all collected from same locality as holotype: MHNLS 18415-16, two adult females collected with the holotype; MHNLS 18375 an adult male, MHNLS 18441 a juvenile, both collected on 14 March 2007 by Gilson Rivas and Tito Barros.

MHNLS 17702, 18455-57 obtained by Gilson Rivas and Marco Natera on 13 February 2006.

Diagnosis: the new species can be distinguished from all congeners by a combination of moderate size, supraciliary spine elongate (Figure 3.3) medial subcaudal scales not distinctly differentiated from adjacent scales on non-regenerated tail, and males with dark grayish brown to black head with round white spots (Figure 3.2). *Gonatodes* sp. 1, together with *G. annularis* and *G. hasemani*, are the only species of *Gonatodes* in which the subcaudal pattern lacks medial differentiated scales. This character state seems fixed in both *G.* sp. 1 and *G. hasemani*, and occurs only at low frequencies in *G. annularis*. From *G. annularis* (character states in parentheses) the new species differs in having an elongate supraciliary spine (absent) and a brown to copper colored iris (blue iris). It differs from *G. hasemani* in having males with a dark grayish brown or black head with white spots as opposed to bright yellow head with or without black reticulations.

Description of holotype: an adult male, with snout-vent length of 43.3 mm. Tail length 49.6 mm, complete and not regenerated. Head 1.2 times longer than wide (HL: 10.4 mm; HW: 8.9 mm). Snout short (2.7 mm), 0.26 times HL, somewhat acutely rounded in dorsal view, sloping toward top of head with an approximate 45º angle. Neck slightly narrower than head and body. Body nearly cylindrical but wider than high; axilla-groin distance 18.1 mm. Limbs well developed with relatively short digits, fourth toe length 4.7 mm, 0.62 times shank length (7.6 mm). Tail round in cross section, tapering toward tip.

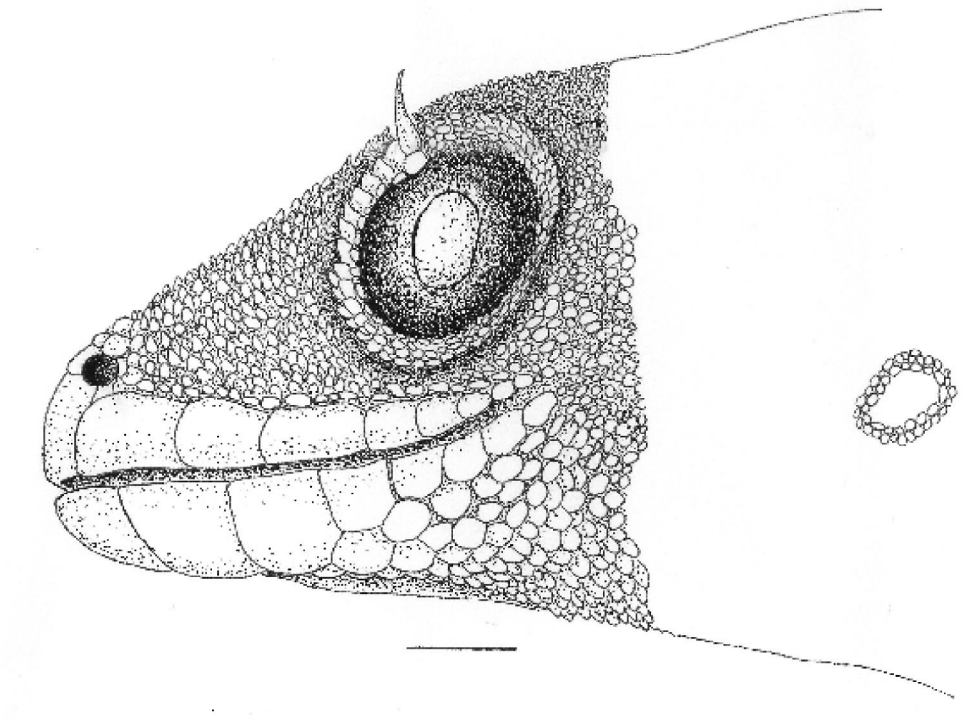


Figure 3.3 Illustration of the head of *G.* sp. 1 in lateral view

Tongue relatively wide with bluntly rounded tip, covered by small, scalelike papillae, which become less defined posteriorly; tip of tongue with a short median cleft. Teeth small, subequal, conical.

Rostral large, visible from above, roughly rectangular but with small, medial indentation on top margin from which a short cleft extends forward halfway through rostral. Four postrostrals, lateral ones (supranasals) distinctly larger than two median, which in turn are slightly larger than adjacent scales between anterior margin of the orbit and the postrostrals. One of the median postrostrals is partially contained in the indentation of the rostral scale, the other is shifted towards the left. Nostril bordered by rostral, four postnasals, lateral postrostral (supranasal), and first supralabial. Uppermost

postnasals noticeably larger than lower three and scales on the loreal region; lower three postnasals about same size as scales on the loreal region. Scales on top of snout roughly round and granular, juxtaposed, gradually becoming more oval-shaped and conical towards loreal region. Loreal scales number about 11 (both sides) in a line between postnasals and anterior margin of orbit. Scales decrease slightly in size from the postrostrals toward posterior part of head. Scales on supraorbital region similar (in size and shape) to and continuous with those on top of head. Supraciliary flap poorly developed, with an elongate supracilliary spine anterior to level of center of eye; supracialliary scales small, granular posterior to spine, somewhat larger, flattened and slightly protruding laterally anterior to spine. Pupil round. Supralabials 5/6 to level of center of eye of which the first is the largest; last supralabial followed by about 8/8 much smaller scales along the lip to rictus of mouth, of which the last four are granular and barely larger than scales on temporal region. Scales on temporal region similar to those on posterior top of head. Ear opening (0.9 mm) much smaller than eye (2.3 mm), obliquely oval.

Mental large, roughly rhomboidal. Postmentals 2, distinctly larger than adjacent posterior scales. Scales on chin small and polygonal directly behind postmentals, granular and tiny posteriorly, a few larger, polygonal scales adjacent to infralabials, juxtaposed; Infralabials 4/4 to level of center of eye, decreasing in size posteriorly, first two very large and projecting onto ventral plane. Supralabials and infralabials with many minute tubercles.

Scales on nape and on sides of neck granular anteriorly, becoming somewhat conical posteriorly. Scales on throat smooth, imbricate, with round posterior margin, with a short transitional area with the granular scales on chin and gular area.

Dorsals subconical, oval-shaped at base, slightly larger than scales on top of head, somewhat projecting posteriorly to confer an almost imbricate appearance; dorsolaterally and on flanks about same size as dorsally but slightly becoming larger proximal to ventrolateral region. Transition between scales on flanks and ventrals somewhat abrupt but not clearly demarcated. Ventral region with scales distinctly larger than dorsals, slightly smaller on chest than on belly, smooth, with round posterior margin for the most part except at level of hind limbs on which the posterior margin is somewhat acuminate, imbricate (each scale overlapping anterior portion of scale lying posteriorly); ventrals in oblique rows, on belly also forming rather regular longitudinal rows, with 42 scales along the midventral line between anterior margin of forelimbs and vent. Scales around midbody about 82, of which 18 are ventrals. Scales on preanal plate similar to ventrals, excepting border of vent, which has minute scales arranged in three rows. Escutcheon not evident.

Scales dorsally on tail slightly larger than in body, flat, inbricate, with posterior margin rather acuminate; a short transition area between the subconical body scales and the caudal scales just described, extends posteriorly about 4 mm from base of tail. Underside of tail with smooth, flat, imbricate scales, with round posterior margin, increasing in size toward midventral line; the medial subcaudal scales are slightly larger

than the adjacent scale rows but they are not clearly differentiated as in the majority of species in the genus (see Diagnosis).

Scales on limbs granular and juxtaposed on dorsal and posterior surfaces, otherwise scales are smooth, flat, somewhat rounded and imbricate. Lamellae under first (**I**) through fifth (**V**) finger (infraproximals in parentheses): **I**: 10/10(3/3), **II**: 14/13(4/4), **III**: 13/14(3/4), **IV**: 15/15(5/5), and **V**: 13/13(5/5), respectively. Lamellae under first through fifth toe (infraproximals in parentheses): **I**: 9/10(3/3), **II**: 12/12(4/4), **III**: 15/16(5/6), **IV**: 17/18(8/7), and **V**: 17/18(6/7), respectively. Fingers and toes with three ort four lateral rows of scales distally. Claws exposed, non-retractile, between two basal scales (dorsal an ventral).

Color in preservative: Dorsally the head is very dark grey, densely overlaid with small but well defined, irregular white spots, each encompassing 1 to 8 scales, but most frequently between 3 and 4 scales. When examined under the dissecting scope the area around the spots is generally darker (almost black) than the intervening areas, which appear dark grayish brown. Under high magnification, the white spots appear as groups of white scales with very few but well defined spot-like melanophores, surrounded by dark scales with a higher density of large melanophores. The head pattern extends posteriorly onto the anterior half of the body and forelimbs, but becomes progressively less defined and fades at the level of the midbody into the roughly uniform grayish brown coloration of the remainder of the dorsal surfaces of the body, hindlimbs and tail. The underside of the head is a continuation of the dorsal coloration, but with a black background and with the white spots becoming slightly larger and more distinct owing

to being almost completely devoid of melanophores, and mostly surrounded by scales that are uniformly black, increasing the contrast of the pattern. This color pattern degrades on the throat, where the white spots become scarce and poorly defined, coinciding with the transition between the granular scales on the gular region and the imbricate scales posteriorly. The imbricate scales of the throat and anterior part of the chest are mostly cream-color suffused with black on the posterior portion, conferring a "salt and pepper" pattern in the area. The black markings end somewhat abruptly at the level of the forelimbs. Venter and ventral surfaces of the limbs and tail very pale smoke gray somewhat darker on tail and towards midbody. Palms and soles darker than venter, most notably on the subdigital lamellae, which are suffused with dark grey.

Variation in paratypes: the paratypes are two adult males (MHNLS 18456, 18375), four adult females (MHNLS 17702, 18415, 18416, 18457), and two unsexed juveniles (MHNLS 18441, 18455). The largest specimen in the type series is an adult female (MHNLS 18457) measuring 44.5 mm in SVL. The smallest specimen is an unsexed juvenile (MHNLS 18441) measuring 18.4 mm in SVL. There are 5-6 supralabials to the level of the center of the eye, followed posteriorly by 1-2 slightly smaller polygonal scales and multiple small granular scales to the rictus of the mouth. There are 3-5 infralabials to the level of the center of eye, followed posteriorly by 2-3 slightly smaller scales and multiple small granular scales. Five of the paratypes have 4 postrostrals as in the holotype, three of them have 3 postrostrals. Scales around the midbody are about 75 in all paratypes of which 16-18 are ventrals. There are 38–46 ventral scales along the trunk. The variation in the number of lamellae under first

through fifth fingers (infraproximals in parentheses): **I**: 8-10(2-3), **II**: 11-14(3-5), **III**: 12-14(3-5), **IV**: 15(4-6), and **V**: 11-14(3-5). The variation in the number of lamellae under the first through fifth toe: **I**: 9-10(2-3), **II**: 12-13(3-4), **III**: 15-16(4-6), **IV**: 17-19(7-8), and **V**: 15-17(5-6). Morphometric variation of the type series is presented on Table 3.1.

Table 3.1 Variation in selected measurements in the type series of *Gonatodes* sp. 1

Specimen number	SVL	TL	AXG	HL	HW	EYN T
MHNLS 17702 (F)	39.5	---	16.3	9.9	7.2	2.5
MHNLS 18375 (M)	42.3	---	18.9	10.3	7.5	2.5
MHNLS 18415 (F)	39.8	32.3	17.0	10.9	7.3	2.5
MHNLS 18416 (F)	35.3	31.8	14.5	9.2	7.6	2.5
MHNLS 18417 (M)	43.3	49.6	18.1	10.4	8.9	2.7
MHNLS 18441 (?)	18.4	18.7	7.5	5.5	3.8	1.2
MHNLS 18455 (?)	27.8	27.0	10.8	7.9	5.3	2.2
MHNLS 18456 (M)	43.3	44.2	16.5	10.9	7.7	2.7
MHNLS 18457 (F)	44.5	---	20.0	10.8	7.2	3.0

The coloration of the adult male paratypes is essentially the same as in the holotype, showing only minor variation with respect to the distinctiveness of the white spots of the head and in the extent to which the head pattern extends on the body.

Distribution and Natural History: known only from the type locality (Figure 3.4) near the city of Puerto Ayacucho, Amazonas State, Venezuela. The Puerto Ayacucho area lies in the western peripheral lowlands of the Guayana Shield. The vegetation in

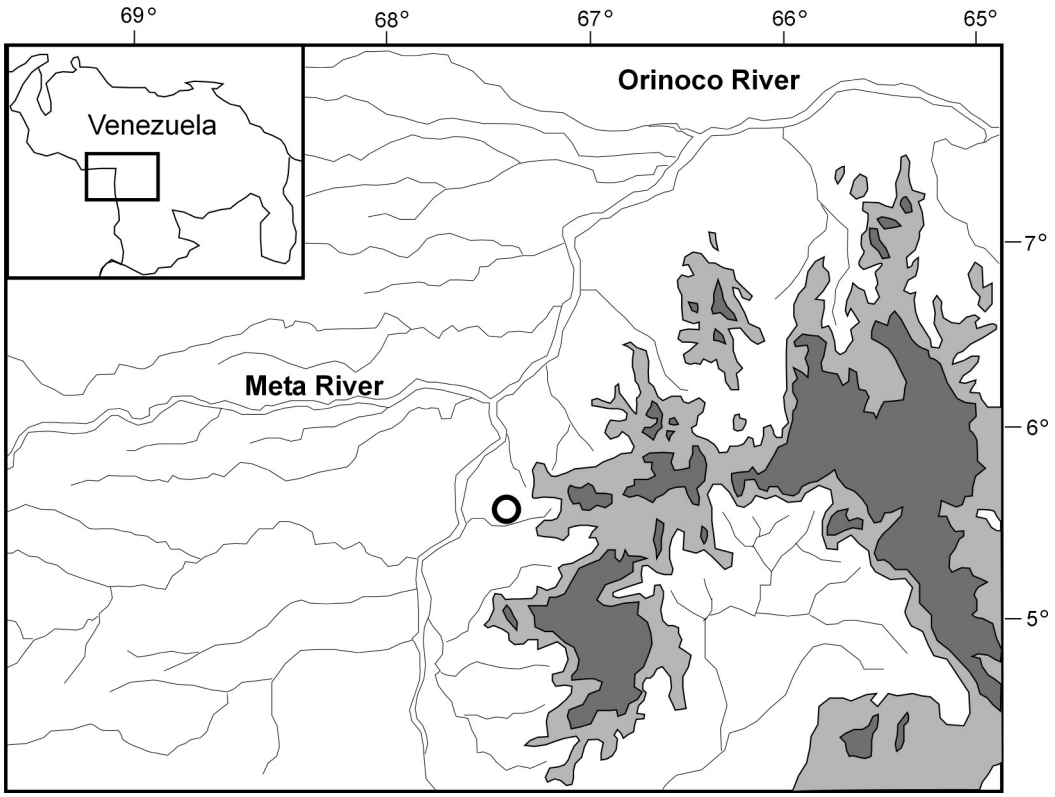


Figure 3.4 Map of Venezuela showing the type locality (circle) of *G*. sp. 1 and *G*. sp. 2

this area represents a transition between the "Llanos", a bioregion dominated by a mix of grasslands and semidecidous forests; and Amazonian rainforest. The original habitat in the area has been severely impacted by logging and agricultural activities leaving a mixture of grasslands with scattered trees and patches of primary and secondary forest. The majority of the individuals were collected or observed on large buttressed trees in patches of secondary forests. When approached they generally fled into holes in the tree or under the bark. When captured the tail was frequently autotomized or parts of the skin were lost easily. Additional observations include a female, a hatchling, and remains of an eggshell obtained from under a rock in the forest. Also, one specimen was

found at night crawling on the ground of a recently burned field; other than this single observation, all other individuals were observed active during the day. An additional specimen was collected in a rural area under the bark of Mango tree, indicating that the species can tolerate human intervention to some extent.

Gonatodes sp. 1 is sympatric with *G. humeralis*, and individuals of both species can occasionally be observed on the same tree. However, *G. humeralis* was consistently observed more often on smaller trees. Furthermore, *G.* sp. 1 is slightly larger and more robust than *G. humeralis*, and there are also differences in head shape between the two species, that might translate into food partitioning. Other species of reptiles that were also observed in the same area were: *Mabuya nigropunctata*, *Kentropix striata*, *Leposoma hexalepis*, *Norops chrysolepis*, *Dactyloa punctata*, *Tropidurus hispidus* and *Phyllodactylus ventralis*.

Gonatodes sp. 2, new species

Holotype: MHNLS 18440 (field number CJF4703), an adult female, collected in Sector El Infierno, 20 km south of Puerto Ayacucho, on road to Gavilan, estado Amazonas, Venezuela: one of two specimens collected on 15 March 2007 by Gilson Rivas and Tito Barros.

Paratypes: All females: MHNLS 18439, specimen with the same collection data of the holotype; UTA R-55378-55379, MHNLS 18397, three specimens from the same locality, obtained by Gilson Rivas, Coleman Sheehy III, Carl Franklin, and Tito Barros on 14 March 2007.

Diagnosis: The new species can be distinguished from all congeners by a combination of very large size, supraciliary spine absent, females with immaculate brown dorsal coloration (Figure 3.4), and a subcaudal pattern (only in the unregenerated tail) of a single medial row of wide scales which laterally contact either two or three adjacent caudal scales in an 1:1 alternating fashion. *Gonatodes* sp. 2 is to our knowledge the largest species of sphaerodactyl gecko, with adult females reaching about 65 mm in SVL, which readily separates this species from the much smaller *G. antillensis*, *G. albogularis*, *G. atricucullaris*, *G. caudiscutatus*, *G. eladioi*, *G. humeralis*, *G. petersi*, *G. tapajonicus* and *G. vittatus*, neither of which exceed 45 mm in SVL (data from: Avila-Pires, 1995; Rivero-Blanco, 1979). The new species is also unique in the genus in having females with a coloration devoid of any well defined markings, a character state that separates it from all other species of comparable size (and also from the smaller species), namely *G. annularis*, *G. alexandermendesi*, *G. ceciliae*, *G. concinnatus*, *G. falconensis*, *G. hasemani*, *G. ocellatus*, *G. purpurogularis*, *G. seiglei* and *G. taniae*, all of which have females with well defined and conspicuous dark markings on the head and the body. Among Amazonian/Guayanan species, *G.* sp. 2 further differs from *G. alexandemendesi* and *G. hasemani* in lacking an elongated supraciliary spine. Finally, *G.* sp. 2 differs from all other species of *Gonatodes*, except *G. eladioi* (a much smaller species) in the subcaudal pattern of the unregenerated tail (described above). All other species of *Gonatodes* have subcaudal pattern (for a description of the different character states and taxonomic distribution see Rivero-Blanco, 1979) in which one of the following patterns occur: a) the medial scales that

Figure 3.5 Female in life of *Gonatodes* sp. 2

contact three scales laterally occur every two medial scales that are in contact with two scales laterally, b) with a divided medial scales every two single medial scales, c) proximally with a pattern as described in *G.* sp. 2, but switching to the pattern described in "b" distally, or d) with medial scales not differentiated from adjacent lateral scales.

Description of holotype: an adult female, with snout-vent length of 56.2 mm. Tail length 74.5 mm, complete and not regenerated. Head 1.5 times longer than wide (HL: 14.5 mm; HW: 10.0 mm). Snout 4.1 mm long, acutely rounded in dorsal view, gently sloping toward top of head. Neck slightly narrower than head and body. Body nearly cylindrical but wider than high; axilla-groin distance 24.3 mm. Limbs well

developed with relatively long digits, fourth toe length 9.1 mm, 0.85 times shank length (10.8 mm). Tail round in cross section, tapering toward tip.

Tongue relatively wide with bluntly rounded tip, covered by scalelike papillae which become smaller posteriorly; tip of tongue with a short median cleft. Teeth small, subequal, conical.

Rostral large, with two small, closely spaced vertexes on upper margin, visible from above, with a median cleft extending forward from posterior margin to near tip of snout. Three postrostrals, lateral ones (supranasals) distinctly larger than medial, medial postrostral slightly larger than adjacent scales between anterior margin of the orbit and the postrostrals. Nostril bordered by rostral, three postnasals, lateral postrostral (supranasal), and in point contact with first supralabial. Postnasals slightly larger than adjacent loreals. Scales on snout and on loreal region roughly round, granular, juxtaposed, but somewhat conical posteriorly. Loreal scales number about 15 (both sides) in a line between postnasals and anterior margin of orbit. Scales decrease slightly in size from the postrostrals toward posterior part of head. Scales on supraorbital region similar (in size and shape) to and continuous with those on top of head. Supraciliary flap not developed and without an elongate supracilliary spine, supracialliary scales conical, somewhat larger and protruding laterally on anterior half of row. Pupil round. Supralabials 5 (both sides), first largest, second through fifth roughly subequal, anterior portion of fifth scale below center of eye. Scales on temporal region similar to those on posterior top of head. Ear opening (1.3 mm) much smaller than eye (3.0 mm), obliquely oval.

Mental large, with round anterior margin following lower lip, posteriorly with three crooked edges of which the laterals run obliquely posteromedially from lip to vertex with the medial transverse edge. Postmentals 2, distinctly larger than adjacent posterior scales. Scales on chin small and polygonal directly behind postmentals, granular and tiny posteriorly, a few larger, polygonal scales adjacent to infralabials, juxtaposed; a few larger, polygonal scales adjacent to infralabials, juxtaposed. Infralabials 7 (both sides), decreasing in size posteriorly, first two very large and projecting onto chin.

Scales on nape and on sides of neck granular, continuous with those on head and body. Scales on throat smooth, imbricate, with round posterior margin, with a short transitional area with the granular scales on chin and gular area.

Dorsals granular, on the vertebral area similar in size to scales on snout; dorsolaterally and on flanks slightly larger. Transition between scales on flanks and ventrals somewhat abrupt but not clearly demarcated. Ventral region with scales distinctly larger than dorsals, slightly smaller on chest than on belly, smooth, with round posterior margin, imbricate (each scale overlapping anterior portion of scale lying posteriorly); ventrals in oblique rows, on belly also forming rather regular longitudinal rows, with 56 scales along the midventral line between anterior margin of forelimbs and vent. Scales around midbody about 115, of which 20 are ventrals. Scales on preanal plate similar to ventrals, excepting border of vent, which has minute scales arranged in three rows. Without escutcheon area on abdomen.

Scales dorsally on base of tail suddenly become flat, smooth, rounded in shape, imbricate (rather than conical), just posterior to level of vent. Underside of tail with smooth, flat, imbricate scales, increasing in size toward midventral line; first 8 small subcaudals posterior to vent on midventral row increasing in size posteriorly but not clearly differentiated from adjacent laterals, followed by a single longitudinal row of significantly enlarged, roughly hexagonal, medial subcaudals; each medial subcaudals wider than long with anterior and posterior margins parallel and in transverse position, lateral two margins converging distally forming a somewhat sharp vertex, lateral margins contacting two or three adjacent scales laterally in alternating fashion.

Scales on limbs granular and juxtaposed on dorsal and posterior surfaces, otherwise scales are smooth, flat, roundish, imbricate. Lamellae under first (**I**) through fifth (**V**) finger (infraproximals in parentheses): **I**: 11/11(2/2), **II**: 17/16(5/5), **III**: ?/20(6/7), **IV**: 22/21(8/8), and **V**: 18/17(6/6), respectively. Lamellae under first through fifth toe (infraproximals in parentheses): **I**: 12/11(3/2), **II**: 18/17(6/5), **III**: 21/20(6/6), **IV**: 25/24(10/10), and **V**: 19/19(5/5), respectively. Fingers and toes with four lateral rows of scales distally, with occasional reduction to three rows in some sections, especially near the claw. Claws exposed, non-retractile, between two basal scales (1 dorsal, 1 ventral).

Color in preservative: dorsum and sides of body and tail uniform grayish brown with top of head and limbs becoming slightly paler. Microscopically each dorsal scale is cream-colored with many evenly spaced, well-defined melanophores. Interstitial dorsal skin more densely covered with melanophores than individual scales. Venter pale

grayish brown, becoming paler towards vent and pectoral region. Microscopically the ventral scales are cream-colored with evenly spaced melanophores throughout most of the venter, but towards the vent the melanophores are found only bordering the posterior margin of each scale which accounts for the change in color saturation described above. In the pectoral and gular region the melanophores occur at smaller densities per scale. Ventral surface of tail slightly paler than dorsal surface. Anterior half of tongue dark gray, posterior half white.

Variation in paratypes: the paratypes are four adult females ranging in SVL from 57.2 to 65.5 mm. Both supralabial and infralabial counts vary from 5 to 7. The paratyes have 3 postrostral as in the holotype, except MHNLS 18439 which has four (two medial small scales between large paired supranasals). Scales around the midbody ranges from 95 to 105 in three specimens (not counted in MHNLS 18439 due to body skin loss) of which 20–22 are ventrals. There are 58–60 ventral scales along the trunk. The variation in the number of lamellae under first through fifth fingers: **I**: 10-12(2-3), **II**: 17-22(5-6), **III**: 21-24(7-9), **IV**: 21-24(7-9), and **V**: 16-18(5-6). The variation in the number of lamellae under the first through fifth toe: **I**: 10-13(2), **II**: 16-19(5-7), **III**: 21-25(6-8), **IV**: 23-28(10-12), and **V**: 19-20(5-6). The regenerated tail (described on UTA R-55378) has a subcaudal pattern that differs significantly from the original tail. In the regenerated tail the subcaudal scales form a single row of roughly rectangular, wide, but very short plates (longest side is transversal to longitudinal axis of tail), each of which extends laterally to the ventrolateral surface of tail. These plates come into contact

laterally with small scales that look just like those in the original tail. Morphometric variation of the type series is presented on Table 3.2.

Table 3.2 Variation in selected measurements in the type series of *Gonatodes* sp. 2

Specimen number	SVL	TL	AXG	HL	HW	EYN
MHNLS 18373	65.6	---	26.4	15.3	11.0	4.2
MHNLS 18374	57.4	74.6	25.2	13.7	10.0	3.8
MHNLS 18397	61.0	---	24.3	14.9	10.7	4.1
MHNLS 18439	57.1	---	23.8	14.3	9.7	3.8
MHNLS 18440	56.2	74.5	24.3	14.5	10.0	4.1

The coloration in the paratypes is essentially as in the holotype with only minor variation in color saturation owing to differences in melanophores densities. Two specimens, MHNLS 18373 and 18397, are noticeably dark compared to the rest of the type series, and especially on the venter which is not as distinctly paler than the dorsum as in the other specimens.

Coloration in life: In life all specimens were dull brown dorsally. All specimens had a vaguely distinct, suffused yellow coloration on the top of the head that was lost in preservative.

Distribution and Natural History: known only from the type locality nearby the city of Puerto Ayacucho, Amazonas, Venezuela. All the specimens were collected in large, dark, granitic, isolated inselbergs that stand out abruptly from the surrounding plains, and which are locally known as “lajas”. Specimens were collected directly from

the rock walls of two nearby and relatively small inselbergs (both about 3 m tall). The area where the specimens were collected was recently burned by indigenous people of the Piaroa tribe, with the purpose of preparing the land for agricultural use in the following rainy season. Three of the specimens were collected between 19:00-20:00, from an inselberg consisting of three large rocks placed in such a way that they formed a small "C" shaped refuge on the ground, which protected the enclosed area from fire and also helped retain water in it. In this small "oasis" of about 40 m^2 several specimens of *Dendrobates leucomelas* and *Pristimantis* sp. were found, in addition to two *Bothrops atrox*. The two other specimens were collected at about 18:00 on the top inner surface of the entrance of a cave-like, horizontal hole on a nearby inselberg. The hole was located about 40 cm above the ground, had an opening of about 30 cm in diameter and was more than 1 m deep. In the same area two *Bothrops atrox* were observed at the entrance of holes at the base of inselbergs. Because different animals were observed dead or agonizing (including two snakes in the genus *Chironius*) in the recently burned, surrounding area, the above observations indicate that inselbergs may act as refuges that protect animals from fire.

Statistical analysis: only two principal components (PC) were retained with eigenvalues higher than one. These first two PC (PC1 and PC2) explained 51.1% and 16.5% of the variance, respectively. Component loadings were high for all limb elements on PC1 whereas HDP was the only variable with high loadings on PC2 (see Table 3.3). *Gonatodes* sp. 2 had, on average, the highest factor scores on PC1 (Figure 3.5), indicating that this species in general has relatively longer limb elements compared

Table 3.3 Component loadings obtained from a PCA on five species of *Gonatodes* using seven morphometric variables

Variable	PC1	PC2
FFL	0.737	0.186
FTL	0.858	-0.112
ULN	0.698	0.216
HUM	0.746	-0.390
HD	-0.090	-0.935
TIB	0.742	0.173
FEM	0.833	0.066

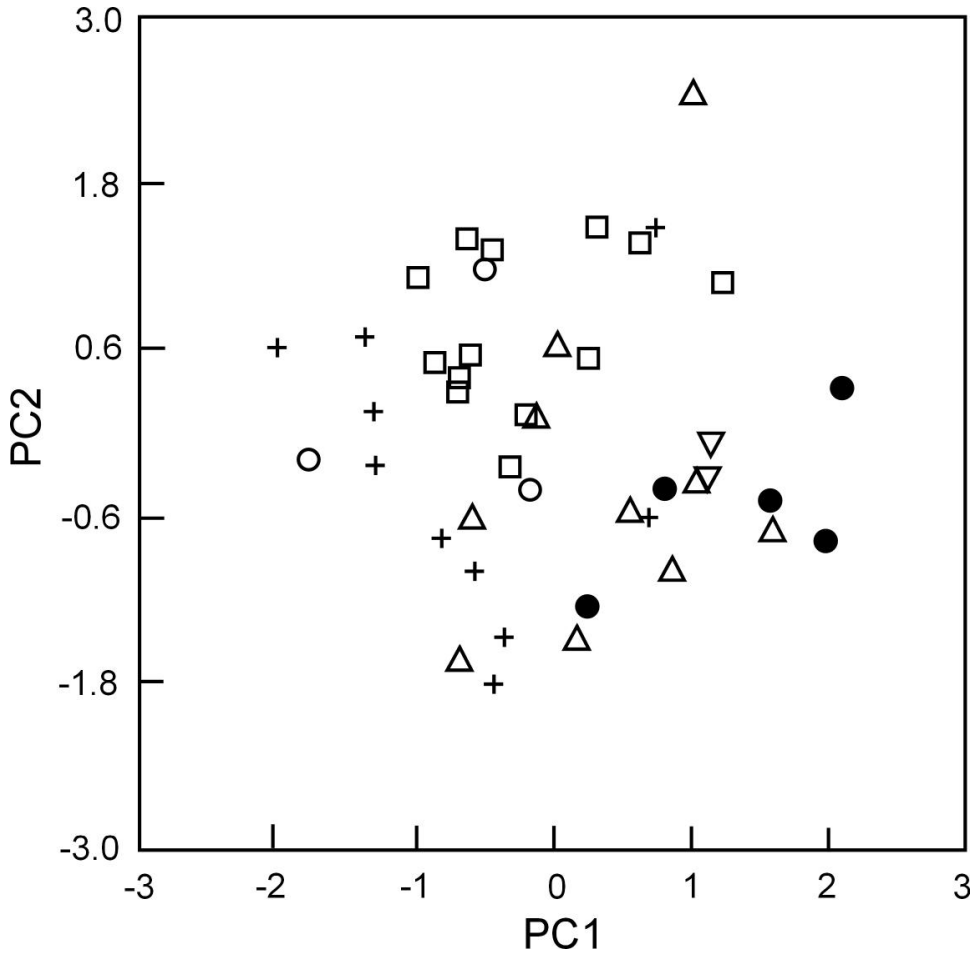


Figure 3.6 Principal component scores for specimens of *Gonatodes* sp. 2 (solid circles), *G. alexandermendesi* (inverted triangles), *G. annularis* (squares), *G. ceciliae* (crosses), *G. concinnatus* (triangles), and *G. taniae* (open circles)

to the other species included in the analysis. Although there are species-specific differences in PC2 (e.g. between *G*. sp. 2 and *G*. *annularis*), *G*. sp. 2 does not seems to be particularly distinct within the genus in terms of HDP.

Remarks: The few known specimens of *Gonatodes* sp. 2 were collected from Inselbergs in the type locality, whereas *G*. sp. 1 and *G*. *humeralis* were found in the surrounding forested areas. There are two morphological features in *G*. sp. 2 that might represent adaptations to life on inselbergs. The first is the unique uniform, background-matching, coloration in this species, which might confer better crypsis than a color pattern with markings (as in all other species of *Gonatodes*), when contrasted to the generally uniformly colored rock surface of the Inselbergs that they inhabit. The other trait, as evidenced on the PCA, is relatively longer limb elements when compared to other congeners. Revell *et al* (2007) suggested that longer limbs in rock-dwelling species would be beneficial to prevent individuals from rolling along the long axis of the body and would also confer greater sprint speed while maintaining the body close to the locomotion surface. Furthermore, based on this functional explanation Revell *et al* (2007) predicted that selection on limb elongation in rock-dwelling species would be strongest on the femur. The high component loading for FEM (0.833) on PC1 is consistent with Revell et al's (2007) prediction, yet FTL (component loading on PC1=0.858) accounted for most of the variation observed in size-corrected limb element measurements. Interestingly, the first thing I noticed upon examination of *G*. sp. 2 was the long digits (quantified in the analysis with FFL and FTL) of this species compared to other *Gonatodes*. Revell et al. (2007; see also Vitt *et al* 1997) also indicated that

some rock-dwelling lizards have exceptionally flatter heads and bodies as this would allow them to retreat to narrow crevices to avoid predator attacks. However, this does not seem to be the case with *G*. sp. 2 as it does not differ significantly from other congeners in relative HDP. This result is actually not unexpected, as specimens of *G*. sp. 2 were not found associated with crevices but with large holes that result from a characteristic erosion phenomenon known as "pseudocarst", which creates a series of channels, gullies and depressions on these rock formations (Gröger, 2000).

If we assume that the above observations do indeed indicate that *Gonatodes* sp. 2 is specialized to live on inselbergs, it is logical to expect that the distribution of this species is limited in part by the distribution of these particular rock formations. Inselbergs are common physiographic elements throughout the peripheral lowlands of the Guayana Shield, from French Guiana in the east to southeastern Colombia on the west (Gröger, 2000). However, Gröger (2000) found that there is hardly any inselberg-endemic species of plant occurring in both the western (Colombia, Venezuela) and the eastern part (French Guiana, Suriname) of the Guayana Shield, and indicated that this is probably the result of past climatological events such as a Pleistocene dry belt crossing the Guayana Shield diagonally. Interestingly, the type locality of *G*. sp. 2 falls in a region that represents a center of endemism and diversity (the "Atures" center following Gröger, 2000) of inselberg associated plant species within the western peripheral lowlands of the Guayana Shield. This Atures center of endemism occurs at the intersection of two phytogeographic units that were defined based on different inselberg-endemic species that extend in distribution to either north or south from the

Atures center. This pattern seems to result from contrasting climatological conditions, especially precipitation, north (dry) and south (humid) from the Atures center. Based on these observations I believe that *G*. sp. 2 is restricted to the western peripheral lowlands of the Guayana Shield, and within this region, it is possible that this species follows one of the patterns exhibited by plant species associated to Inselbergs (i.e. endemic to the Atures center, or extending either north or south from there).

Although males of *Gonatodes* sp. 2 are yet to be collected I speculate about their coloration based on some features observed in specimens available and the fact that some of the dark and pale markings found in females of other species of *Gonatodes* seem homologous (based on topographical similarity) with the distinct coloration ornaments found in males. As mentioned above, *G*. sp. 2 is unique in the genus in having females with uniform body coloration devoid of any markings; thus, it is possible that males of *G*. sp. 2 are not as sexually dichromatic as most other species in the genus. However, the fact that in life all specimens of *G*. sp. 2 had more of a yellowish hue on the head relative to the body might be an indication that males have a distinct yellow hood such as in many other species of *Gonatodes*. Collecting and studying male specimens should be the immediate focus of future studies of *G*. sp. 2, as this would potentially shed lights on the relationships of this species to other members of this genus as well as revealing how sexual dichromatism has been affected by selection towards cryptic coloration in a novel environment.

3.5 Discussion

The present study shows evidence for recognizing at least five species of *Gonatodes* that are endemic to the Guayana Shield. The fact that three of these five species represent new species to science is testimony of how the Guayana Shield is a biodiversity "hotspot" for which much more study is still needed. This observation is even more compelling if we consider that all five species recognized herein are both easily diagnosable morphologically (i.e. none of them is a cryptic species) and also highly divergent genetically (all of them diverge by more than 5% in the 12s gene). I expect that further fieldwork in the region in combination with fine scale studies will likely reveal additional species of *Gonatodes*.

Perhaps the most interesting result of this study is that all Guyana Shield endemics, together with *G. hasemani*, form a monophyletic group. This clearly indicates that the diversity of *Gonatodes* in the Guayana Shield has resulted from within region diversification from a single ancestral lineage, and adds to the growing evidence showing that the high level of diversification and endemism within the region is not necessarily confined to the highlands (e.g. tepui summits) and can also be observed in some groups found mostly on mid- and low elevations.

Gamble et al. (2008) recently conducted a phylogenetic study of *Gonatodes* in which *G. alexandermendesi* (identified as *G.* sp. in Gamble et al. and determined herein to be *G. alexandermendesi*), *G. annularis*, and *G. hasemani* were included. Their study also found support for a close relationship between those three species, and also noted that they all share a single, unique deletion of four codons in the C-mos sequence,

which they proposed as a synapomorphy for this clade. Herein I found that all species in the Guayana Shield clade have this deletion, which is not shared with any other currently known species of *Gonatodes* nor the outgroup (*Lepidoblepharis*). To our knowledge this major deletion is the only known unambiguous synapomorphy supporting the Guayana Shield clade.

Gamble et al. (2008) also used a penalized likelihood method, calibrated with an amber-preserved *Sphaerodactylus* specimen, to estimate divergence times in *Gonatodes*. They estimated divergence time for the node uniting *G. alexandermendesi*, *G. annularis* and *G. hasemani* at the early Miocene. Because *G.* sp. 2 and *G.* sp. 4, are the most basal species of the Guayana Shield clade, it is possible that the divergence time estimate for this clade is even older. However, the topology supported herein is incongruent with Gamble et al. (2008) as they found *G. hasemani* to be sister to *G. alexandermendesi* + *G. annularis*, whereas our analysis suggest a sister relationship between *G. annularis* and *G. hasemani*. It is not possible at this point to speculate much about the nature of this incongruence in topology. Gamble et al. (2008), used a larger character dataset including several nuclear genes; however, the present study includes a much more comprehensive taxon sampling throughout the genus.

Interestingly, some species pairs, such as *G.* sp. 1 and *G.* sp. 2, and *G. annularis* and *G. alexandermendesi*, are found in sympatry but in different habitat. Both *G.* sp. 1 and *G.* sp. 2 have been found in the same locality near Puerto Ayacucho, but the former is associated with large trees in forest areas, whereas *G.* sp. 2 has been found associated with large rock outcrops in more open areas. *Gonatodes annularis* and *G.*

alexandermendesi have been both found in sympatry in the Sierra de Lema, yet the former seems to be associated to trees or rocks near water bodies or in flooded forest (Avila-Pires, 1995; Rivas and Molina, 2004), whereas the latter was found also on trees and rocks but never close to water bodies (Cole and Kok, 2006). Moreover, when examined in a phylogenetic context, divergence in habitat seems to follow a model of ecological character displacement and is consistent with "stage 1" of evolutionary radiations (Streelman and Danley, 2003). Streelman and Danley (2003) noted that in the early stages of evolutionary radiations in vertebrates, species have the tendency to diverge mostly in preferred habitat ("stage 1), and in later stages they diverge mostly in trophic morphology ("stage 2") and communication ("stage 3"). In "stage 1" ecological divergence in habitat is thought to occur initially in allopatry due to local pressures and is accelerated greatly following secondary contact (Streelman and Danley, 2003). In the Guayana Shield clade of *Gonatodes* divergence in habitat is clearly associated with the resource partitioning in sympatric species. The only species in the Guayana Shield clade that does not occur sympatric with another species in this clade is the most generalist in habitat, as *G. hasemani* has been found in rocks and tress in open areas, terra firme forests, and flooded forest. All other Guayana Shield species have been found in sympatry with the one other species that seems most divergent in preferred habitat.

CHAPTER 4

SPECIES BOUNDARIES IN A GROUP OF MONTANE DIURNAL GECKOS (*GONATODES*) IN NORTHERN SOUTH AMERICA

4.1 Introduction

In light of the current biodiversity crisis taxonomic research in the tropics has become as ever important given that the regions within this zone generally contain the highest biological diversity and are also suffering the most rapid rate of habitat destruction. Whereas in most temperate regions the rate of discovery of new taxa in many important groups (e.g. vertebrates) has become close to cero, in the tropics this rate does not seem to be slowing down as many new species are discovered every year for most major groups and recent molecular evidence suggest high levels of cryptic diversity (Bickford et al., 2007; Fouquet et al., 2007), indicating that we might not be even close to complete full species accounts for most higher taxa. Furthermore, most of the biological diversity in the tropics has been described by means of traditional taxonomy, which in terms of practice has not changed considerably in the last two centuries. Although traditional taxonomy practices remains vital to rapidly describe the biological diversity of tropical regions, it is not without serious limitations. In most traditional taxonomy studies a typological species concept is implicitly used and species limits decisions are made inductively in arbitrary and ad hoc ways. There is generally no ontological consideration to what a species is, and little epistemological justification for the

discovery operations used to delimit species boundaries. Traditional taxonomy basically translates into defining a species as a group of individuals in a somewhat defined geographic area that posses an arbitrary amount of differences when compared to other such species. As a consequence, current taxonomy might not closely reflect the limits between the theoretical entities (e.g. evolutionary species concept) that we now equate the concept of species to (Frost and Kluge, 1994; de Queiroz, 1998). Therefore, there is a need for incorporating into taxonomic research more sophisticated and scientifically rigorous discovery operations that are consistent with the ontological status of the entities we try to delimit (i.e. species).

Unlike the decades old, ongoing debate on species concepts, it is only in recent years that there has been an increased interest on empirical methods for delimiting species boundaries (for a review see Sites and Marshall, 2004). Several different methods and criteria have been proposed and used in empirical studies. However, many of these methods have sampling requirements that, for several reasons, might be difficult to attain for groups distributed in tropical regions. Lack of proper funding and resources for research and other logistic problems generally pose serious limitations to conduct the intensive sampling required for some of the most sophisticated empirical methods for delimiting species boundaries (e.g. Wiens and Servedio, 2000; Templeton, 2001). I herein use a simple framework that requires moderate sampling and that uses multiple lines of evidence in a complementary rather than exclusive way to delimit species in a group of neotropical lizards of the genus *Gonatodes*.

The genus *Gonatodes* is a diverse group of neotropical diurnal geckos in the

family Sphaerodactylidae. There are 20 species currently recognized in this genus, which has a collective distribution that includes most of Middle America, tropical mainland South America, and most of the Caribbean Islands. Species of *Gonatodes* also occupy a diverse array of habitats, including arid or semiarid regions, submontane humid forests, cloudforests, and Amazon rainforests. The genus is particularly diverse in north-central South America, specifically in montane humid forest of Venezuela (Rivero-Blanco, 1979) and in the lowlands of the Guayana Shield (Chapter 3). The taxonomy of *Gonatodes* was reviewed by Rivero-Blanco (1979) in his, to date, unpublished PhD. Dissertation. Because published taxonomic work on *Gonatodes* is limited mostly to the original species descriptions, many of which are old and not detailed, Rivero-Blanco's dissertation has become an important reference for the few people who have worked on the taxonomy of this genus during the last two decades (e.g. Esqueda, 2004; Cole and Kok, 2006; Avila-Pires, 1995). Some of the most important results from Rivero-Blanco's dissertation are the discovery of three new taxa from Venezuela and one from Trinidad. All four new taxa discovered by Rivero-Blanco are to be found in montane or submontane humid forest of the Merida mountain range or the Coastal mountain range, in Venezuela, or the Northern mountain range of Trinidad. Unpublished molecular data (Chapter 5), indicates that the species restricted to the humid forests of these mountain ranges, including not just the four new taxa discovered by Rivero-Blanco, but also *G. ceciliae*, *G. falconensis*, *G. purpurogularis*, *G. seiglei*, and *G. taniae*, from Venezuela, as well as *G. ocellata* from Tobago Islands may form a monophyletic group. I herein evaluate the species boundaries in this group

of related species using a combination of morphological, molecular and ecological niche modeling data, in a framework that is simple and is realistic given the funding and resource limitations for taxonomic research in tropical countries. I also feel that the strength of this approach is that it uses the different types of data in a complementary way, and not as potentially conflicting discovery operations that might support different species limits (therefore different taxonomic arrangements). The approach is based on the notion that the properties of species that we traditionally use to delimit their boundaries (e.g. diagnosability, reproductive isolation) are contingent and not prescriptive, and therefore, it is consistent with our current understanding of the ontological status of species.

4.2 Materials and methods

4.2.1 Philosophical and operational approach to delimiting species

Following the work of some philosophers of the species problem (see below) I herein contend that the solution to the species problem has been determined and that the ongoing debate on species concepts is coming to an end as this solution becomes widely accepted. The solution to the species problem is not to be credited to any single author or published work, yet in my opinion it has been presented must clearly and thoroughly by Frost and Kluge (1994), Mayden (1997) and de Querioz (1998; 1999). Instrumental to the solution of the species problem is the thesis of "species as individuals" set forth by Ghiselin (1974) and Hull (1976; 1978). These authors proposed that species are real entities (=individuals in the philosophical sense) in nature that exist independently of our ability to detect or delimit them in practice. Frost and Kluge (1994), grounded on

the species as individuals' thesis, indicated that the species problem mainly results from conflating and confounding the simplifying assumptions of particular discovery operations with the nature of the entities (=species as individuals) we search for. Therefore, the properties of species on which our discovery operations are based (e.g. diagnostic characters, reproductive isolation, haplotype exclusivity, etc) are only contingent on processes resulting from integration of species, but do not prescribe what a species is. Frost and Kluge (1994) went on to equate species to the Evolutionary Species Concept (ESC), which they restated as the largest integrating lineages below the level of non-integrating clade. That is, species are the entities at the uppermost limit in which its constituent parts are related tokogenetically and the lower-most level to participate in phylogenetic relationships. Mayden (1997) presented an evaluation of 22 species concepts based on the criteria of theoretical significance, generality, and applicability, and concluded that the ESC is the only theoretical concept appropriate for species. Furthermore, the ESC is a primary concept essential to the structuring of our ideas and perceptions of real species in the natural world. The remaining species concepts evaluated by Mayden (1997) were considered secondary concepts equivalent to operational tools to discover the entities in accord to the primary concept. Finally, de Queiroz (1998) indicated that all the modern species concepts equate species explicitly or implicitly to segments of population (=metapopulation; sensu de Queiroz, 2005) level evolutionary lineages. He called this view the *general lineage concept of species* and, based on arguments similar to those by Frost and Kluge (1994) and Mayden (1997), suggested that differences or incompatibilities between modern alternative concepts are

mostly attributable to these concepts treating properties acquired by diverging population lineages as necessary properties of species. Therefore, if we recognize the common thread uniting species concepts (i.e. species are metapopulation level lineages = ESC) and we drop certain contingent properties of lineages as necessary properties of species the species problem is solved (de Queiroz, 1998; 1999; 2005).

Having accepted a purely theoretical concept of species (the ESC) the next logical step is to formulate the discovery operations that will allow us to detect species in practice. The theoretical nature of the ESC implies that no single discovery operation will guarantee detecting all existing species (Frost and Kluge, 1994). Furthermore, different discovery operations may have different data requirements and have different strengths and limitations (Sites and Marshall, 2003); however, they are not necessarily mutually exclusive. Thus, it seems like the best option to delimit species is by using multiple complementary discovery operations (sensu Grant, 2002) that look at different properties contingent on species being evolutionary lineages (for discussion of contingent properties of species see de Queiroz, 2005). In doing so I herein use a combination of character-based and tree-based approaches (Wiens and Penkrot, 2002) in combination with information on geographic distribution/barriers to delimit species (see Wiens and Graham, 2005).

To make species-level decisions I follow the framework outlined in Figure 4.1. Following this framework implies that, for species to be recognized, at least two independent lines of evidence out of the possible three (morphology, molecules, and geographic barriers) should support the distinctiveness of focal species. Focal species

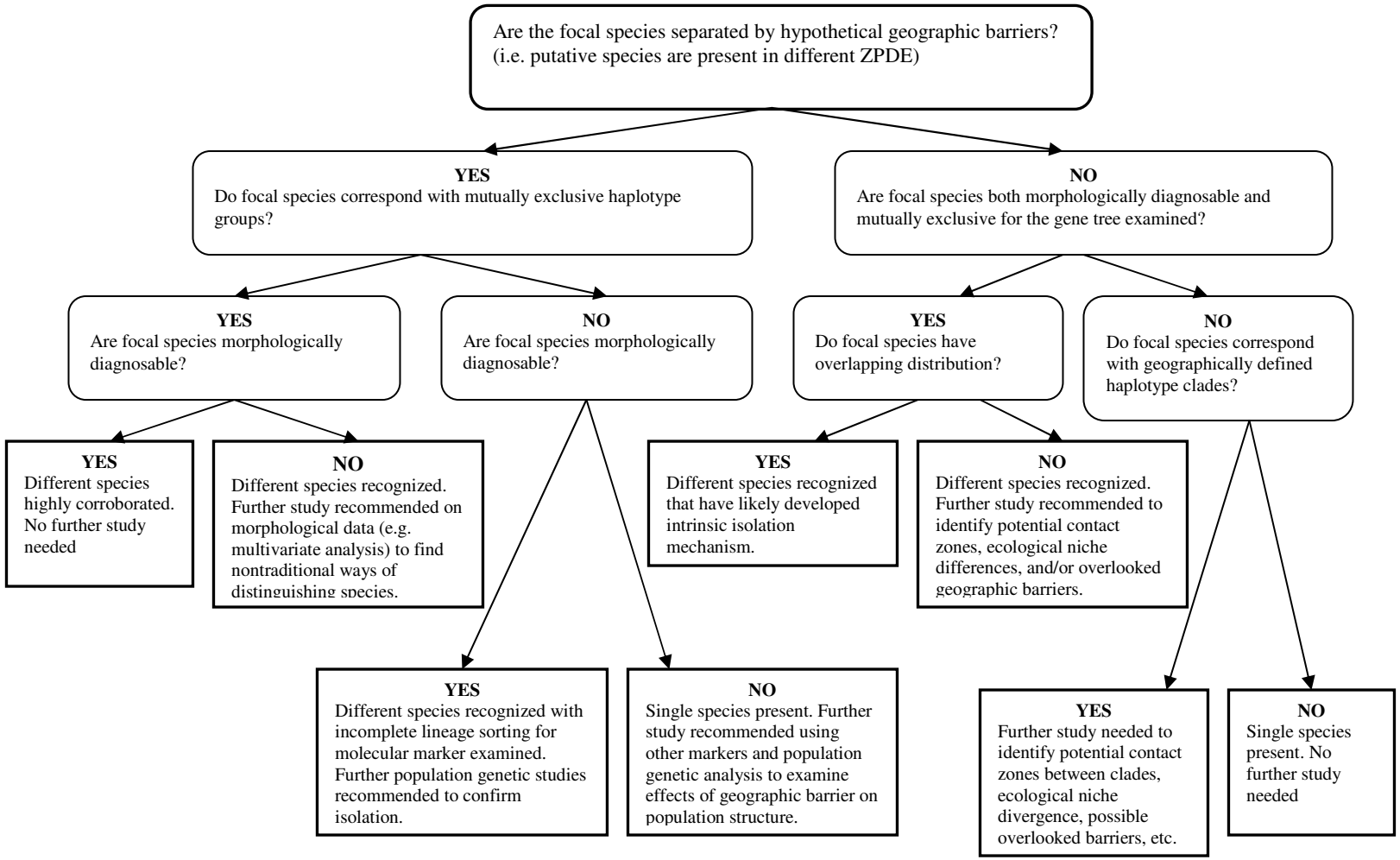


Figure 4.1 Framework used for making species limits decisions in montane *Gonatodes*

are starting hypotheses of species limits, which can be set forth based on previous taxonomy, presence of putative geographic barriers, new morphological characters or phylogenetic results of the molecular data. In the framework outlined all possible pairs of focal species can be evaluated but emphasis is made on sister or closely related species. Morphological characters will be judged as fixed (or diagnostic) if at least five individuals per focal species have been examined without showing alternate states for the character being examined. This minimum sample size is certainly small to statistically evaluate if a characters state is fixed (see Wiens and Servedio, 2000); however, because some focal species are known only from only a few specimens having a bigger sample size requirement will limit the use of morphological data for evaluating the species validity. In addition to this, the consequences of erring in underestimating diversity can be far worst (e.g. good species subsumed into another one will be overlooked in conservation efforts) than when diversity is overestimated. For the molecular data, exclusivity (sensu Baum, 1992) of mitochondrial haplotypes corresponding with focal species will be the criteria to support species delimitation. The more explicit statistical method of Nested Clade Analysis (Templeton et al., 1995) can also be used to infer species limits; however, this methods requires far more intensive sampling than what has been obtained for this study, and in general we find this to be a important limitation in using this approach for most tropical taxa (see Sites and Marshall, 2003, for a brief discussion of limitations of different methods for delimiting species).

The presence of geographic barriers as evidence for species recognition requires

some explication. A geographic barrier is used here in a general sense and does not include only physical barriers but also includes ecological barriers such as unsuitable habitat for a given species. Obviously, geographic barriers are mostly associated with allopatric speciation, which is not the only possible mode of speciation. However, empirical evidence supports allopatric speciation as the most common mode of speciation (Mayr, 1942; 1963; Lynch, 1989; Chesser and Zink, 1994; Lynch, 1999; Barraclough and Vogler, 2000; Hall and Harvey, 2002), while theoretical analyses show that parapatric and especially sympatric speciation are possible only under some relatively stringent conditions (Coyne and Orr, 2004). Furthermore, vicariant speciation seems to be the general rule at least for montane terrestrial vertebrates (Lynch, 1999). Despite this, the presences of impassable geographic barriers are traditionally only used implicitly or in an ad hoc fashion by taxonomist to delimit species. This is mainly because, unless geographic barriers are very obvious (e.g. a mountain range, large river, etc), it is generally difficult to infer them. In recent years the development of GIS-based ecological niche modeling techniques seem to provide a powerful tool to infer species geographic ranges and also the presence of potential geographic barriers (Peterson et al., 1999; Soberón and Peterson, 2004). The idea is that by using locality records for species and maps of climatic layers, the "climatic niche envelope" of a species can be delineated geographically with the aid of computer algorithms. Thus, if we are examining two focal species and we find that areas that are outside their climatic niche envelopes separate them, gene flow between them would be unlikely because it would require individuals crossing unsuitable habitat (Wiens and Graham, 2005). Finally, if

we assume we have good sampling and because we know that the molecular dataset, morphological characters and geographic distribution are independent of each other, concordance between them will indicate effective isolation and historical individuality (see Kluge, 1990).

4.2.2 Specimens examined and *morphological characters*

Except for size, coloration and a few squamation characters, species of *Gonatodes* are otherwise morphologically conservative. Rivero-Blanco (1979) used mostly characters from the sexually dimorphic coloration of males and the subcaudal pattern of the regenerated tail to distinguish among species of *Gonatodes*. I herein focus mostly on these two character systems, since further examination of specimens did not reveal any new useful characters. A list of specimens examined can be requested from the author.

4.2.3 Lab protocols and molecular analyses

Tissues (liver or muscle) were obtained from freshly killed specimens in the field (Appendix C) and were preserved in 95-99% ethanol, and permanently stored at –70°C. Total genomic DNA was isolated using the DNAeasy Blood and Tissue Kit (Qiagen) following the manufacturers protocol. DNA Sequences were amplified from the mitochondrial ribosomal small unit (12S; primers: 12a: 5'-CTG GGA TTA GAT ACC CCA CTA-3'; 12b: 5'-TGA GGA GGG TGA CGG GCG GT-3') using standard polymerase chain reaction (PCR) protocols. The PCR products were purified using the ExoSAP-IT^{tm} kit (United States Biochemical) and used as templates in sequencing reactions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems).

Sequencing reactions were cleaned-up using ETOH/Sodium acetate precipitations and were run on a ABI 3100xl automated capillary sequencer. Contigs were assembled and edited in Sequencher 4.1 (Genes Code Corps., Inc).

Sequences were aligned online in T-COFFEE 5.56 (Notredame et al., 2000) using the regular option. This service provided through a web server (http://www.tcoffee.org) of the Swiss Institute of Bioinformatics (see Poirot et al., 2003). A Maximum Parsimony (MP) analysis of the concatenate dataset was performed on TNT 1.2 (Goloboff et al., 2000) using the default parameters and options of the traditional search.

4.2.4 Ecological niche model inference of ecological barriers

I used the program Maxent version 3.2.1 (Philips et al., 2006) to generate a map of the climatic niche envelop for montane species of *Gonatodes*. Maxent uses a maximum entropy statistical approach to generate a probability distribution of species presence as a function of environmental variables. This method has been shown recently to outperform other widely used methods for ecological niche modeling (e.g. BIOCLIM, GARP; Elith et al., 2006). The program takes for input a list of geographic coordinates for presence-only localities of a given species and georeferenced grid-type files containing environmental layers for the area of interest. In the analysis I used 24 localities including mostly those where montane species of *Gonatodes* were collected directly by the author but I also included a few additional localities associated to museum specimens not collected by myself (Appendix D). Geographic coordinates were generally obtained in the field using a handheld GPS. When this equipment was

not available or when localities came from museum specimens, the geographic coordinates where obtained from cartographic maps only when the localities could be located with precision on these maps, otherwise, they were excluded from the analyses. The environmental layers used were altitude and all the 18 bioclimatic variables (1 km^2 spatial resolution) that are made available at www.worldclim.org (Hijmans et al., 2005). I used DIVA-GIS version 5.4 to crop the environmental layers to encompass an area that includes mostly the northern half of Venezuela. Because I am interested only in major ecological barriers that affect the group under study, and not on accurately predicting the distribution of individual species, I combined localities for different focal species in the analysis. Because I am combining multiple species, some of which might have diverged in their ecological niche, the distribution of the montane group of *Gonatodes* will likely be overpredicted in the study area; therefore, I expect this approach to be conservative and will identify only the major ecological barriers. I also consider this approach to be the appropriate null model for identifying ecological barriers because it does we not make any *a priori* assumptions about groupings. I ran Maxent with the cumulative frequency option and default parameters to generate a new layer of relative suitability. To divide the layer into areas suitable and unsuitable for montane species of *Gonatodes* I used as a threshold the lowest value of suitability associated to a locality included in the analysis. Ecological barriers were defined as unsuitable areas separating suitable areas by a minimum distance of 5 km. Areas interconnected by suitable habitat isolated by ecological barriers from such other areas are what I called zones of potential demographic exchangeability (ZPDE).

4.2.5 Starting taxonomic framework

The focal species examined herein are based on the taxonomic arrangement proposed by Rivero-Blanco (1979), which includes four undescribed species that I will refer to as sp. "A", sp. "B", sp. "C", and sp. "D" ("D" was proposed by Rivero-Blanco as a subspecies of *G. ceciliae*). I have also included the recently described *G. purpurogularis* and two additional focal species (sp. "E", and sp. "F") for populations that could not be assigned based on morphology (putative diagnostic character were found based on the criterion defined above) to any of the species in Rivero-Blanco (1979). All focal species in the starting framework are listed in Table 4.1 and most of them are shown in life in Figure 4.2. The distribution of some of these species has been expanded with my own unpublished data. For example, *G. falconensis*, which in the literature is known only from Falcon Sate, is a species that I have collected from throughout the Cordillera de Merida, Sierra de Aroa, and the western part of the Cordillera de La Costa. I also recently collected *G. purpurogularis*, which was known only from the type locality in Calderas, from the southern tip of the Cordillera de Merida. Finally, specimens referred to *G.* sp. "C" were also obtained from Peninsula de Paria (previously known only from Turimiquire Massif and Margarita Island). A map with the approximate distribution of all the focal species in the initial taxonomic arrangement is shown in Figure 4.3.

Table 4.1 Focal species examined for the montane group of *Gonatodes*

Species	Type locality and distribution as known in the literature. Only distribution is presented for putative new species.	References
ceciliae	Cerro Azul, Peninsula de Paria. Found throughout humid forests of the Peninsula de Paria	Donoso-Barros (1966), Rivero-Blanco (1979), Rivas et al. (2006)
falconensis	Pauji, Falcon State. Currently known only from the type locality, Sierra de San Luis, and P. N. Cueva del Toro.	Shreve (1947), Rivero-Blanco (1979), Mijares-Urrutia and Arends (1995).
ocellatus	Tobago Island	Rivero-Blanco (1965)
purpurogularis	Calderas, Barinas. Known only from the type locality.	Esqueda (2004)
seiglei	Las Puertas de Miraflores. Known only from two localities in the Oriental Coastal Cordillera of Venezuela	Donoso-Barros (1965), Rivero-Blanco (1979)
taniae	Estacion Biologica "Rancho Grande", Aragua, Venezuela. Known from two localities in the Central Coastal Mountain Range	Roze (1963), Rivero-Blanco (1979)
sp. A	La Azulita, Merida.	Rivero-Blanco (1979)
sp. B	P. N. Guatopo	Rivero-Blanco (1979)
sp. C	Margarita Island and Cordillera de la Costa Oriental	Rivero-Blanco (1979)
sp. D \	Northern Range of Trinidad Island. This focal species is currently under *G. ceciliae*.	Rivero-Blanco (1979), considered this population to be a subspecies of *G. ceciliae*.
sp. E	Hacienda La Elvira, P. N. Guatopo, Guarico, Venezuela. Known only from this locality.	This study
sp. F	La Pena, Trujillo, Venezuela	This study

Figure 4.2 Specimens in life (from top to bottom, left to right) of *G.* sp. "B", *G. ceciliae*, *G. falconensis*, *G.* sp. "E", *G. purpurogularis*, *G.* sp. "C", *G. seiglei*, and *G.* sp. "F"

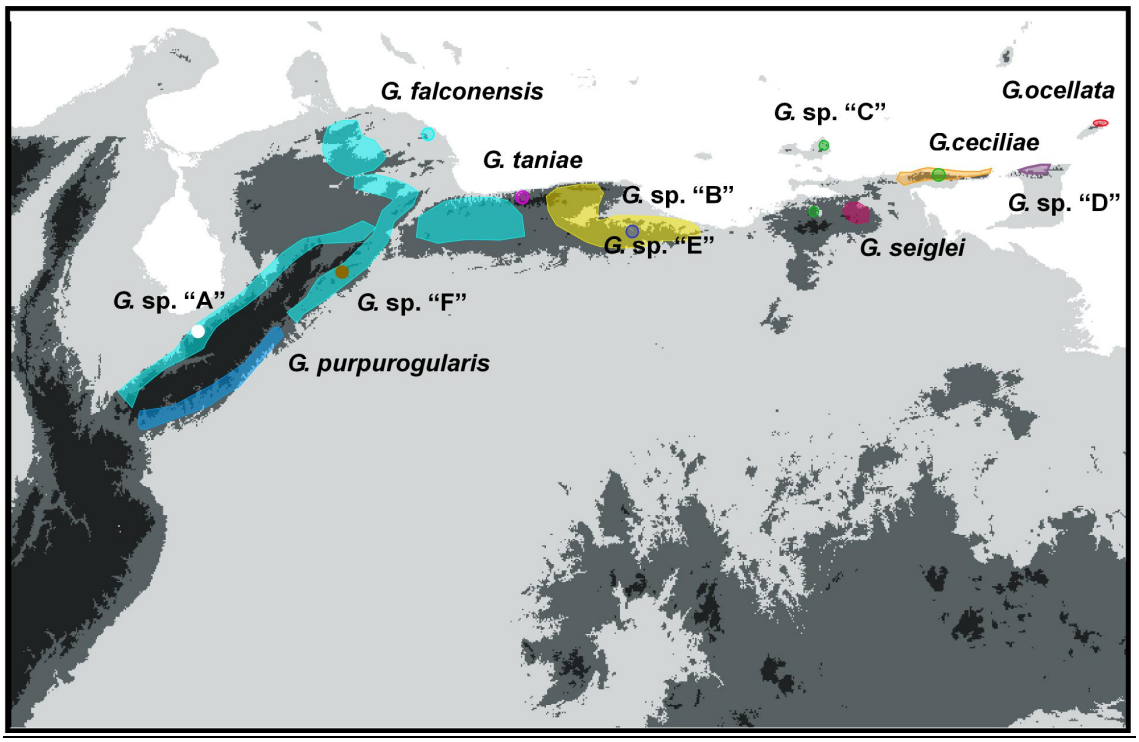


Figure 4.3 Map of the approximate distribution of the focal species of the Montane Group of *Gonatodes*

4.3 Results

4.3.1 Ecological barriers

The ecological niche modeling approach identified two ecological barriers for montane *Gonatodes*: the Unare Depression and the San Felipe Depression. Consequently, three ZPDE are recognized in mainland Venezuela: The Cordillera of Mérida + the Lara-Falcon system, the Central Coastal Mountain Range, and the Oriental Coastal Mountain Range. The other ZPDE, which are isolated by sea, are Margarita Island, Trinidad Island, and Tobago Island. A map of mainland Venezuela showing habitat suitability, geographic/ecological barriers and the localities included in the model is shown in Figure 4.4.

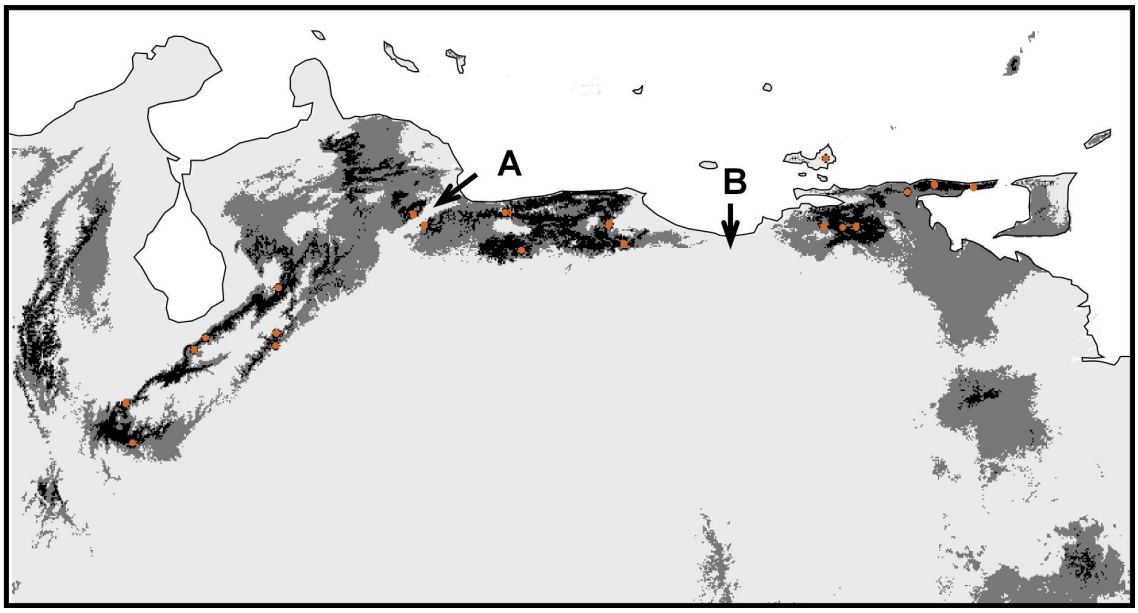


Figure 4.4 Map of habitat suitability (pale grey: unsuitable, medium grey: moderately suitable, dark grey: very suitable) for montane *Gonatodes*. The two ecological barriers are Depression de San Felipe (A) and Depression de Unare (B)

4.3.2 Phylogenetic analysis

The MP analysis resulted in 60 equally parsimonious trees with tree length of 923 steps. A strict consensus tree recovered (shown partially in Figure 4.5 and Figure 4.6) that the Montane Group is non-monophyletic and members of this group fall in two different clades, except *G.* sp "B", which falls sister to one of these clades + *G. antillensis* and *G. concinnatus*. However, nuclear data (see Chapter 5) is incongruent with the 12s tree and has moderate support for a monophyletic Montane Group. Because herein I am only interested in defining species boundaries in the Montane Group, and because the phylogenetic relationships of members of this group is treated in Chapter 5, I will here only focus on how the 12s dataset supports or rejects species

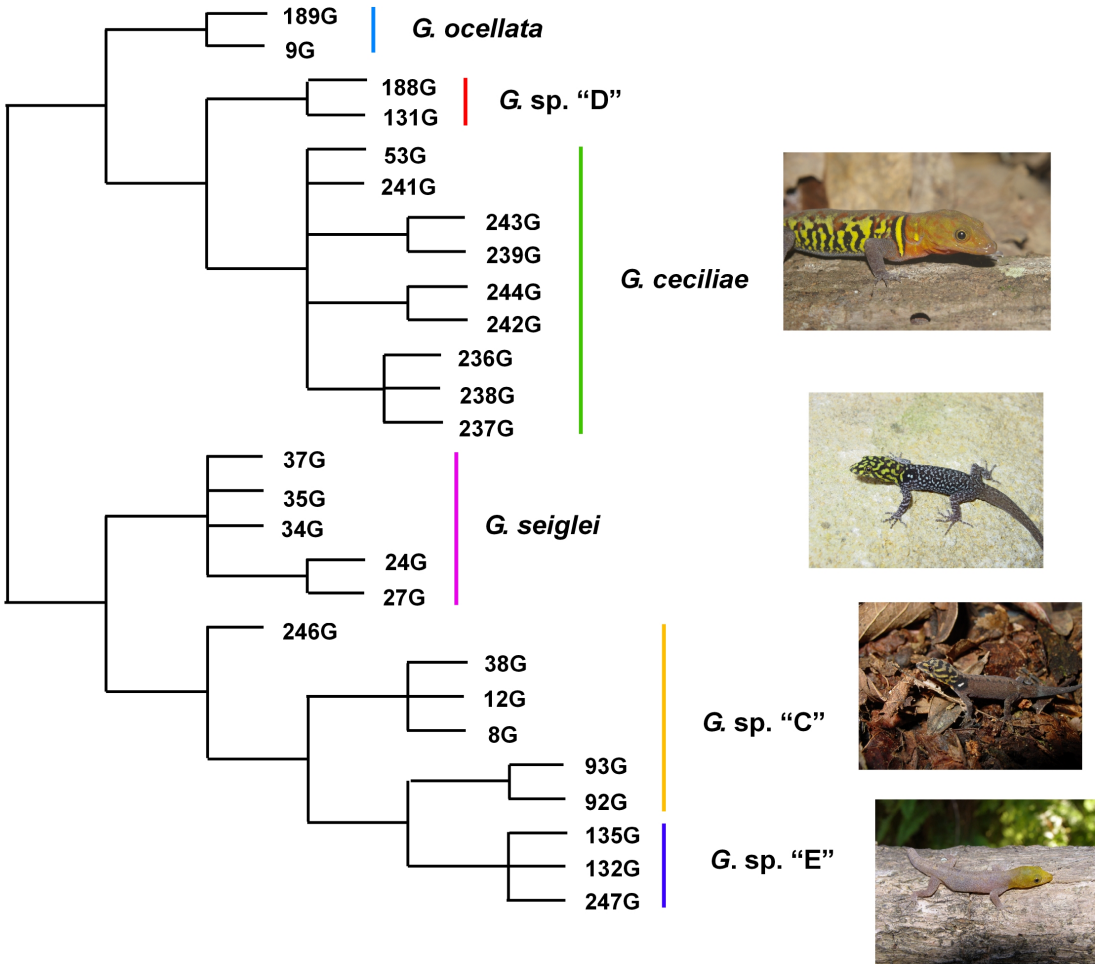


Figure 4.5 Clade of montane *Gonatodes* recovered in a phylogenetic analysis of the genus using the 12s mitochondrial gene

based on haplotype exclusivity. Therefore, I only show the topologies of the two clades that contain presumably closely related species in the Montane Group.

4.3.3 Species boundaries

Mitochondrial haplotypes of *G.* sp. "B" fall in the tree removed from all other species in the MG and it is also morphologically diagnosable (see Rivero-Blanco, 1979), thus it is corroborated as a valid species. The three putative species (*G. ceciliae*, *G. ocellata*, and *G.* sp. "D") in the top branch of the clade shown in Figure 4.5, are

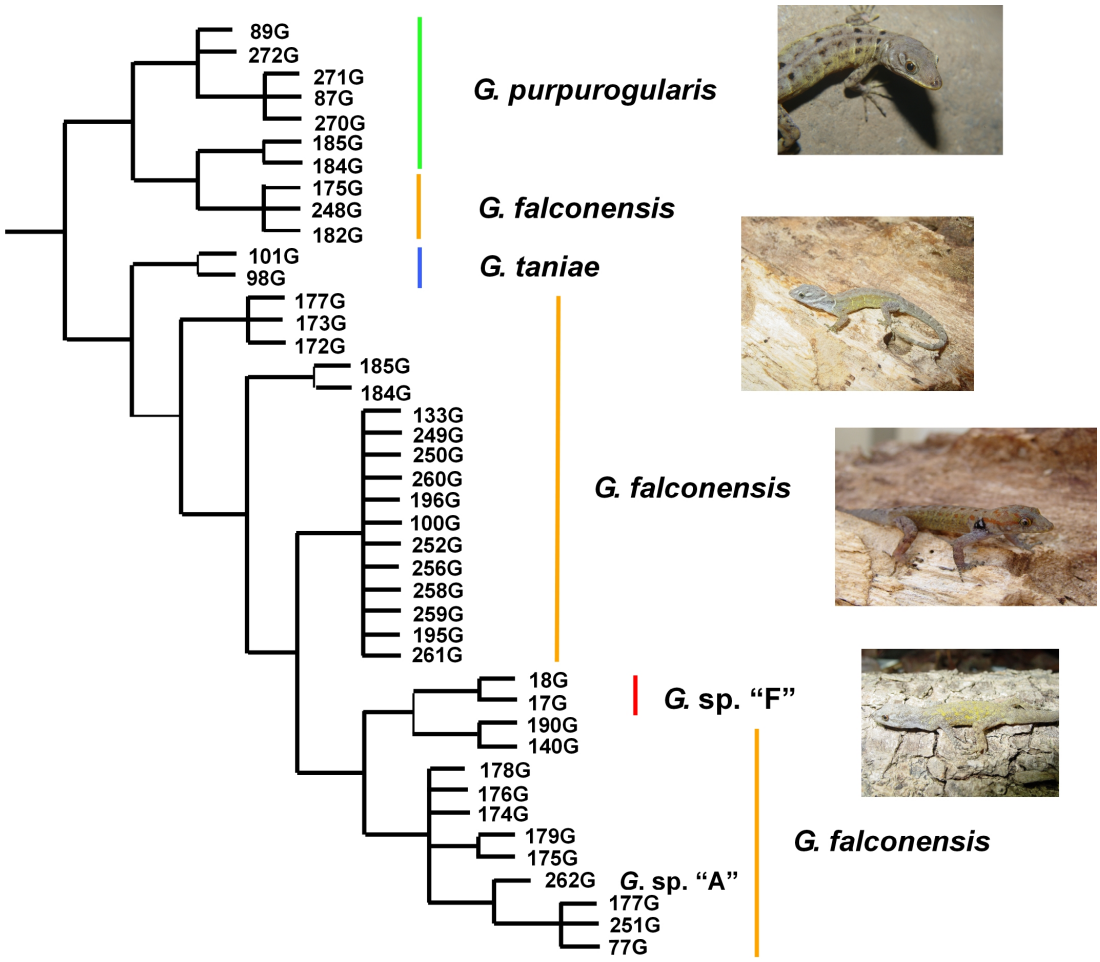


Figure 4.6 Clade of montane *Gonatodes* recovered in a phylogenetic analysis of the genus using the 12s mitochondrial gene

exclusive for their mitochondrial haplotypes, separated by geographic barriers and morphologically diagnosable, and as such are well corroborated species. In the bottom branch of this clade, *G. seiglei* is morphologically diagnosable and exclusive for its mitochondrial haplotypes, but occurs in the same ZPDE as *G.* sp. "C", yet they have not been found in sympatry. *Gonatodes seiglei* is corroborated as a valid species based on congruence between morphology and molecules; however, the framework recommends further studies to identify contact zones between *G. seiglei* and *G.* sp. "C". *Gonatodes*

sp. “C” is in turn closely related to *G*. sp. “E”, which occurs in a different ZPDE. Because both species are morphologically diagnosable and are separated by a major ecological barrier (the Depression de Unare) they represent distinct valid species. However, these two focal species are not mutually exclusive for their mitochondrial haplotypes so the framework recommends further population genetics studies to confirm isolation.

The clade shown in Figure 4.6 contains *G. falconensis*, *G. purpurogularis*, *G.* sp. “F”, and *G.* sp. “A”. New specimens of *G. falconensis* obtained in this study show that *G.* sp. “A” falls within the morphological variation of the former and they both fall within the same ZPDE; therefore, they are considered conspecific. Neither, G. *purpurogularis* nor *G. taniae* are supported as different from *G. falconensis* (the older available name) because there is not congruence between morphology with either mitochondrial exclusivity or separation by geographic barriers. As a consequence *G. purpurogularis* and *G. taniae* are considered conspecific and junior synonyms of *G. falconensis*. However, the framework used herein recommends further studies at contact zone between haplotype clades and between morphologically diagnosable populations. A summary of the taxonomy proposed by the framework used herein is presented in Table 4.2.

4.4 Discussion

The framework proposed herein seems to have worked well in defining species boundaries in the montane group of *Gonatodes*, and includes reasonable recommendations for future studies to corroborate or reject the taxonomic decisions that

Table 4.2 Corroborated species of montane *Gonatodes* and future study recommendations

Corroborated species	Putative species included within	Further study recommended to confirm species limits?
ceciliae	None	No
falconensis	*G. purpurogularis*, *G. taniae*, *G.* sp. "A", and *G.* sp. "F".	Yes. Further studies at potential contact zones between haplotype clades or between morphologically different populations are recommended.
ocellatus	None	No
seiglei	None	Yes. Further study recommended to identify potential contact zones, ecological niche differences, and/or overlooked geographic barriers in relation to *G.* sp. "C".
sp. "B"	None	No
sp. "C"	None	Yes. Further population genetic studies recommended to confirm isolation from *G.* sp. "E". See also recommendations for *G. seiglei*.
sp. "D"	None	No.
sp. "E"	None	Yes. See recommendations for *G.* sp. "C".

resulted from using it. There were cases in which current taxonomy underestimated (e.g. *G. ceciliae*) as well as overestimated current diversity in the group (e.g. *G. purpurogularis* and *G. taniae*). It also corroborated (e.g. *G.* sp. "E") and rejected (*G.* sp. "F") putative new species. Therefore, the results varied across the whole spectrum of possible decisions included.

I believe that using independent datasets in complementary (see Grant, 2002) rather than competing ways is the main strength of the framework proposed herein. As a matter of fact, species of *Gonatodes* in the Montane Group were supported by different combinations of the three lines of evidence used herein, indicating that a "morphology only" or "molecular only" approach would have proposed different and conflicting taxonomic arrangements.

I also think that combining independent lines of evidence in complementary ways is consistent with advances on the understanding of the ontological status of species. The notion that species are real entities (individuals) that are defined theoretically by the Evolutionary Species Concept (i.e. species are the largest integrating lineages), seems to be carrying the day in the "species problem" discussion (see papers in "Species Delimitation" Symposium in issue 6 of Systematic Biology 2007). Accepting this idea implies that the properties (e.g. diagnosability, reproductive isolation, etc.) of species that we use to delimit them empirically are only contingencies and do not defining attributes of species (Frost and Kluge, 1994). Moreover, there is not even a valid epistemological justification for claiming global superiority of one single discovery operation over the others for inferring species boundaries. All contingent properties of species represent potentially important evidence for delimiting species that, when found in congruence with each other, are strong indicatives of effective isolation and historical and /or current integration processes of species.

To my knowledge this is the only framework proposed for delimiting species that not just helps in testing species limits but also provides explicit recommendations

of future studies when these are deemed necessary. It is also possible to make a "second pass" of the framework once a "first past" has provided an initial test of species limits, before carrying out any additional study that would require further sampling. For example, the current dataset can be analyzed again focusing only on *G. falconensis*, *G. purpurogularis* and *G. taniae*. Because these are closely related or conspecific as suggested herein, we can redo the ecological niche modeling approach limiting the localities used in MAXENT to only those where individuals referred to this putative species have been found. This will likely improve the model prediction and might uncover ecological barriers that were overlooked by the model when pooling together the collecting localities of all the species examined herein.

CHAPTER 5

GENERAL DISCUSSION

5.1 Phylogenetic relationships of *Gonatodes*

In this Chapter I present the full phylogenetic tree obtained but shown partially in Chapter 3. This tree is based on a Maximum Parsimony analysis of DNA sequences of the 12s mitochondrial gene and the C-mos nuclear gene (see Chapter 3 for details). The analysis recovered five major clades within *Gonatodes* (Figure 5.1). To my knowledge there are no morphological characters unique to any of these clades, the closest exception being the "Small, heliothermic species clade" for which four (five if the SMLB species is included) out of the six currently recognized species in it are the only ones in the genus with a conspicuous pale middorsal stripe. However, most members of a given clade generally have in common inhabiting same geographic regions and habitat. The "Small, heliothermic species clade" as the name suggests is comprise of generally small species relative to other *Gonatodes*, many of which prefer open type of habitat. The *G. vittatus* Complex is endemic to extreme northern South America but the other species in this clade are found in different regions including Middle America, Ecuador, Peru and Brazil.

Another clade contains *G. antillensis*, *G. concinnatus*, and *G. humeralis*. The phylogenetic position of *G. antillensis* has some interesting evolutionary implications,

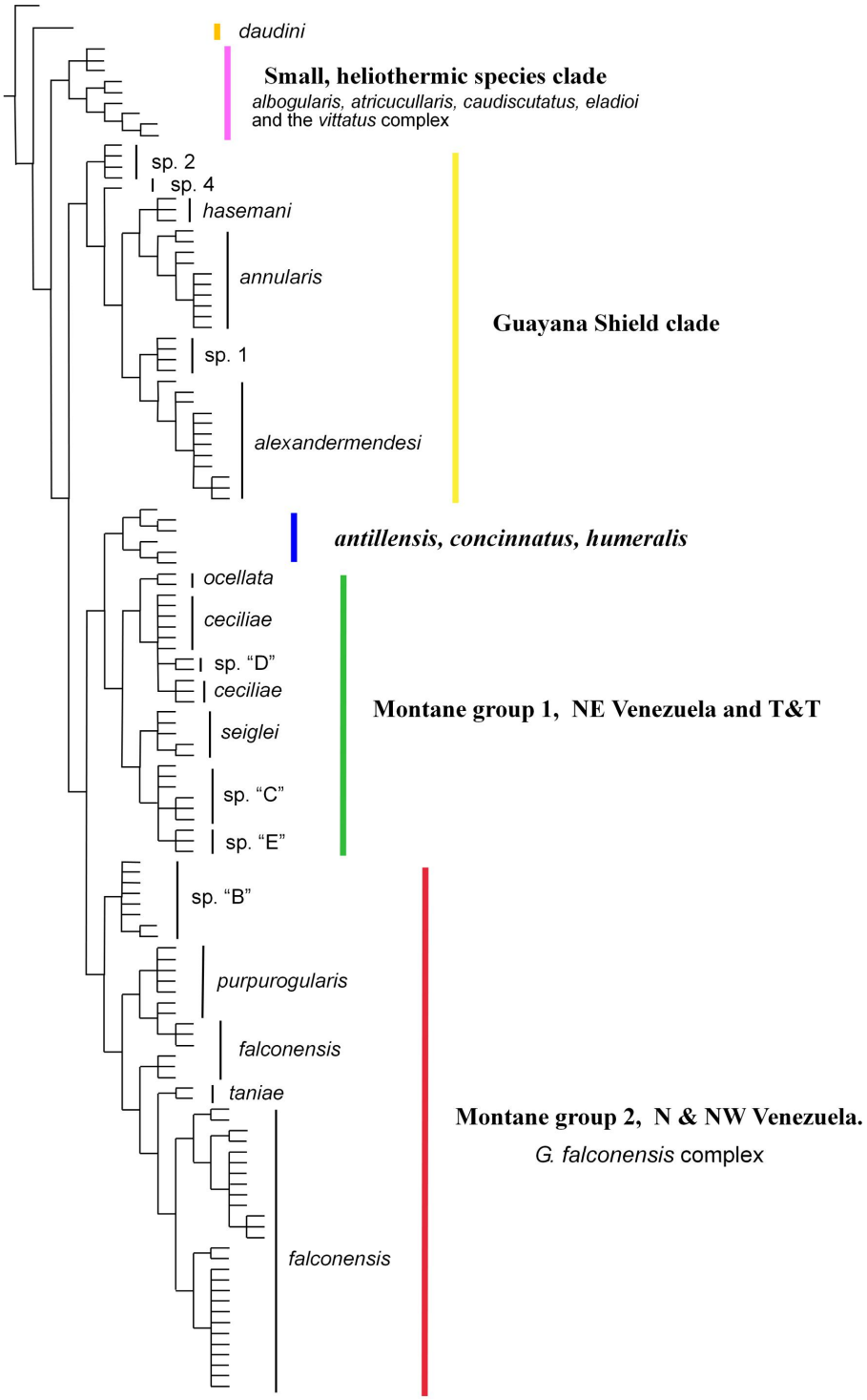


Figure 5.1 Strict consensus tree of 21 equally parsimonious trees for the genus *Gonatodes*

which are discussed below, but it is curious that these three closely related species have little in common. *Gonatodes humeralis* is a small species that is widespread in low elevation Amazonian Rainforest. *Gonatodes concinnatus* is a larger species found along the eastern slopes of the Andes, from Venezuela to Peru. *Gonatodes antillensis* as discussed below is unique in many ways and it is a moderate sized species of *Gonatodes*, found only in a few islands off the coast of Venezuela. This small clade is sister to one of the clades of montane species of *Gonatodes*.

Species in the montane group of *Gonatodes* examined in Chapter 4 fall in two different clades. The first of these clades (Montane group 1) is sister to the clade containing *G. antillensis*, *G. concinnatus* and *G. humeralis*, and is restricted in distribution to northeastern Venezuela and the nearby islands of Trinidad and Tobago. Only one species, *G.* sp. "E", is found on the Central Coastal Range of Venezuela, three species (*G. ceciliae*, *G. seiglei* and *G.* sp. "C") are found in the Oriental section of Coastal Range, and *G.* sp. "D" and *G. ocellata*, are endemic to Trinidad and Tobago, respectively. *Gonatodes ceciliae*, *G.* sp. "D" and *G. ocellata* are closely related, a result consistent with a recent connection between Trinidad and Tobago and mainland Venezuela, as has been suggested before. The relationships between these three species [(*G. ceciliae* + *G.* sp. "D") + *G. ocellata*] suggests that the connection between Trinidad and Tobago was broken before the connection between Trinidad and Venezuela.

The other clade containing montane species of *Gonatodes* (Montane group 2) is restricted to the Coastal Mountain range of Venezuela and the Cordillera de Merida. This clade contains three species currently recognized, namely *G. falconensis*, *G.*

purpurogularis, and *G. taniae*. Both *G. purpurogularis* and *G. taniae* were rejected as valid species following the framework used in Chapter 4; however, further study including additional samples is needed for a more rigorous evaluation of species limits in this small group, for which I propose the name of *G. falconensis* complex. All three currently recognized species in this complex are unique among *Gonatodes* in being only weakly sexually dimorphic in coloration relative to other species in this genus. *Gonatodes* sp. 2 is sister to the *G. falconensis* complex and is a well-corroborated species, which is also very different in morphology relative to the *G. falconensis* complex. It is one of the most interesting species of *Gonatodes* with a least four very different color morphs found in males, all of which might be found in sympatry at some localities (Rivero-Blanco, 1979).

Finally, there is the "Guayana Shield clade" which contains six species, all but one, *G. hasemani*, are restricted to Guayana region as discussed in Chapter 3. Also noteworthy is the fact that *G. daudini* was found to be sister to all other *Gonatodes*. This small species endemic to Union Island is also unique in the genus in having large granular scales and a series of large colorful ocelli on the sides of the body and also a single one in the parietal region. The phylogenetic position of this species is controversial as Gamble et al. (2008) found it to be nested within the genus, specifically sister to *G. albogularis* and *G. vittatus*. As mentioned before, discrepancies were found between the results shown herein and those obtained by Gamble and collaborators. It is not possible to determine the cause of these discrepancies in results but they are likely due to the different character and taxon samplings in the two studies.

The phylogenetic tree presented herein is almost the same as the one obtained using just the 12s gene data. This makes sense because C-mos has little variation relative to 12s. A Maximum Parsimony analysis with only C-mos (Figure 5.2) shows support for the same major clades supported in the combined dataset analysis but the relationship among them is not resolved in a strict consensus tree. However, is the strict consensus tree is relaxed to a 50% majority rule consensus trees some interesting changes are observed. The first is that *G. daudini* falls sister to *G. albogularis* + *G. vittatus* complex in agreement with the results obtained by Gamble and collaborators. The other noteworthy change is that the montane group appears as monophyletic in contrast to the results of the combined analysis. Because taxon sampling is intensive in the present study with almost all species of *Gonatodes* sampled, resolving these discrepancies in results will most likely need increased character sampling including characters systems not explored to date (e.g. morphology). To this end, I am currently working on increasing both the mitochondrial and nuclear datasets as well as generating a matrix of morphological characters for the genus.

5.2 Phylogenetic position of the only nocturnal *Gonatodes*

The Gekkota is the only major clade of mostly nocturnal species of lizards. Nocturnal behavior has allowed this clade to exploit what is a novel ecological niche for lizards, and one in which they have diversified significantly due to a release in competition from other groups of lizards (Pianka and Vitt, 2003). Two of the most distinctive traits that have been associated with the evolution of nocturnality in Gekkotans are an elliptical pupil, which allows for better vision at night, and the ability

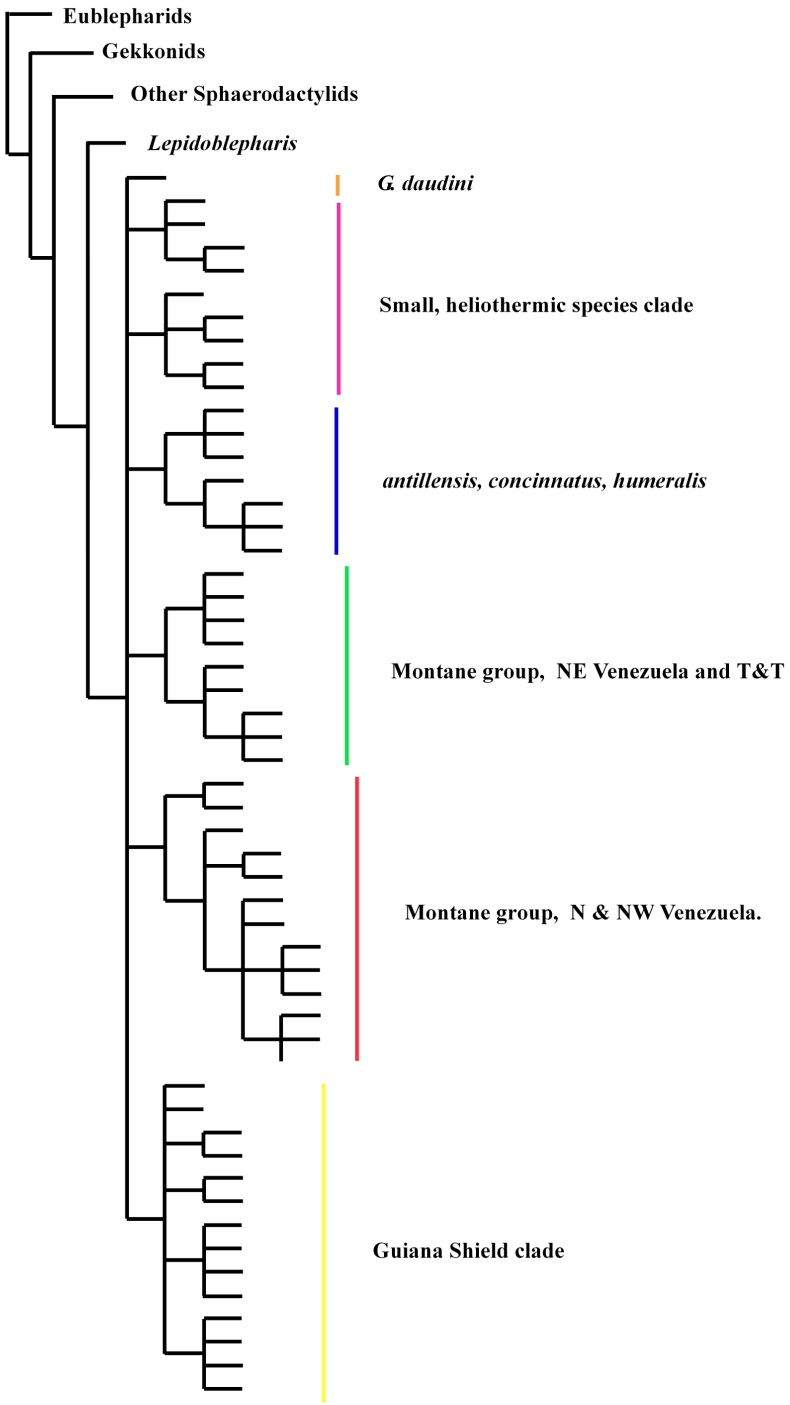


Table 5.2 Strict consensus tree of a Maximum Parsimony analysis using only the C-mos dataset

to vocalize or produce “calls”, which is used during social interactions (courtship and territorial encounters). As mentioned before, the evolution of vocalizations in gekkotans seems to compensate for the limited used that the visual displays might have in the limited light available at night (Marcellini, 1977). Within Gekkota, nocturnal activity is presumably the ancestral state (Vitt and Pianka, 2003) with diurnality having evolved secondarily in several groups including Sphaerodactylids. Almost all species of *Gonatodes* are diurnal and show traits associated with diurnal activity, including a round pupil, the use of visuals displays, and having striking sexual dichromatism. Additionally, none of the diurnal species of *Gonatodes* are known to produce vocalizations. *Gonatodes antillensis* is the only species in the genus known to be strictly nocturnal. Interestingly this is also the only species of *Gonatodes* with an elliptical pupil and known to produce vocalizations (Donoso-Barros, 1968; own unpublished data). However, the phylogenetic position of this species was somewhat controversial. Rivero-Blanco (1964) first placed this species in *Gonatodes* but later Donoso-Barros (1968) included it in the genus *Gymnodactylus.* Peters and Donoso-Barros (1970) followed Rivero-Blanco in allocating this species to *Gonatodes* and this position has not been challenged ever since. My results confirm that *G. antillensis* falls within *Gonatodes*, and this holds true with the C-mos dataset including several other Sphaerodactylids and Gekkonids (family in which *Gymnodactylus* is currently placed) and rooting the tree with Eublepharids. This results implies that *G. antillensis* has re-evolved the most conspicuous traits associated with nocturnality, such as the elliptical pupil and the ability to vocalize. Notably it has also retained the sexual dichromatism characteristic

of other *Gonatodes*. Males of *G. antillensis* posses a yellow hood like most other *Gonatodes*.

5.3 Final remarks

Although the present study has provided an improved understanding of the systematics of the genus *Gonatodes*, research on the subject is far from complete. Discrepancies with previous studies indicate the need for increasing character sampling. Additionally, there is still one currently recognized species in the genus, namely *G. tapajonicus*, which has not been sampled for molecular analyses. Some species groups still require additional study to clarify species limits (e.g. *G. falconensis* complex). Also, surveys especially in the Guayana Shield and in the northern Andes will likely reveal additional new species of *Gonatodes*.

A full understanding of the systematics of this group will provide the foundations for additional studies of this interesting genus. As stated in Chapter 1, *Gonatodes* has many characteristics that make this group ideal for studies of sexual selection, display evolution, biogeography, evolution of color polymorphism.

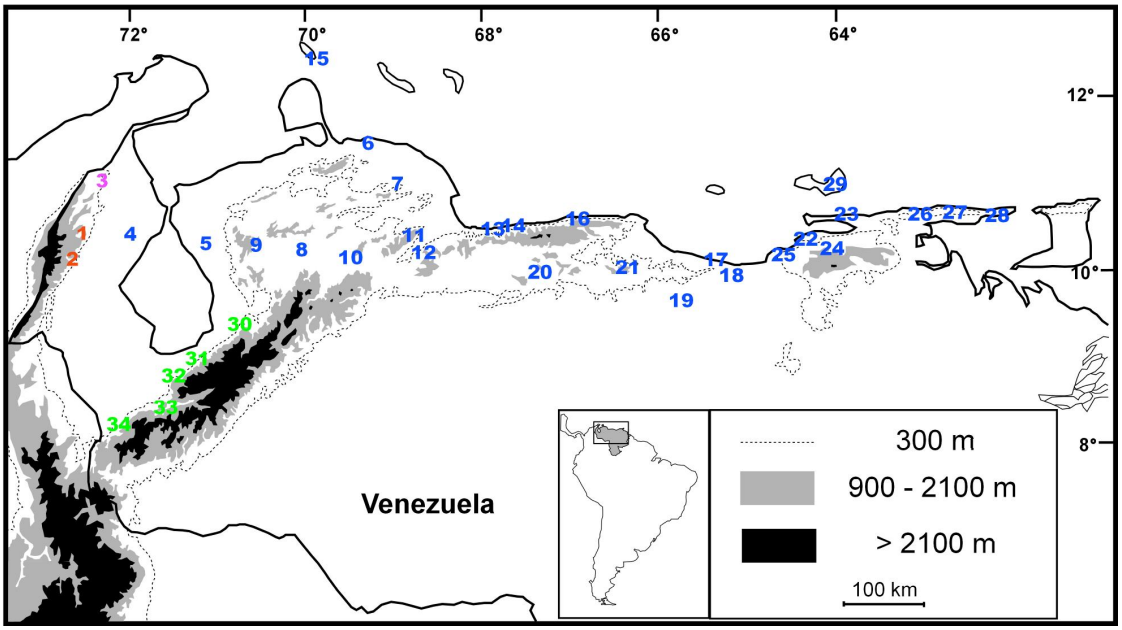
APPENDIX A

SAMPLES USED IN THE MOLECULAR ANALYSES OF THE *G. VITTATUS* COMPLEX

Locality #	Taxon	Locality	Sample code
1	*petersi*	Zulia: Rio Las Lajas	156G
2	*petersi*	Zulia: Finca el Deseo, Cuenca Rio Negro.	163G, 193G, 194G, 195G
3	*petersi vittatus*	Zulia: Parcelamiento La Orchila, Mara.	55G, 56G, 57G, 64G, 67G 58G, 61G
4	*vittatus*	Zulia: Hacienda Grano de Oro	219G
5	*vittatus*	Zulia: Parque Recreacional Burro Negro.	253G, 254G, 255G
6	*vittatus*	Falcon: Cumarebo	211G, 212G
7	*vitattus*	Falcon: Jacura	204G
8	*vittatus*	Lara: Carora	215G
9	*vittatus*	Lara: Cerron	213G
10	*vittatus*	Lara: Pavia	36G
11	*vittatus*	Yaracuy: San Felipe	94G
12	*vittatus*	Yaracuy: Fila Jaiguao	220G
13	*vittatus*	Aragua: Choroni	208G, 209G, 210G
14	*vitttatus*	Aragua: Playa de Cata	200G
15	*vittatus*	Aruba: Tanki, Leendert.	59G
16	*vittatus*	Distrito Federal: Caracas	74G
17	*vitttatus*	Anzoategui: Boca de Uchire	196G
18	*vittatus*	Anzoategui: Carretera Aguas - Clarines	197G, 198G
19	*vittatus*	Guarico: San Rafael de Laya	201G
20	*vittatus*	Guarico: San Juan de los Morros	203G
21	*vittatus*	Guarico: Hacienda La Elvira, P. N. Guatopo	202G
22	*vittatus*	Sucre: Playa Cumana	102G
23	*vittatus*	Sucre: Chacopata	199G
24	*vittatus*	Sucre: Las Trincheras. Yoroco	91G
25	*vittatus*	Sucre: posada La Alquimia	207G
26	*vittatus*	Sucre: Balneario Rio Guyana	218G
27	*vittatus*	Sucre: Rio Grande Arriba	205G, 206G, 217G
28	*vittatus*	Sucre: carretera Guiria Macuro	216G
29	*vittatus*	Nuevas Esparta: Isla de Margarita, Copey	10G, 11G
30	SMLB	Trujillo: Betijoque	160G, 161G
31	SMLB	Merida: Carretera Las Mercedes	226G, 229G

Locality #	Taxon	Locality	Sample code
32a	SMLB	Merida: Carretera La Azulita – Santa Elena	227G, 228G
32b	SMLB	Merida: La Azulita	224G, 225G
33	SMLB	Merida: San Felipe	1G, 221G, 222G, 223G
34a	SMLB	Tachira: Rio La Blanca	155G, 158G
34b	SMLB	Tachira: Socorro	154G, 157G

The approximate location of these collecting localities is shown below.



APPENDIX B

SAMPLES USED FOR MOLECULAR WORK IN THE PHYLOGENY OF *GONATODES*

Species	**Locality**	**Sample code**
G. alexandermendesi	Venezuela: BO: Rio Uei, Sierra de Lema	137G, 138G
G. alexandermendesi	Venezuela: BO: Paragua	117G, 118G
G. alexandermendesi	Venezuela: AM: Jaua Tepui	116G
G. alexandermendesi	Guyana: Tukeit trail, Kaieteur N. Park.	149G, 151G
G. alexandermendesi	Guayana: Trail to Amu, Kaeieteur N. P.	148G
G. alexandermendesi	Guayana: Magdalen's Creek	146G
G. alexandermendesi	Guyana: Trib. Of Elinkwa River, Kaieteur N. P.	150G, 152G
G. annularis	French Guyana: Bakra Mountains	31G
G. annularis	Venezuela: DA: Isla Tobebuja	69G, 153G
G. annularis	Guyana: Berbice River	145G
G. annularis	Venezuela: BO: Sierra de Imataca	167G, 168G, 169G
G. annularis	Venezuela: BO: Rio Uei, Sierra de Lema	122G, 129G, 144G
G. albogularis	Venezuela: BA: Barinitas	41G
G. albogularis	Honduras	54G
G. sp. 1	Venezuela: AM: Pto. Ayacucho - Gavilan	127G, 143G, 170G, 171G
G. sp. 2	Venezuela: AM: Pto. Ayacucho - Gavilan	126G, 139G, 140G, 141G
G. hasemani	Peru: Lodge Pantiacolla, P.N. Manu	73G
G. hasemani	Brasil: Rondonia: Rio Formoso, Guajara-Mirim	105G
G. hasemani	Brasil: Amazonas:Rio Ituxi, Madeirera Scheffer	113G
G. daudini	St. Vincent, Union Island	119G
G. caudiscutatus	Ecuador: Esmeraldas	297G
G. vittatus	Venezuela: DF: Caracas	74G
G. petersi	Venezuela: ZU: Finca El Deseo	163G
G. sp. 4	Guyana	231G

Species	**Locality**	**Sample code**
G. humeralis	Brasil: Amazonas	115G
G. humeralis	Venezuela: DA: Isla Tobebuja	68G
G. humeralis	Guayana	52G
G. antillensis	Venezuela: Isla Los Roques	136G
G. concinnatus	Ecuador: Reserva Faunistica Cuyabeno	109G
G. concinnatus	Venezuela: BA: Barinitas	14G
G. ocellata	Tobago Island	9G, 189G
G. ceciliae	Venezuela: SU: Las Melenas	53G, 239G, 241G
G. ceciliae	Venezuela: SU: Rio Guayana	242G, 243G, 244G
G. ceciliae	Venezuela: SU: Guiria – Macuro	236G, 237G, 238G
G. seiglei	Venezuela: SU: Cueva del Guacharo	24G, 27G
G. seiglei	Venezuela: SU: Miraflores	34G, 35G, 37G
G. purpurogularis	Venezuela: BA: Calderas	89G, 272G
G. purpurogularis	Venezuela: BA: Altamira - Calderas	271G
G. purpurogularis	Venezuela: TA: Rio Negro	184G, 185G
G. taniae	Venezuela: AR: Rancho Grande	98G, 101G
G. falconensis	Venezuela: ME: Colon – La Grita	75G
G. falconensis	Venezuela: ME: Rio La Blanca	182G, 248G
G. falconensis	Venezuela: AR: Sector Riitos	172G, 173G, 177G
G. falconensis	Venezuela: FA: Qda. Cueva del Toro	84G, 85G
G. falconensis	Venezuela: GU: Cerro Platillon	133G, 249G, 250G
G. falconensis	Venezuela: YA: Cocorote	95G, 96G, 100G, 261G
G. falconensis	Venezuela: YA: Sierra de Aroa	258G
G. falconensis	Venezuela: YA: Fila de Jaiguao	252G, 256G, 259G

Species	**Locality**	**Sample code**
G. falconensis	Venezuela: PO: El Chorreron	40G, 90G
G. falconensis	Venezuela: TR: Flor de Patria – Santa Ana	174-176G, 178G, 179G
G. falconensis	Venezuela: ME: Rio Frio	251G
G. atricucullaris	Peru: Cajamarca	165G
G. sp. A	Venezuela: ME: La Azulita	77G, 262G
G. sp. B	Venezuela: MI: Guatopo	62G, 65G, 266G, 267G
G. sp. B	Venezuela: MI: Rio Urva	268G
G. sp. B	Venezuela: MI: La Elvira	264G, 265G
G. sp. C	Venezuela: SU: Las Melenas	246G
G. sp. C	Venezuela: SU: Las Trincheras	92G, 93G
G. sp. C	Venezuela: NE: Cerro Copey	8G, 12G, 38G
G. sp. D	Trinidad Island	131G, 188G
G. sp. E	Venezuela: GU: La Elvira	132G, 135G, 247G
L. xanthostigma	Nicaragua: Rio San Juan	110G

APPENDIX C

LOCALITIES USED IN THE ECOLOGICAL NICHE MODEL

Species	Locality	Latitude	Longitude
ceciliae	VEN:SU:Balneario Rio Guayana	10.5908	-62.9408
ceciliae	VEN:SU:carretera Guiria - Macuro	10.6511	-62.1517
ceciliae	VEN:SU:Las Melenas	10.6856	-62.6167
sp. C	VEN:SU:Yoroco	10.1831	-63.9356
sp. C	VEN:SU:Copey	10.9994	-63.9114
purpurogularis	VEN:BA:Calderas	8.9178	-70.4506
purpurogularis	VEN:TA:Rio Negro	7.6075	-72.1608
purpurogularis	VEN:BA:carretera Barinitas - El Cacao	8.7625	-70.4572
sp. B	VEN:MI:P.N. Guatopo	10.1975	-66.5153
sp. B	VEN:MI: P.N. Guatopo	10.1972	-66.4931
sp. B	VEN:MI: P.N. Guatopo	10.2161	-66.4853
sp. B	VEN:GU:Hda. La Elvira	9.9764	-66.3075
seiglei	VEN:SU:Cueva del Guacharo	10.1842	-63.5447
seiglei	VEN:SU:Miraflores	10.1725	-63.7044
taniae	VEN:AR:Rancho Grande	10.3494	-67.6847
taniae	VEN:AR:Los Riitos	10.3492	-67.7213
falconensis	VEN:YA:Fila Jaiguao	10.2003	-68.69
falconensis	VEN:YA:Sierra de Aroa	10.3367	-68.8319
falconensis	VEN:TR:carretera Santa - Flor de Patria	9.4569	-70.4258
falconensis	VEN:GU:Santa Rosa del Sur	9.9028	-67.5292
falconensis	VEN:GU:Cocorote	10.3283	-68.8192
falconensis	VEN:ME:La Azulita	8.7153	-71.4308
falconensis	VEN:TA:Rio La Blanca	8.0794	-72.2372
falconensis	VEN:ME:Rio Frio Alto	8.8578	-71.2939

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