UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

FILOGEOGRAFÍA DE LAS POBLACIONES DE *Campylorhynchus rufinucha* (AVES: TROGLODYTIDAE).

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

MAESTRO EN CIENCIAS BIOLÓGICAS

(SISTEMÁTICA)

P R E S E N T A

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MÉXICO, D. F. **ENERO 2007**

Esta tesis fue elaborada gracias a las siguientes fuentes de financiamiento: Becas de Posgrado de Excelencia, **Consejo Nacional de Ciencia y Tecnología (CONACYT)** y **DGEP-UNAM**. Apoyo para estancia en el extranjero 2005, **Posgrado en Ciencias Biológicas, UNAM**. Proyecto Fondo Sectorial **Semarnat-CONACYT C01-0265**.

El planteamiento, formulación y realización de este trabajo fue supervisado por mi comité tutoral, integrado por la **Dra. Blanca Estela Hernández Baños,** la **Dra. Gabriela Parra Olea** y el **Dr. Adolfo Gerardo Navarro Sigüenza**.

This project was also developed at the **Omland Laboratory**, Department of Biological Sciences, **University of Maryland Baltimore County**. Research was supported by **National Science Foundation grant DEB-0347083**. The Omland laboratory is a participant in the **Smithsonian Ornithology group**.

Este proyecto no hubiera sido posible sin la intervención de muchas personas, a todas ellas mi más infinito agradecimiento y mi reconocimiento:

A mi madre, a Anita y a mi familia, por estar siempre conmigo y apoyarme en todo sentido.
A la familia Sosa López, que me trataron como una parte de ellos e hicieron todo esto posible.
A mi tutor Dr. Adolfo Navarro, por ser la muestra viviente de que paciencia y constancia tienen un límite casi infinito. Gracias por no claudicar conmigo.
Al Dr. Kevin Omland por permitirme aprender de él y enseñarme nuevas formas de pensar. Gracias por la ayuda desinteresada a los estudiantes mexicanos.
A mi comité tutoral: Dra. Blanca Hernández, Dra. Gabriela Parra y Dr. Adolfo Navarro, por guiarme en cada etapa del desarrollo de este proyecto, por compartirme su conocimiento y por aguantarme.
A la Dra. Ella Vázquez y al Dr. Octavio Rojas, por sus valiosos comentarios, sugerencias y todos sus consejos.
A Roberto Sosa, Vicente Rodríguez, Samuel López y Adolfo Calderón, por su amistad y sufrir conmigo los piquetes de insectos, el calor extremo y mi forma de ser. A todas las personas de Chiapas, Oaxaca, Guerrero y Veracruz que nos permitieron trabajar en sus tierras. A todas las autoridades por su atención y los permisos otorgados.
A Nanda por su destreza y magia en la "cocina", gracias por compartir el pan, la sal y la suerte molecular. A los miembros del Omland Lab (UMBC) por todo su apoyo. A Blanca Hernández y Laura Márquez por permitirme usar sus laboratorios en el MZFC e IBUNAM.
A todos los colectores de aves que colaboraron directa o indirectamente a este proyecto.
A las colecciones científicas y curadores que nos dieron muestras de tejidos: John Klicka (UNLV), Mark Robbins y A. Townsend Peterson (KU), David Willard (FMNH), Sharon Birks (UWBM), Paul Sweet (AMNH), Robb Brumfield y Donna Dittman (LSU). Gracias a A. Townsend Peterson, James Dean y Paul Sweet por permitirme revisar sus colecciones en KU, USNM, y AMNH respectivamente.
A mis amigos Paula, Rodrigo, Yénika, Mitsue, Betsa, César, Héctor, Ayim, Laila, Tanya, Luis A., Erick, Gordillo, Fanny, Gaby, Magali, Iván, Bertha, Jerónimo, Chris, Beatrice, Jeff, Diana, Sandra, Sussette, Beto, Manolo, y Sara, por hacer la vida divertida y placentera.
A las fuentes de financiamiento que me permitieron hacer esta tesis.
A aquellos que deban estar aquí y que no haya recordado.

A las personas que quiero.

A los colectores de aves.

A los capichochos, teconchis, pachecos, arroceros, chancuacos, chupahuevos, cucarachitas, gualcachías y cucaracheros que donaron su DNA y alma.

Nos cuenta la señora Josefina Gutiérrez López en la localidad “La Chole”, La Soledad, Petatlán, Guerrero, lo siguiente:

... cuando el Señor les dio el canto a las aves,

el Capichocho brincaba y brincaba a su alrededor

pidiéndole un nombre y un canto con mucha insistencia.

Esto desesperó al Señor y le dijo que dejara de estar molestando.

Cansado, el Señor le dijo al Capichocho que cantara como se le diera la gana,

Por eso son muy groseros y dicen:

“Capichocho, capichocho, capichocho, ya te vi, ya te vi, ya te vi”

(Nota: este escrito no está relacionado con las inclinaciones espirituales del autor de la tesis).

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ÍNDICE DE CUADROS Y FIGURAS

INTRODUCCIÓN

Dentro de las regiones más biodiversas del planeta se encuentra el Neotrópico, y con respecto a las aves, es la región con el mayor número de especies (aproximadamente unas 3715, Navarro y Sánchez-González 2003). La formación de especies en aves es un proceso que ocurre generalmente por el aislamiento y divergencia de poblaciones (Newton 2003). Con el uso de caracteres morfométricos, moleculares y de vocalizaciones, entre otros, se ha podido documentar que varias poblaciones de aves neotropicales, a pesar de ser similares en apariencia, están diferenciadas (Isler *et al.* 2002).

El estudio de las diferencias fenotípicas y genotípicas en las poblaciones de una misma especie a lo largo de su distribución es conocido como variación geográfica (Futuyma 1998). Varias inferencias evolutivas, como la selección natural y la diferenciación alopátrida, entre otras, y su efecto en las poblaciones, se han realizado comúnmente por medio de la variación morfológica. Por otra parte, puede no existir variación en la morfología o es difícil de percibir. Debido a ello y a otros factores, se ha intensificado recientemente el uso de métodos moleculares que puedan revelar la asociación entre la geografía y la variación genética. Con los patrones de variación genética se pueden hacer inferencias sobre los procesos evolutivos en la fragmentación de poblaciones y el papel del flujo génico en poblaciones estructuradas (Zink 1994).

Una de las formas en que puede estudiarse la variación genética de las especies y sus poblaciones es la filogeografía, la cual analiza los principios y procesos que gobiernan la distribución geográfica de los linajes genealógicos, utilizando componentes históricos y filogenéticos de la distribución espacial de los linajes de genes (Avise 2000). Por sus profundas raíces en la biogeografía histórica y la genética de poblaciones se le ha llegado a considerar como puente entre los procesos micro y macroevolutivos (Bermingham y Moritz 1998).

La existencia de una amplia variación genética en diferentes especies de aves de México y Centroamérica (Honey-Escandón 2002, Baker *et al.* 2003, Cortés-Rodríguez 2003, García-Deras 2003, González *et al.* 2003, García-Moreno *et al.* 2004), está relacionada principalmente con su aislamiento geográfico y con la compleja historia geológica de Mesoamérica. El complejo de la matraca nuca rojiza *Campylorhynchus rufinucha/capistratus*, presenta una marcada variación morfológica y una amplia distribución que abarca desde el estado de Colima en México por la vertiente del Pacífico hasta el norte de Costa Rica y presenta una población aislada en el centro del estado de Veracruz (Selander 1964, AOU 1998).

A pesar de las evidentes diferencias morfológicas entre las subespecies, Selander (1964, 1965) reporta que existe una estrecha área de hibridización entre *C. r. humilis* y *C. r. nigricaudatus*,

ubicada en el Istmo de Tehuantepec. Los valores de hibridización de Selander (1964, 1965) muestran formas morfológicamente intermedias entre esas subespecies. La reciente filogenia molecular del género *Campylorhynchus* (Barker 1999), no abarcó la mayoría de la distribución de esta especie ni la zona de hibridización. No se conoce con certeza si dicha área representa una zona de contacto secundaria de dos formas que han evolucionado independientemente o si se trata de un continuo con formas graduales intermedias a lo largo de toda la distribución. Por otro lado, la monofilia de *C. rufinucha* quedó parcialmente demostrada (Barker 1999), ya que no se muestreó la población disyunta de Veracruz y las relaciones filogenéticas de dicha población todavía no se han analizado.

El uso de marcadores moleculares ha permitido estudiar la hibridización ya que éstos proveen de más caracteres que la morfología por sí sola. Se han descrito zonas híbridas no conocidas para diferentes especies y se ha podido caracterizar la extensión de dichas zonas, así como la introgresión de genes entre especies (Masta *et al.* 2002). Dentro de los marcadores moleculares, los mitocondriales son los más usados en estudios filogeográficos debido a que el genoma mitocondrial es haploide y sus genes son más variables que los nucleares (Avise 1998, Bermingham y Moritz 1998).

UBICACIÓN FILOGENÉTICA

El complejo taxonómico *Campylorhynchus rufinucha* está ubicado dentro de la familia Troglodytidae (matracas), en el orden Passeriformes, la cual comprende desde 75 hasta 90 especies dependiendo de la taxonomía utilizada, distribuidas en Europa, Asia y América (Brewer y MacKay 2001). La diversidad más alta de especies ocurre en el Continente Americano, donde el mayor número se ubica cerca del Ecuador y va disminuyendo conforme se acerca a los polos. Brewer y MacKay (2001) reconocen 15 géneros, donde sólo una especie, *Troglodytes troglodytes*, tiene distribucion holártica. Las relaciones filogenéticas de dicha familia reconstruidas usando hibridización de DNA-DNA (Sibley y Ahlquist 1990) y usando secuencias de DNA (Barker 2004, Barker et al. 2004) muestran que su grupo hermano es la familia Sylviidae (perlitas), y la familia Certhiidae (trepadores) es hermana a esas dos. La única diferencia entre dichas técnicas moleculares es que Barker (2004) excluye al género *Donacobius* de la familia Troglodytidae.

El género *Campylorhynchus* contiene 13 especies reconocidas (Barker 1999, Brewer y MacKay 2001, aunque Selander (1964) incluyó a *C. chiapensis* como subespecie dentro de *C. griseus*, reconociendo sólo 12 especies). Todas se distribuyen en el Continente Americano, de las cuales 12 son enteramente neotropicales, cinco son endémicas a México (*C. jocosus*, *C. gularis*, *C.*

yucatanicus, C. chiapensis y *C. megalopterus*), cuatro a Sudamérica (*C. griseus, C. turdinus, C. nuchalis* y *C. fasciatus*) y *C. rufinucha* a Mesoamérica; dos especies se comparten entre Mesoamérica y el norte de Sudamérica (*C. albobrunneus* y *C. zonatus*) y una se comparte entre México y el suroeste de Estados Unidos (*C. brunneicapillus*).

Las relaciones filogenéticas dentro del género *Campylorhynchus* (Barker 1999, Fig. 1) muestran al complejo *C. rufinucha/capistratus* como monofilético, en un clado estadísticamente bien soportado con *C. yucatanicus, C. chiapensis, C. griseus, C. jocosus* y *C. gularis* en todas las reconstrucciones hechas con distintos métodos filogenéticos. Con relación a otros troglodítidos, el género *Campylorhynchus* es un grupo monofilético con *Thryomanes* y con *Thryothorus ludovicianus*, hermano a otro clado monofilético con varias especies de *Thryothorus, Henicorhina, Cyphorhinus y Cinnycerthia* (Barker 2004, Fig. 2).

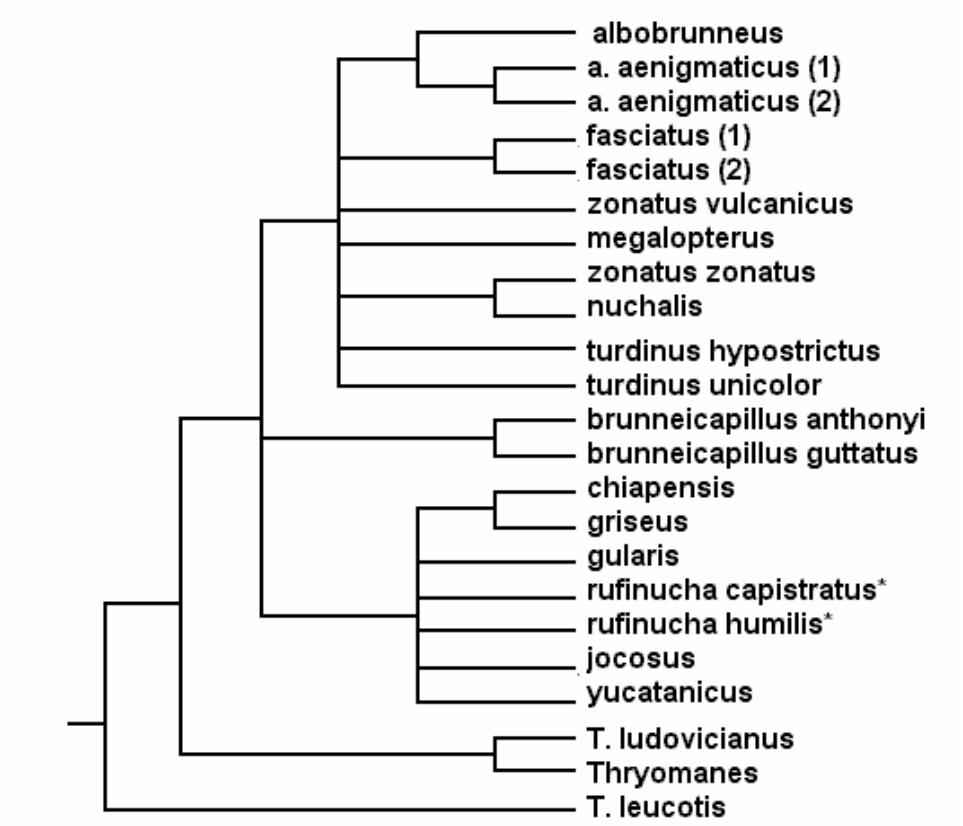

Fig. 1. Relaciones filogenéticas de las especies del género *Campylorhynchus* (Barker 1999). El cladograma es un consenso estricto de 145 árboles con la misma longitud, el cual no contradice ninguna de las otras hipótesis de relación entre sus especies obtenidas por otros métodos filogenéticos.

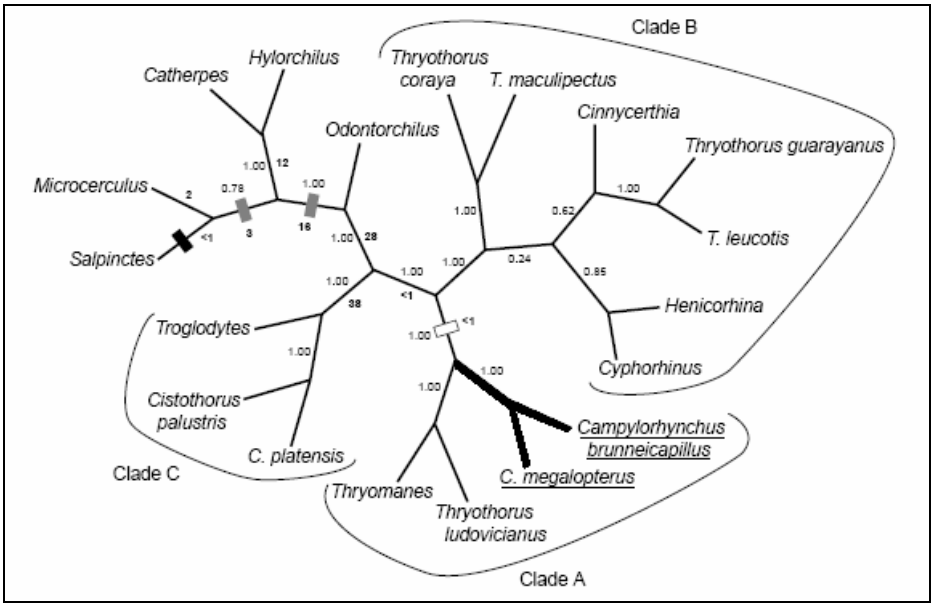

Fig 2. Posición filogenética del género *Campylorhynchus* dentro de la familia Troglodytidae. Las especies de dicho género se encuentran resaltadas por líneas más gruesas y subrayadas. Modificado de Barker (2004).

En este trabajo, exploré la variación genética dentro del complejo taxonómico *Campylorhynchus rufinucha.* Usé diferentes métodos filogenéticos para reconstruir las relaciones evolutivas entre los diferentes haplotipos. Por medio de parámetros de génetica de poblaciones, estimé valores de diferenciación poblacional. Finalmente, comparé la filogenia obtenida con la de otros taxones para buscar patrones biogeográficos en la región.

TAXONOMÍA Y VARIACIÓN GEOGRÁFICA

El complejo *C. rufinucha*, distribuido por la costa del Pacífico en el oeste de México hasta el norte de Costa Rica, con una población disyunta en Veracruz (Fig. 3). Comprende entre una y tres especies con varias subespecies (Fig. 4) con una longitud corporal entre 15 y 19 cm (Brewer y MacKay 2001). El trabajo más amplio sobre la variación del complejo (Selander 1964) reconoce tres formas distintas (Fig. 5), dos con una sola subespecie y una con dos. Las tres formas distintas son tomadas como tres especies evolutivas diferentes por Navarro y Peterson (2004).

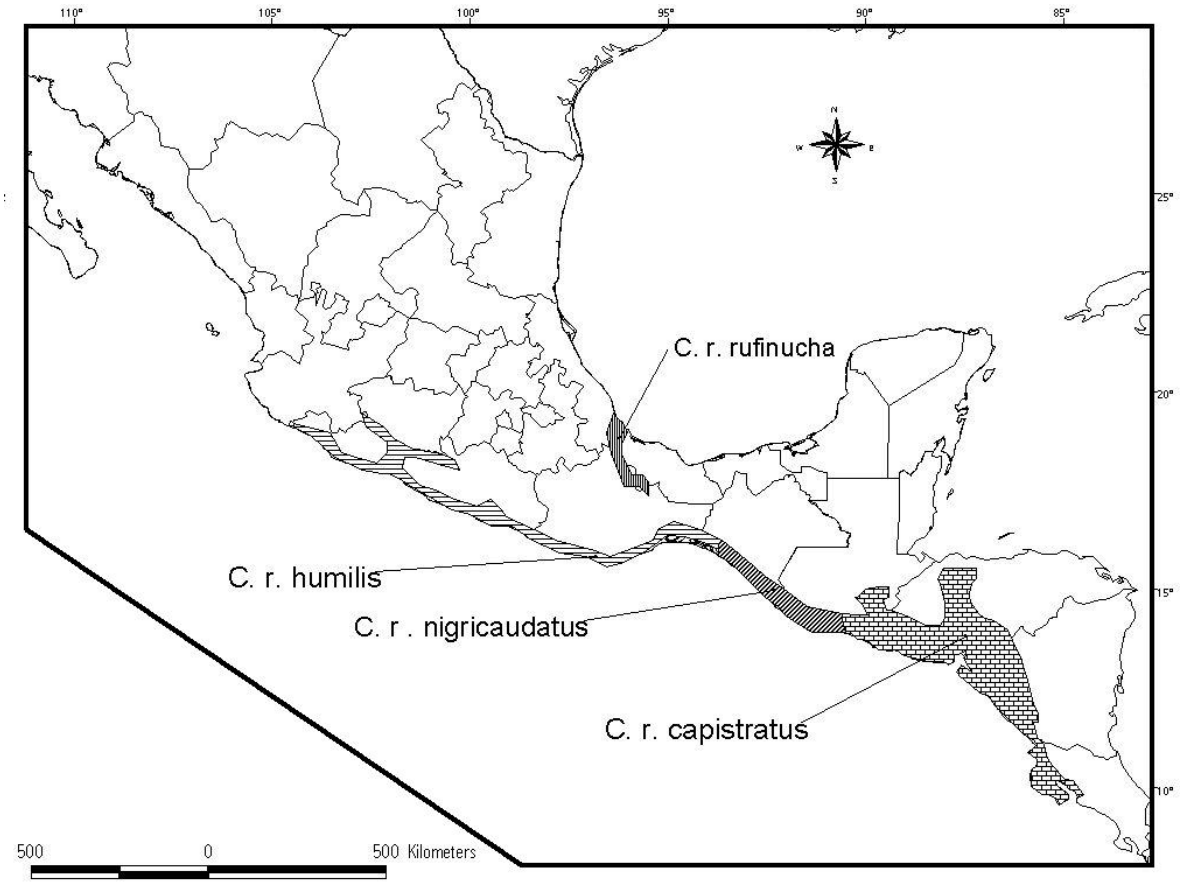

Fig. 3. Distribución del complejo *C. rufinucha sensu* Selander (1964) con las subespecies correspondientes. Navarro y Peterson (2004) mencionan a cada una como especies evolutivas, considerando a las más sureñas como una sola.

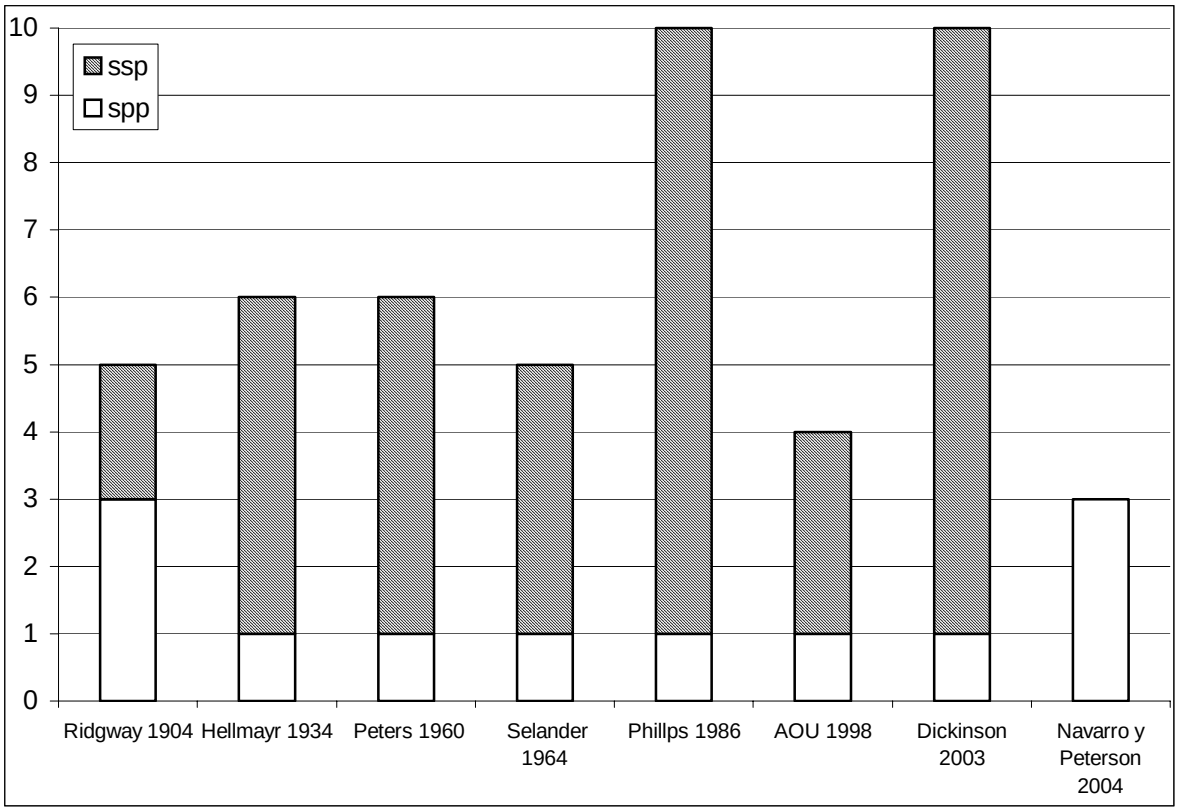

Fig. 4. Taxonomías del complejo *C. rufinucha* en orden cronológico. Las barras blancas representan las especies reconocidas, las barras grises las subespecies. AOU (1998) considera un complejo taxonómico con tres grupos. Navarro y Peterson (2004) utilizan el concepto evolutivo de especie, las demás autoridades el concepto biológico.

La taxonomía de este complejo es muy variable, por lo que para el presente trabajo se considera que el complejo es una sola especie, *Campylorhynchus rufinucha***,** con varias poblaciones distribuidas en la Vertiente Pacífica de Mesoamérica y una población disyunta en Veracruz.

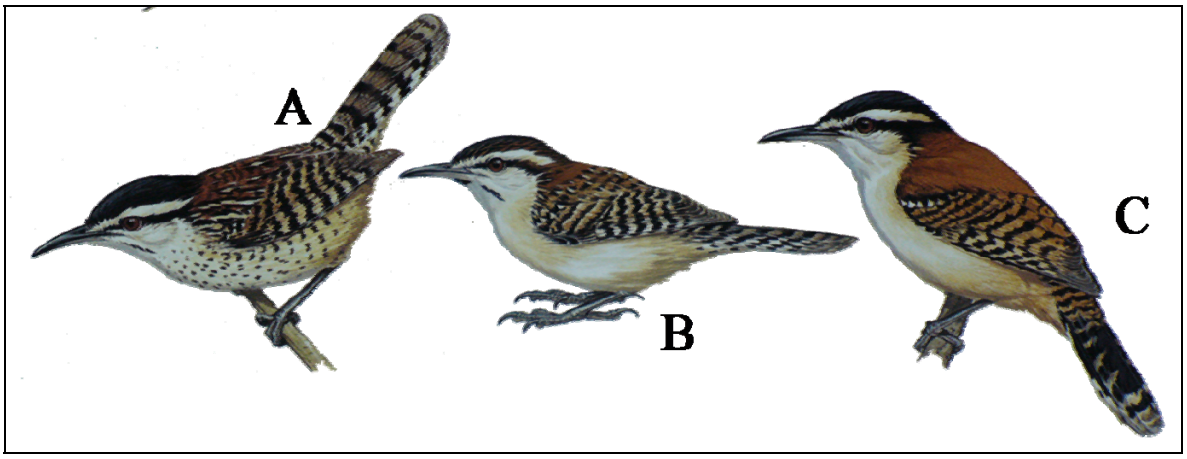

Fig. 5. Variación morfológica dentro del complejo *C. rufinucha sensu* AOU (1998). A se refiere a *rufinucha*, B se refiere a *humilis* y C se refiere *capistratus*. Tomado de del Hoyo et al. (2005).

OBJETIVOS

Objetivo general

Reconstruir las relaciones filogenéticas de las poblaciones del complejo *Campylorhynchus rufinucha/capistratus.*

Objetivos particulares

- Determinar si dicho complejo es monofilético.
- Reconstruir los patrones filogenéticos y filogeográficos de las poblaciones de dicho complejo.
- Analizar la variación genética intra e interpoblacional con los genes mitocondrial (ND2).
- Determinar si existe relación entre la variación genética y morfológica en términos geográficos.
- Comprobar si existe una zona de hibridización entre Chiapas y Oaxaca.
- Caracterizar genéticamente la naturaleza de dicha zona de contacto.

PHYLOGEOGRAPHY OF THE RUFOUS-NAPED WREN COMPLEX (*CAMPYLORHYNCHUS RUFINUCHA*): SPECIATION AND HYBRIDIZATION IN MIDDLE AMERICA

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ABSTRACT.

The Rufous-naped Wren (*Campylorhynchus rufinucha*) is a highly sedentary, morphologically variable species complex distributed in the dry forests of Mesoamerica. It ranges from Colima, Mexico south to Costa Rica along the Pacific slope, with a disjunct population in central Veracruz. Populations of two morphotypes on the Pacific slope intergrade in Chiapas, apparently as a result of a secondary contact zone. We used mtDNA to explore phylogeography and hybridization in this species complex. We found three divergent lineages, corresponding to the two sides of the Isthmus of Tehuantepec plus the disjunct Veracruz population. F statistics and AMOVAs also are consistent with genetically distinct populations. However, the geographic distribution of haplotypes shows secondary gene introgression from west of the Isthmus to eastern populations. Additional morphological and behavioral evidence also support the existence of independent evolutionary lineages. We recommend recognizing three distinct species, two of which hybridize in a narrow contact zone.

RESUMEN

El Chancuaco (*Campylorhynchus rufinucha*) es un complejo de especies con variación morfológica marcada, muy sedentario, asociado a las selvas secas de Mesoamérica. Se distribuye en la vertiente del Pacífico desde Colima, México a Costa Rica, con una población disyunta en el centro de Veracruz. Las poblaciones de dos morfotipos se sobrelapan en el extremo oeste de la costa de Chiapas, aparentemente en una zona de contacto secundario. Usamos DNA mitocondrial para explorar la filogeografía y la hibridización en este complejo de especies. Encontramos tres linajes divergentes, correspondientes al Istmo de Tehuantepec y la población disyunta de Veracruz. Los estadísticos F y las pruebas de AMOVA también son consistentes con poblaciones genéticamente distintas. Sin embargo, la distribución geográfica de los haplotipos muestra introgresión secundaria

de genes de las poblaciones del Oeste del Istmo al Este. Evidencias adicionales, tanto morfológicas como conductuales también apoyan tres linajes evolutivamente distintos. Recomendamos reconocer tres especies de reciente divergencia, dos de las cuales hibridizan en una zona de contacto estrecha.

INTRODUCTION

Speciation, defined as the formation of new species (Wiley 1981) is a central focus of evolutionary biology (Hewitt 2001). The most commonly observed mechanism for speciation is the geographical isolation of populations (Newton 2003), commonly characterized by vicariance or by dispersal (Chesser and Zink 1994). Mexico and Central America (known as Mesoamerica) contain a wealth of geographical barriers, such as isthmuses and extensive mountain chains (Ferrusquía 1993), granting this region notable altitudinal gradients and a large number of fragmented areas, resulting in an impressive number of species and endemic taxa. Physiographic and climatic fluctuations over geological time have also provided this region a wide variety of habitats for new forms to occupy (Graham 1993). Bermingham and Martin (1998, and references therein) emphasized the influence of the Panamanian Isthmus on the evolution of Neotropical biota. However, speciation of Mesoamerican taxa near the Isthmus of Tehuantepec has been only recently explored from a molecular perspective (García-Paris et al. 2000, Mulcahy and Mendelson 2000, Sullivan et al. 2000, Castoe et al. 2003, García-Moreno et al. 2004, Hasbún et al. 2005, García-Moreno et al. 2006).

Mesoamerican birds show high levels of endemism, and many species display geographic variation (Navarro and Sánchez-González 2003). The wrens (family Troglodytidae), are among the most speciose groups of Neotropical insectivorous perching birds. They are characterized by a sedentary behavior and complicated social structure (Brewer and MacKay, 2001). The species in the genus *Campylorhynchus* are the largest members of the family and are among the best known cooperative breeders in birds (Rabenold 1990), having Mesoamerica as their center of diversification (Brewer and MacKay 2001). One typical species in this group is the Rufous-naped Wren *Campylorhynchus rufinucha*, a tropical dry forest specialist. The highly marked morphological and song variation exhibit by this wren (Selander 1965) has resulted in a wide variety of different taxonomies. Some recognize it as three different species (Ridgway 1904; also Navarro and Peterson 2004, using the evolutionary species concept, ESC). Others recognize a species complex (AOU 1998), comprising a single species with a variable number of subspecies (Hellmayr 1934; Peters 1960, Selander 1964; Phillips 1989; Dickinson 2003) based on the biological species concept (BSC). The official AOU

Checklist (AOU 1998) recognizes three groups (in here referred as morphotypes). Selander recognized four subspecies (1964). The species complex is distributed from Western Mexico to Northwestern Costa Rica on the Pacific slope and an isolated population on the plains of Central Veracruz near the Gulf of Mexico (Fig. 1).

These discrepancies among taxonomies stem from opposite points of view. Initially, conspicuous morphological differences among the three morphotypes granted them the status of full species (Ridgway 1904). The morphological variation in this species or group of species, as well as its ecology and distribution, was extensively studied by Selander (1964). Using nine morphometric and six color characters, Selander encountered deep gaps between morphotypes. However, he discovered intermediate individuals that he showed graphically as an nearly perfect morphometric cline. Museum specimens within this cline were scored as hybrids using trait index scores. These specimens come from a highly localized zone in southwestern Chiapas (Fig. 1) (Selander 1964, further surveyed in Selander, 1965). Selander's results can be briefly summarized as follows: extensive and marked morphological differentiation exists within this wren complex; nevertheless, there is strong morphometric evidence of hybridization between two of the subspecies. (Note that he did not analyze these data using multivariate statistics.)

Morphometric techniques are useful approaches to assess geographic variation. Currently, other approaches use molecular markers. Many species that exhibit noticeable morphological differences also display deep genetic variability. A way to analyze such variability is phylogeography, defined as the study of within species genetic variation in a geographic context (Avise et al. 1987). It is used to study variation from the population and individual levels to speciation and phylogeny of closely related species (Avise 2004), suggesting that phylogeography could be the link to address many issues in systematics and population genetics (Avise et al. 1987). Phylogeography relies on both phylogenetic analyses and genetic statistics, although the degree on which each approach is used varies largely, either differentiating phylogeography from species level historical biogeography or considering them as synonyms (Zink 2002). The "phylogeographic explosion" of studies in the last decade has ranged from approaches to genetic differentiation, comparing histories of different taxa, to examing hybridization events among lineages (Hewitt 2001). The significance of hybridization to evolution has been issue of debate in many fields of study,

especially with regard to speciation and species recognition (Barton 2001). Then, phylogeography is a useful approach to study hybridization and species limits in many different taxa (e.g. Cooley et al. 2001, Walton et al. 2001, Pierpaoli et al. 2003, Vianna et al. 2006) including birds (e.g. Saetre et al. 2001, Brumfield 2005).

In recent years phylogeographic studies of wrens with wide distributions and morphological variation show that genetic patterns are heavily influenced by geography (González et al. 2003; Drovetski et al. 2004). However, hybridization has not yet been studied from such perspective in this group of birds. Due to its interesting geographic distribution, controversies surrounding taxonomic issues and the possibility of hybridization, we set out to study the Rufous-naped Wren complex.

We had four main goals in this study: 1) use phylogenetic trees and networks to investigate relationships among populations; 2) analyze population genetics parameters to assess degrees of population differentiation; 3) determine whether gene introgression among populations has happened as suggested by morphology; and 4) compare speciation patterns in this wren to other taxa to explore general historical biogeographical patterns in Mesoamerica.

METHODS

Specimen collecting and tissue loans

We collected wren specimens from across the species range in Mexico. These specimens are deposited at the Museo de Zoología "Alfonso L. Herrera", UNAM (MZFC, see Appendix 1). Muscle, heart and liver samples were preserved either in pure ethanol or cryogenically frozen in liquid nitrogen. Tissue loans given from Mexican and US bird collections from Central American populations were stored in ethanol. We also obtained samples of additional *Campylorhynchus* outgroup species. To increase the sample size for the Veracruz population, we used skin samples from three museum specimens, giving a total of 129 Rufous-naped Wren individuals and eight outgroups (see Appendix 1).

Lab procedures and protocols

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METHODS

Specimen collecting and tissue loans

We collected wren specimens from across the species range in Mexico. These specimens are deposited at the Museo de Zoología "Alfonso L. Herrera", UNAM (MZFC, see Appendix 1). Muscle, heart and liver samples were preserved either in pure ethanol or cryogenically frozen in liquid nitrogen. Tissue loans given from Mexican and US bird collections from Central American populations were stored in ethanol. We also obtained samples of additional *Campylorhynchus* outgroup species. To increase the sample size for the Veracruz population, we used skin samples from three museum specimens, giving a total of 129 Rufous-naped Wren individuals and eight outgroups (see Appendix 1).

Lab procedures and protocols

We extracted genomic DNA from muscle and liver using a phenol-chloroform protocol and using DNeasy kits (Qiagen) following manufacturer instructions. Polimerase chain reaction (PCR) products from the first half of the mtDNA gene NADH2 (ND2) were amplified using the forward primer 5'-TATCGGGCCCATACCCCGAAAAT-3' (Hackett 1996) and a specific reverse primer 5'-GGAGATKGAGGAGAAGGCTA-3' (designed in PRIMER3 http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi, by J. Peters). We amplified DNA using the following PCR protocol: initial phase at 92°C for 3 minutes, denaturing at 92°C for 1 minute, annealing at 50°C for 1 minute, elongation at 72°C for 1 minute, with a final extension at 72°C for 3 minutes for 38 cycles. The skin samples were processed in a molecular genetics lab in a different building (see ACKNOWLEDGEMENTS) using brand new reagents to avoid contamination following a similar PCR protocol. PCR products were purified using QiaQuick columns (Qiagen) following manufacturer instructions. We used BigDye 3.1 termination reaction and its sequence cycle profile (Applied Biosystems) following recommended guidelines. Excess sequencing reaction reagents were eliminated using ethanol/EDTA precipitation (Applied Biosystems) technique for microfuge tubes using suggested directions. The sequencing process was done in an ABI 3100 sequencer, with assembly carried out using Sequencher 4.5 (Gene Codes Co. 2005). The ND2 protein coding gene has no indels, therefore it was easy to align.

Phylogenetic analyses

We used NETWORKS 4.1.1.1 (http://www.fluxus-technology.com) using the median joining algorithm, Bandelt *et al.* 1999), to obtain a parsimony (MP) network, with all groups joined. Parsimony trees with 1000 replicates bootstrap support were constructed in NONA 2.0 (Goloboff 1993) with WinClada 0.9.99 (Nixon 2000) using TBR algorithm with additive characters and 100 replicates keeping 10 initial trees. Using PAUP* 4.10 (Swofford 2002) maximum likelihood (ML) trees were constructed using TBR algorithm with 100 replicates keeping one initial tree using a GTR+I+G model selected by hierarchical Likelihood Ratio Test (hLTR) in MODELTEST 3.7 (Posada and Crandall 1998). We also calculated models by codon to obtain a partitioned data set. First and second codon

positions acted under a JC model, and the third codon positions acted under a GTR+I+G model. To estimate bayesian (BY) trees and posterior probabilities we used the partitioned data set in MRBAYES 3.1 (Huelsenbeck and Ronquist 2001) with flat prior probabilities, two runs and four chains each for 2,000,000 generations sampling every 100th generation.

Genetic determination of possible hybrids

We determined hybrid individuals by comparing mtDNA and morphology. Using our mtDNA trees, we compared voucher specimen's morphotype (Fig. 1) to the phylogenetic position of their mtDNA. If there is geographic and morphologic structure on the tree, haplotype placement and morphology should match. A lack of correspondence between morphotype of the specimen and its mtDNA assignation was considered evidence of hybridization (as employed by Brumfield 2005, such specimens referred from this point forth as mtDNA hybrids).

Population delimitation and genetic parameters

From the 129 Rufous-naped Wren ingroup samples we delimited 12 populations based on the following criteria. We lumped all localities within a 10 km radius, then considered all populations containing eight or more individuals (localities with fewer than four individuals were discarded, Fig. 5). For each of the 12 populations included, we inferred haplotype (h) and nucleotide diversities (π), pairwise F statistics and exact test of population differentiation (ETPD, Raymond and Rousset 1995) in ARLEQUIN 3.01 (Excoffier et al. 2005). We defined three groups of populations for an analysis of molecular variance (AMOVA, Excoffier et al. 1992) design based on each sample's correspondence to any of the three main morphotypes (A, B or C, Fig. 1). Then we calculated two AMOVA tests. The first excluding putative hybrid populations and the second including all populations. All statistics were calculated with a significance cutoff at $p<0.05$.

RESULTS

Phylogenetic analyses

The final editing of the sequences yielded a 547 bp product of ND2 for all 137 samples. We detected 35 unique haplotypes from the 129 Rufous-naped Wren samples. The haplotype network (Fig. 2) shows five main haplotype groupings (I-V) with the following correspondence to morphology (Fig. 1):

1) Morphotype C samples corresponds to haplotype group "I", containing all the samples from the east of the Isthmus of Tehuantepec from Mexico and three from Guatemala; and haplotype group "II", including all samples from Nicaragua, El Salvador, Costa Rica and two from Guatemala (Central America).

2) Morphotype A samples correspond to haplotype group "III" from the isolated Veracruz population.

3) Morphotype B samples: haplotype group "IV" has all individuals from the center of the Isthmus of Tehuantepec and five individuals from Laguna la Joya populations in Chiapas Mexico; and haplotype group "V" contains all the haplotypes from the west Pacific coast of Mexico.

Mapping the five different haplotype groups ("I-V") on their corresponding localities (Fig. 3), reveals strong geographic structuring. The geographically isolated group "III" does not show evidence of overlap with any other group. Similarly, none of the localities belonging to "V" show intergradation with any other haplotype group. In contrast, the mixture of different haplotype groups in the same localities occurs in two places: "IV" intergrades with "I" in the Laguna la Joya (Chiapas Mexico), and "I" haplotypes are mixed with "II" in the westernmost part of Guatemala.

The MP, ML and BY trees all recovered the Rufous-naped Wren populations as monophyletic in mtDNA relative to the eight outgroup species. The BY tree (Fig. 4a) has a –LnL of 2309.482 with a sequence divergence Standard Deviation of 0.006. (The ML tree had a –LnL of 2429.9004, with the same topology as the BY tree, therefore is not shown.) The MP tree (Fig. 4b) is a strict consensus of 24 equally parsimonious cladograms with a length of 600 steps, CI of 0.57, and RI of 0.75. The ML scores estimated in PAUP for all

trees show a non significant superiority of the ML tree (SH test $p>0.4$, Shimodaira and Hasegawa 1999) over the MP and BY trees. Similarly, the MP length scores for the ML (601) and BY (602) trees show little superiority of the MP tree.

There are well-supported similarities in all trees. The BY (Fig. 4a) and the MP (Fig. 4b) generally resemble the haplotype network (roman numerals depict haplotype groupings, Fig. 2). In both trees, haplotype groups "III", "IV", and "V" form a well supported clade. Haplotype groups "I" and "II" are more distantly related to the other three groups. These two mentioned sets of haplotype groupings correspond to a geographic distribution west of the Isthmus of Tehuantepec and east of that isthmus, respectively. Haplotypes in "I", "III" and "IV" are monophyletic in both trees with high posterior probabilities and bootstrap support. Each specimen had the same morphotype assignment as described for the network (Fig. 2). Every specimen from "III" belongs to morphotype A. Specimens having haplotypes from "I" and "II" all belong to haplotype C. All specimens from "V" and "IV" belong to morphotype B, with the exception of haplotype *h*23 individuals (marked with and asterisk *) with belonging to morphotype C.

However there are some inconsistencies between the trees within the haplotype groups. These inconsistencies are not well-supported. In the BY tree (Fig. 4a), "V" haplotypes are a paraphyletic grade relative to "III" and "IV" in an unresolved polytomy with low posterior probabilities. In contrast, in the MP tree (Fig. 4b), haplotype group "V" individuals are monophyletic, although with low bootstrap values. However, parsimony shows haplotypes in "II" are paraphyletic to haplotypes in "I". Minor differences are also low supported.

Hybrid individuals determined by mtDNA

The vast majority of specimens demonstrated an agreement between morphology and their phylogenetic (Figs. 4a,b) and network (Fig. 2) positions. However, several specimens did not. Five individuals in three localities near the lagoon "Laguna la Joya" in Chiapas Mexico, which should match group "I" (based on plumage) were nested in group "IV". All individuals collected for this study from the Laguna la Joya surroundings display a clear morphological correspondence to morphotype C (Fig. 1). From this comparison, we were

able to determine five mtDNA hybrids (see asterisk [*] marked samples in the Appendix 1). These hybrid individuals are restricted to the Laguna la Joya (Fig. 5). In addition, two specimens from Retalhuleu (Guatemala) correspond to group "II" and one nests within group "I". We were not able to inspect the voucher specimens for the samples from Guatemala we used. This fact and the low sample sizes did not allow us to compare morphology and genetics to better understand these Guatemala samples.

Phylogeographic patterns and genetic diversity

Descriptive statistics. Samples determined to be mtDNA hybrids are all included in the JOY population (for codes see Fig. 5). The population with the highest haplotype diversity is MAN, followed by JOY, NIC and SAL. Since all individuals from TUX and TAP populations were identical they showed no diversity (Fig. 6A). A similar pattern is observed in the π diversity. However, the JOY hybrid population in this case had the highest nucleotide diversity (Fig. 6B).

Population differentiation. In the two AMOVAs more than half of the variation is explained by differences among morphotypes (Fig. 7). The second largest source of variation is differences between populations among morphotypes, and the third source is within population variation. The relative level of source contribution for total variation does not change much in both AMOVAs. Variation among morphotypes decreased about 7% when including the JOY hybrid population, and the variation within populations increased 8%. Variation due to differences between populations among morphotypes remained similar in both tests. The fixation index excluding hybrids ($\Phi_{st} = 0.96$, $p<0.00001$) shows almost complete genetic differentiation among groups. Even including the mtDNA hybrids the result still shows high genetic differentiation ($\Phi_{st} = 0.88$, $p<0.00001$).

Pairwise ETPD ($p<0.00001$, Table 1B) results demonstrate that Φ_{st} values (Table 1A) between VER and all the other populations are significant. Comparing populations between distinct haplotype groups (I-V, Fig. 2), all the Φ_{st} differences are also significant. Comparing populations in the same haplotype group, populations in group "I" have very low levels of genetic differentiation and are not significantly different. Populations

belonging to group "V" have low to intermediate levels of genetic differentiation. Those differences are significant comparing populations on either side of group "V" distribution but none exist between close populations. There are no differences between populations within group "IV". Comparing all populations with JOY, all Φ_{st} are intermediate and significant, except for Φ_{st} values between JOY and "I" populations. Between morphotypes, there are large levels of differentiation between morphotypes (p uncorrected distances). Between A and B is 2.9%, for A and C is 4.6% and B and C there is 3.6%.

DISCUSSION

Phylogenetic relationships and patterns among morphotypes.

Our mtDNA haplotype network (Fig. 2) shows three divergent groups, which correspond to the three main divisions of the AOU (1998), or three different previously proposed species (Ridgway 1904, Navarro and Peterson 2004). Despite the differences in topologies, all trees show that morphotypes A and B are sister taxa and morphotype C is sister to A and B morphotypes. There is morphological correspondence with that phylogenetic relationship. Both A and B morphotypes are smaller than morphotype C, have whiskers and patterned backs, underparts and tails (Fig. 1). Morphometric differences (Selander 1964) are similar to our differentiation Φ_{st} values among morphotypes. When morphological and molecular changes coincide they are considered to be correlated (Omland 1997). There are cases of deep genetic divergence within low or null phenotypic variation (reviewed in Avise 2000, Omland et al. 2000) and others with marked morphological variation and little genetic differentiation (Baker et al. 2003, Kondo et al. 2004). Ecological adaptations and selection of behavioral traits (such as female preference) are commonly used to explain those cases (Hofmann and Omland 2006). Our AMOVAs showed that more that half of the variation is explained by differences among morphotypes. This means that the morphological variation in this species complex has an important genetic base, although it is very likely that ecology played a significant role for this group variability (Selander 1964). Other neotropical wrens also show morphology and molecular coupling, reflecting taxonomic propositions (González et al. 2003). That is not the case of the holarctic wrens (Drovetski et al. 2004). It

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(González et al. 2003). That is not the case of the holarctic wrens (Drovetski et al. 2004). It is possible that holarctic taxa have a higher tendency to cryptic variation than do neotropical species, although more comparative data is needed to detect a consistent pattern.

We found species level paraphyly in our trees. This paraphyletic pattern occurs in both widespread and restricted-range species across the world (Omland et al. 2000). Paraphyly and polyphyly in mtDNA for several taxa appears to be a common phenomenon in nature (Funk and Omland 2003). The Band-backed Wren (*Campylorhynchus zonatus*) is paraphyletic to the Grey-barred Wren (*C. megalopterus*) in mtDNA (Barker 1999). Mann et al. (2006) reported the first case of this pattern for genus level in the family Troglodytidae. The reasons for this phenomenon seem to be gene flow, hybridization or incomplete lineage sorting (see Funk and Omland 2003 for references and detailed discussion).

Geographic patterns and secondary contact

The statistical results indicate that there is genetic differentiation among populations from different haplotype groups, and those differences are significant. The distribution of haplotypes along coastal Pacific plains of Mesoamerica, from the Isthmus of Tehuantepec to Central America, at first glance would suggest a cline of genetic variation. However, there is not enough evidence for such cline. The two intermixed haplotype groups, "I" and "IV", are distantly related. A clinal variation would show little and progressive differentiation between near localities and large differences from one extreme of the distributional range to the other, which is not the case. The highest nucleotide diversity occurs in the hybrid population JOY, in the center of the distributional range. Morphological data lead Selander (1964) to suggest secondary contact from 75 to 100 years ago, to explain the observed morphologic hybridization. Our data is also consistent with this hypothesis of secondary contact. The haplotype of all mtDNA hybrids (*h23*) is different by one mutational step from the most common haplotype "IV" group (*h22*). Although we did not date events in our trees, a different haplotype in the hybrids could mean that secondary contact happened earlier than proposed (Omland et al. 2006). It also possible that

other morphotype B individuals have *h23* haplotype and secondary contact did happen very recently. We would need a larger sample to have a good time estimate of that event.

Specimens with lack of correspondence between genetics and morphology are responsible for the observed pattern. They were collected in the vicinity of the previously reported hybrid zone (Selander 1964, 1965). The nesting of haplotypes and a posterior mapping of the haplotype groups onto their distributional range suggests a possible sequence of events leading to the diversification within this wren. First, an earlier separation into the three major groups (large genetic breaks) followed by secondary contact (intermixing of different haplotype groups. Despite the intergradation of haplotypes in restricted localities, the majority of the haplotype groups are geographically structured. The divisions of the entire distributional range in east of the Isthmus ("I and "II"), west of the Isthmus ("III", "IV" and "V") groups suggests that the Isthmus of Tehuantepec could have influenced speciation in this wren. Several other taxa with similar distribution (Peterson et al. 1999) were affected by the Isthmus. If the proposition of secondary contact among populations is correct, we would expect range expansions of each group. Our network shows star-like haplotype nestings for groups "I" and "V". Star-like phylogenies usually mean recent population expansions (Avise 2000). It is possible that dry forest expansions (Becerra 2005) or recent habitat alterations (Selander 1964) promoted that contact zone, although to truly test whether population expansions have happened (Rogers and Harpending 1992, Harpending 1994), we would need more sampling.

Consequences of hybridization

Hybridization and its implications for systematics depend on the concept used to detect and separate different species. A strict BSC stipulates if reproductive isolation is violated within populations or groups, despite their differences (morphological, genetic or behavioral), those groups most be considered as conspecific (e. g. Remsen 2005, and references therein). Other concepts, such as the PSC, emphasize the differentiation of lineages as a basis of species delimitation (see Zink 2006 for detailed discussion). Our results propose that even though there is differentiation between lineages likely by means of geographic barriers (Isthmus of Tehuantepec), there is also introgression of morphotype B mtDNA to

morphotype C individuals (the same pattern from west to east is observed for Mesoamerican dry forests expansions, Becerra 2005). Therefore, this wrens' taxonomy cannot be considered as completely resolved.

The occurrence of hybridization in nature is well known in many taxonomic groups (Arnold 1992, Riesenberg et al. 2006). For example in mammals, blue whales and fin whales hybridize in nature (Árnason and Gullberg 1993) as well as Dall's and harbour porpoises (Willis et al. 2004), but species status of neither of them is questioned although they are not reproductive isolated as stipulated by a strict BSC. In birds, 9.2% of species hybridize (Grant and Grant 2002). Many backcrosses of species in the same genus occur, and a few intergeneric hybrids within the same family produce fertile hybrids (Price and Bouvier 2002). Generally, these are recognized as "good" species. In addition, different species of North American avian taxa are known to hybridize (Pyle 1997). Some of those birds, such as owls (Hamer et al. 1994), buntings (Baker and Johnson 1998), orioles (e.g. Rising 1996), ravens (Omland et al. 2000) and chickadees (e.g. Curry 2005) have hybrids between non sister taxa (Omland et al. 2000, Klicka et al. 2001, Allen and Omland 2003, Gill et al. 1993, 2005, Barrowclough et al. 2005). Our phylogeny shows that morphotypes B and C haplotypes are not sister clades. To our knowledge, this is the first genetic approach of non sister taxa hybridization of birds in Mexico. If we were to recognize B and C as conespecific, they would be a paraphyletic taxon, since A is sister to B.

Possible secondary contact and gene introgression among other *Campylorhynchus* species has been suggested (Haffer 1975, Ridgely 1989). Our results are the first to confirm genetic hybridization in *Campylorhynchus* wrens. From the pairwise Φ_{st} values is clear that significant differences do exist between the hybrid population and those from the center of the Isthmus of Tehuantepec ("IV"), but no differences exist between the hybrid population and those from central Chiapas ("I"). This means that there is probably more genetic interchange among populations of the same morphotype (mtDNA hybrids and morphotype C). Gene flow between populations belonging to different morphotypes it is very limited (mtDNA hybrids and morphotype B).

Selander (1964: 80) concluded that almost all his 125 surveyed specimens show evidence of mixed ancestry in the vicinity of that lagoon. He also mentioned that he observed pairs of birds with different morphotypes breeding. However none of our new

series of specimens from the hybrid zone show such intermediate phenotype in plumage (vouchers MZFC CHIS113, 114, 137, 320 and 326 in Appendix 1). This suggests that hybrid designation only based on plumage of recent birds could underestimate the real number of hybrids. The proportion of mitochondrially detected hybrids in four localities near Laguna La Joya (Appendix 1) is 45%. Newly collected specimens for this study and several others in museums (Table 2), show typical morphotype B plumage traits, such as whiskers, barred undertail cover feathers or both (also noted by Dickey and Van Rossem 1938: 430-1). Additional specimens also show those traits but generally more subtle. These observations suggest morphometric characters could be detecting hybridization better than coloration or plumage traits. The latter characters could possibly be the result of retained polymorphisms. Ten out of 13 *Campylorhynchus* species have whiskers and barred underparts, specially the closely related ones (Barker 1999), such as the Spotted (*C. gularis*), Yucatan (*C. yucatanicus*), and Boucard's (*C. jocosus*).

We could not detect any morphotype B individuals in the hybrid zone. This area should be evaluated using nuclear markers and field studies to test whether there is an active contact zone with currently interbreeding individuals.

The biogeographic position of central Veracruz

Isthmuses are important in historical biogeography (Marshall and Liebherr 2000, Morrone 2006). The role of the Isthmus of Tehuantepec in Mesoamerican speciation as an east-west driver of speciation has been documented for several taxa (Peterson et al. 1999, Sullivan et al. 2000) due to its complicated geologic history (Barrier et al. 1998). Central Veracruz is an integral part of the Isthmus of Tehuantepec area. However, to our knowledge, no there are no historical biogeographic studies considering this area. The cloud forest specialist Common-bush Tanagers (*Chlorospingus ophthalmicus*) is also distributed across both sides of the Isthmus of Tehuantepec, and also has an isolated population in central Veracruz. These birds exhibit a similar geographic divergence pattern (García-Moreno et al. 2004). First, a division east to west, and a secondary division of west of Isthmus from central Veracruz. This congruence of phylogenetic patterns among wrens and tanagers could mean

that despite cloud and dry forest are completely different habitats, both species groups have been equally influenced by the Isthmus of Tehuantepec.

Taxonomic Implications

Regarding the taxonomy of this species complex, our genetic data revealed three largely distinct lineages. The level of divergence among them is similar to other pairwise values between well-recognized species (Klicka and Zink 1997, and references therein). These lineages are also well differentiated in morphometrics (Selander 1964) and song (Selander 1965, R. Sosa-López pers. comm.). We propose the following taxonomic recommendations for this group:

1) *C. rufinucha* (Lesson 1838) corresponds to the Veracruz ("III") population and morphotype A.

2) A large degree of genetic divergence exists between the Chiapas ("I") and the Central American ("II") populations. Nevertheless they are not reciprocally monophyletic (Fig. 4). There is not a clear differentiation in their morphometrics and song. It is possible that the "II" populations do constitute a separate evolutionary lineage; however, at this point we do not have enough evidence to separate "II" from "I", so we propose that both should constitute *C. capistratus* (Lesson 1842), corresponding to morphotype C.

3) *C. humilis* (Sclater 1856) corresponds to morphotype B, including individuals from the center of the Isthmus of Tehuantepec populations ("IV) and west of the Pacific Coast ("V") populations. It is the same case of paraphyly as with *C. capistratus*, and there is not yet enough additional evidence to separate "IV" from "V".

The coincidence of multiple criteria (de Queiroz 1998, Helbig et al. 2002) tells us that deep lineage distinction exists, and that the fact that there is or was limited hybridization should not negate these distinctions.

CONCLUSIONS

We described the genetic variability and phylogeographic patterns of a wren distributed in Neotropical deciduous forests and presented one of the first studies of speciation and hybridization of Middle American birds. We also provide strong data that could aid others to draw species limits, specially in cases of hybridization. We discovered three well differentiated lineages that match morphometric (Selander 1964) and song data (Sosa pers. comm.). We recommend that those three lineages should be recognized as full species. Our results match classic (Ridgway 1904) and recent taxonomic propositions (Navarro and Peterson 2004). High genetic differentiation values such as ours have also led others to suggest multiple species of wrens previously considered a single species (Drovestki et al. 2004). It is very likely that additional wren species complexes will provide similar findings and will recognize multiple species. We detected the Isthmus of Tehuantepec as probably the main driver of this group speciation which is consistent with biogeographic patterns from other taxa (Peterson et al 1999, Marshall and Liebherr 2000, Morrone 2006). It is also likely that other Mesoamerican taxa have similar historical biogeography.

Aside speciation, we found strong genetic evidence for secondary contact previously suggested by traditional morphometrics (Selander 1964, 1965). Our results support that confirmed or putative hybridization is just a part of the evolutionary phenomenon and should not be the sole criterion for species recognition. Gathering multiple sources of evidence (de Queiroz 1998, Helbig et al. 2002) helps identify evolutionary lineages and opens new insights for future research.

ACKNOWLEDGEMENTS

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process skin samples. We received tissue loans from John Klicka (UNLV), Mark Robbins and A. Townsend Peterson (KU), David Willard (FMNH), Sharon Birks (UWBM), Paul Sweet (AMNH), Rob Brumfield and Donna Dittman (LSU). A. Townsend Peterson, James Dean and Paul Sweet allowed us inspection of their specimen holdings at KU, USNM, and AMNH respectively. Merle Okada, Chris Blake and Mary LeRoy assisted us greatly at the AMNH. Terry Chesser helped us get literature from USNM libraries. We would like to thank especially all bird collectors. Without their hard work this study would not have ever been possible. Our most deep appreciation for their efforts and fellowship to Roberto Sosa-López, Vicente Rodríguez-Contreras, Samuel López de Aquino, Héctor Moya-Moreno, Luis Antonio Sánchez-González, Adolfo Calderón-Zarza, John Klicka, Garth Spellman, Markus Mika, Mark Robbins, A. Townsend Peterson, Néstor Herrera, Carlos Zaldaña, Arpad Nyard, Francisca Delgado, and Francisco Javier Sahagún.

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Fig. 1. Distribution of the Rufous-naped wren, with currently recognized groups (AOU 1998) and subspecies (Selander 1964). Major morphological differences on body are depicted for AOU groups (A: rufinucha, B: humilis, C: capistratus) and tail patterns for subspecies, delimited by dashed lines (-). The putative hybrid zone discovered by Selander (1964) is marked with an asterisk (*).

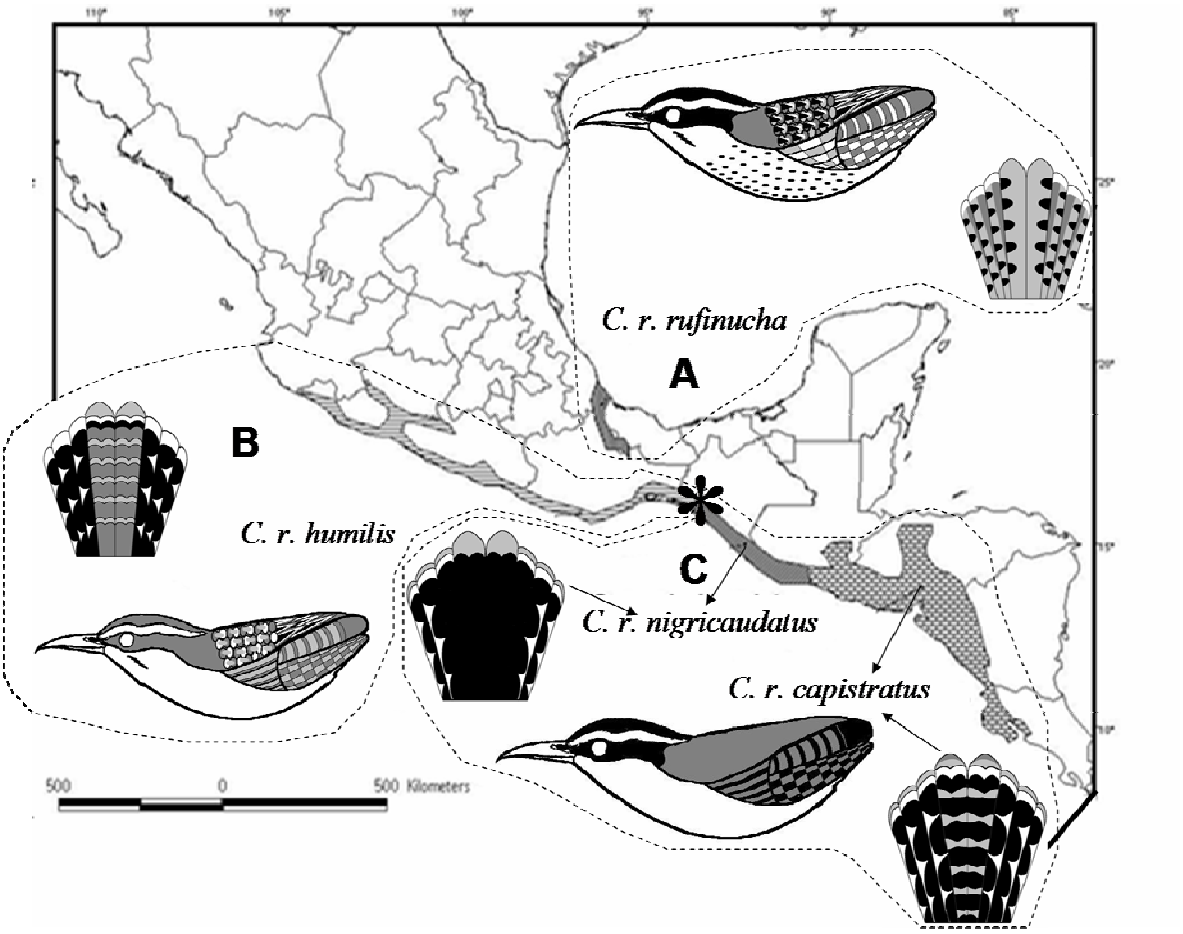

Fig. 2. Minimum spanning network (MP) of all Rufous-naped Wren haplotypes. Roman numerals depict different haplotype groups. I: specimens from Chiapas Mexico and Guatemala; II: specimens from Guatemala, el Salvador, Nicaragua and Costa Rica; III: specimens from Veracruz, Mexico; IV: specimens from Oaxaca And Chiapas, Mexico; and V: specimens from Michoacan, Guerrero and Oaxaca, Mexico. Single numbers on branches depict mutations between haplotypes. Letters and numbers on circles depict vouchers for specimens in the Appendix 1. Capital letters (A,B and C) link haplotype groupings with morphotypes (Fig. 1). (Open circles depict unsampled haplotypes, closed circles depict median vectors.)

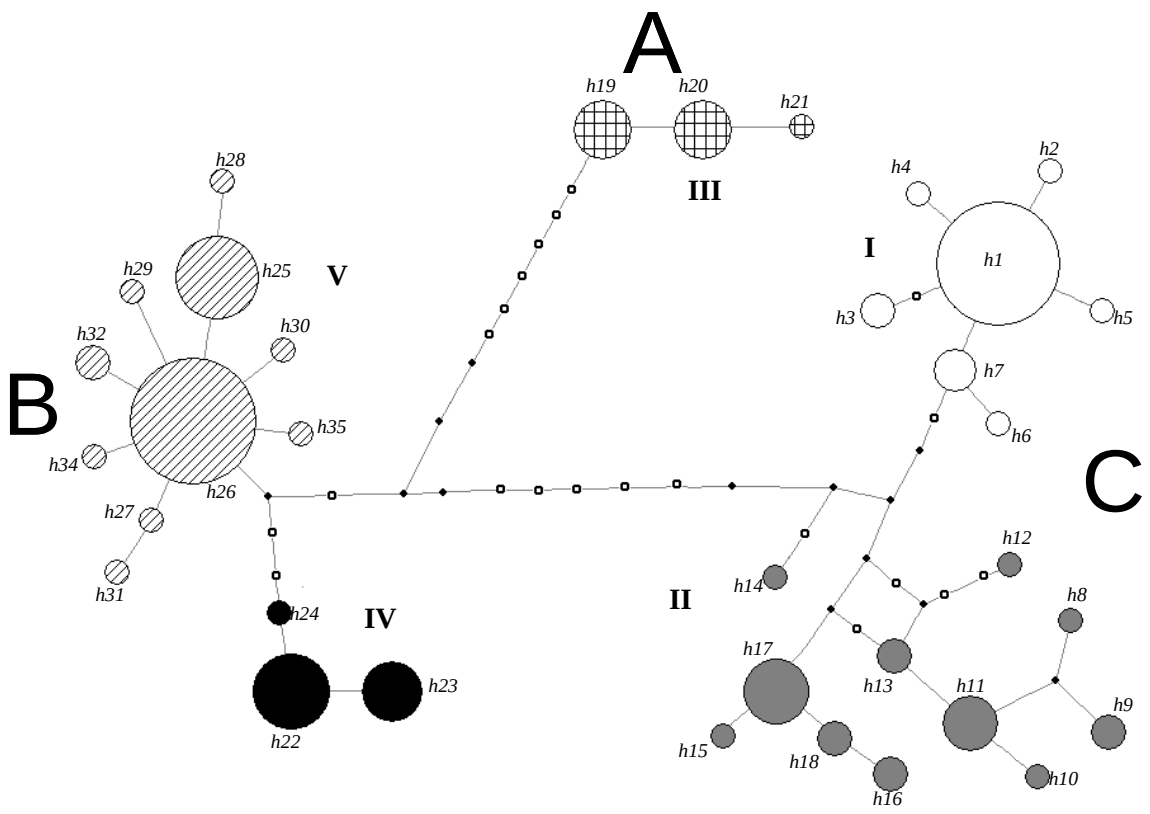

Fig. 3. Geographic distribution of the main five haplotypes groupings found on the network (Fig. 2). Solid white “I” , solid gray “II”, squares “III”, solid black is for the “IV”, and diagonal lines is for the “V” haplotype groups.

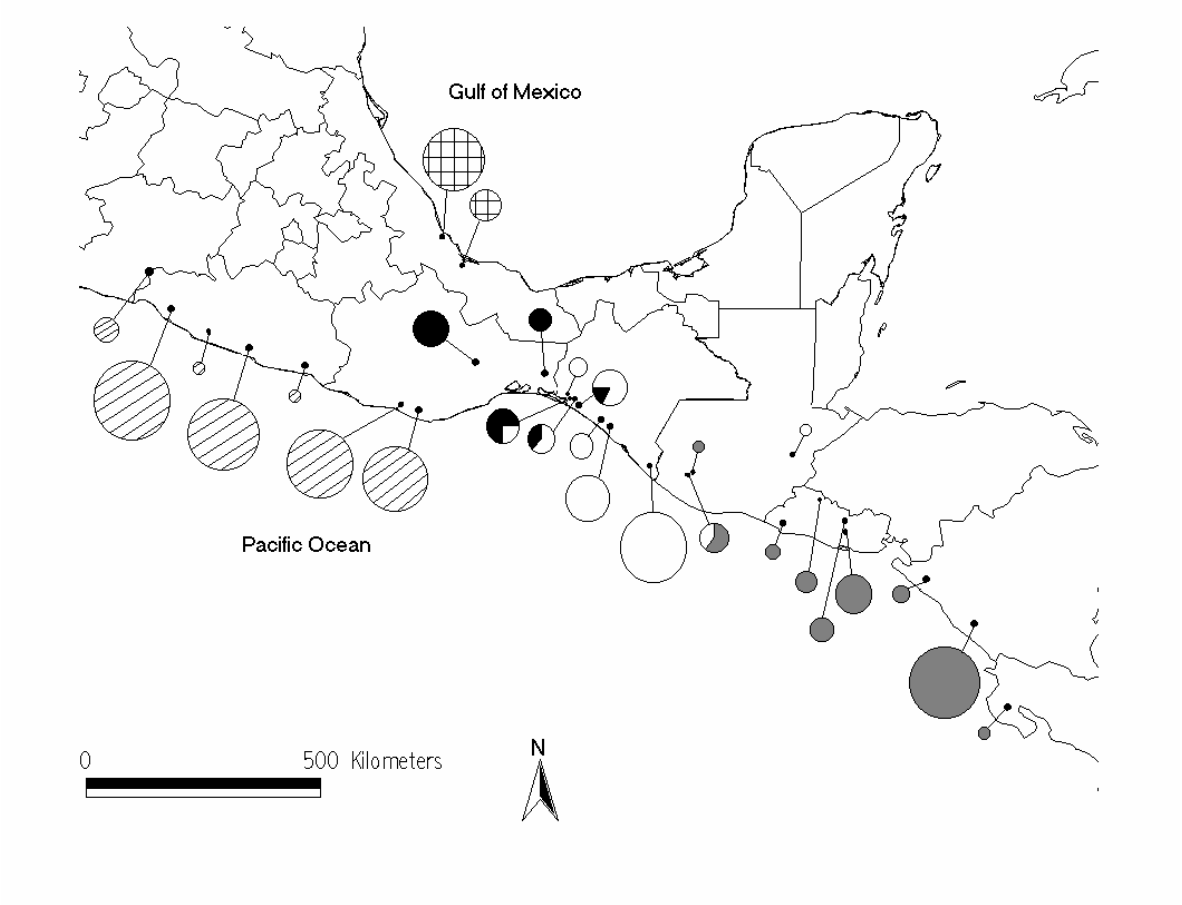

Fig. 4. Phylogenetic relationships of the Rufous-naped Wren unique haplotypes (*h*). Haplotypes marked with an asterisk (*) denote a distinct morphologic assignment. a) Bayesian tree (BY). Branch support depicts posterior probabilities of the clades. b) Parsimony tree (MP). Branch support depicts over 50% bootstrap values for each clades, branches below 50% are shown collapsed. Roman numerals refer to haplotype groupings found on the network (Fig. 2). Particular unique haplotype grouping are delimited by brackets }.

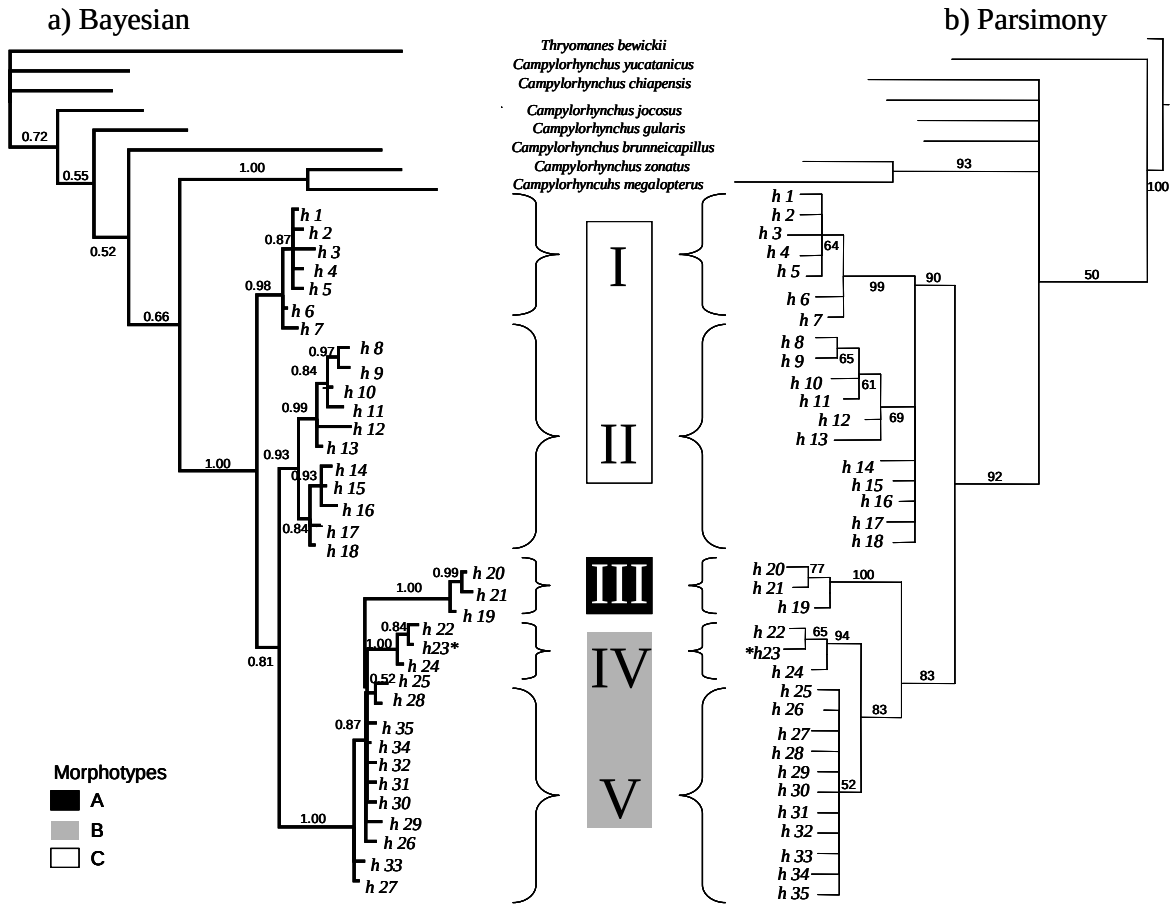

Fig. 5. Map with sampled localities depicting 12 populations/OGUs used in this study. Circles represent localities lumped into OGUs. A three letter code identifies each population as follows: VER, central Veracruz, Mexico; PET, Petatlán Guerrero, Mexico; TEC, Laguna Tecomate Guerrero, Mexico; MAN, Río Manialtepec, Oaxaca, Mexico; PTO, Puerto Escondido Oaxaca, Mexico; CPL, Cerro Piedra Larga Oaxaca, Mexico; TAP, Tapanatepec Oaxaca, Mexico; JOY, Laguna La Joya Chiapas, Mexico; PIJ, Pijijiapan Chiapas, Mexico; TUX, Tuxtla Chico Chiapas, Mexico; SAL, La Paz, El Salvador; NIC, Las Plazulas Granada, Nicaragua.

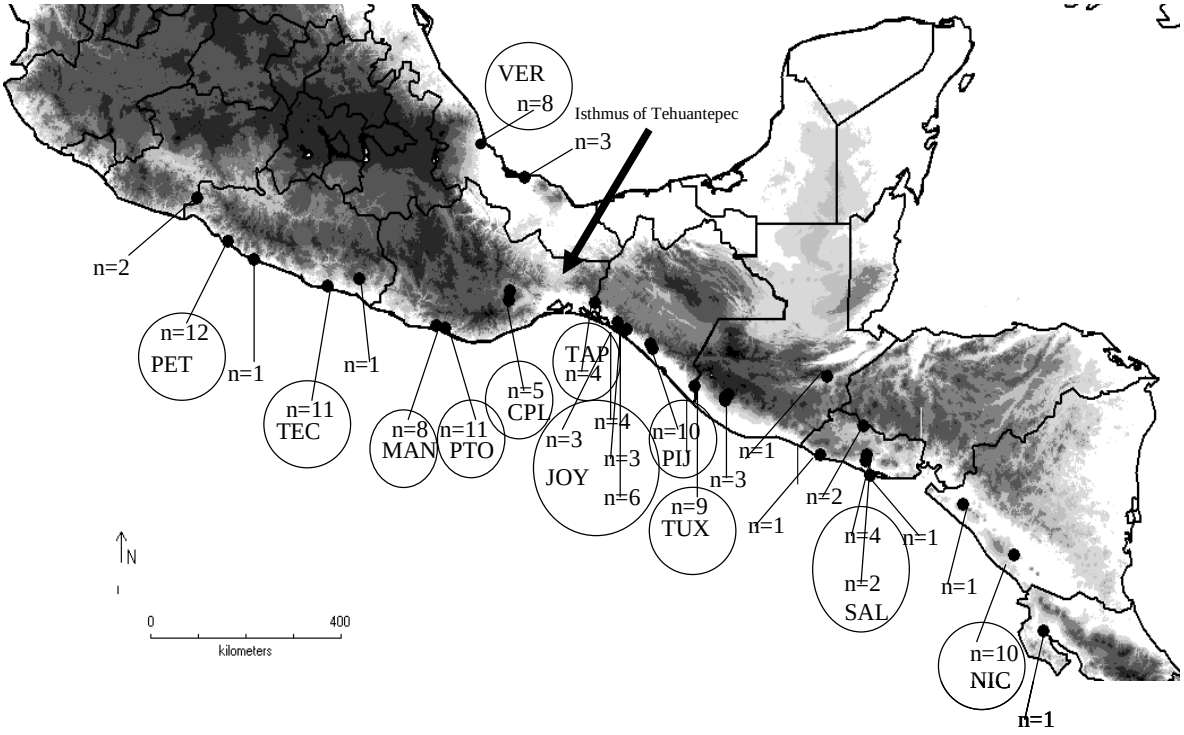

Fig. 6. Descriptive statistics of population variation with standard error bars.

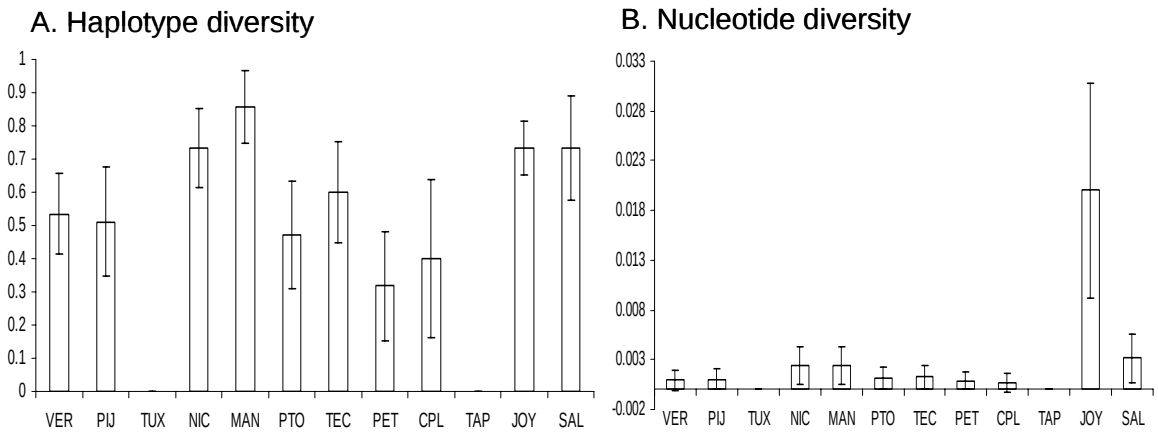

Fig. 7. Representation of the sources of genetic variation from the AMOVA analysis. Legend depicts sources of variation. Numbers refer to percentage of the observed variation (%). a) graphic does not include the JOY population (Fig. 5). b) graphic includes all 12 populations. Groups correspond to morphotypes.

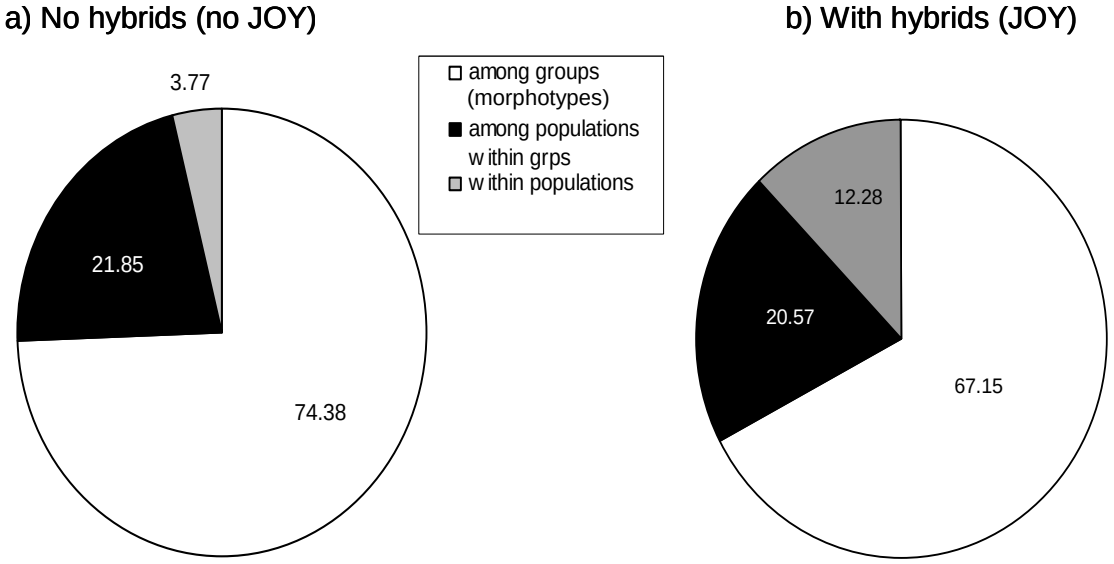

Table 1. A) Pairwise Φst values among populations. B) Pairwise *p* ETPD values among populations with standard deviation. Each population is labeled with its correspondent haplotype group (roman numbers, Fig. 2). Underlined values correspond to comparisons between the population with hybrids (JOY). Bold values correspond to **non** significant *p* values.

A. Φst	VER: III	PIJ: I	TUX: I	NIC: II	MAN: V	PTO: V	TEC: V	PET: V	CPL: IV	TAP: IV	JOY: I, IV
VER: III	-										
PIJ: I	0.97695	-									
TUX: I	0.98854	0.07407	-								
NIC: II	0.95779	0.91195	0.93873	-							
MAN: V	0.93426	0.95242	0.96633	0.92314	-						
PTO: V	0.95801	0.96999	0.98404	0.94430	-0.00403	-					
TEC: V	0.95839	0.96897	0.98288	0.93879	0.18768	0.44615	-				
PET: V	0.96439	0.97359	0.98755	0.94539	0.17520	0.47660	0.02963	-			
CPL: IV	0.97276	0.97895	0.99123	0.95567	0.84833	0.90939	0.89437	0.91089	-		
TAP: IV	0.98458	0.98787	1.00000	0.96638	0.88754	0.94688	0.93559	0.95238	0.00000	-	
JOY: I, IV	0.73896	0.23551	0.25763	0.54630	0.61652	0.63643	0.64157	0.64404	0.65449	0.66818	-
SAL: II	0.95615	0.90645	0.92973	0.74200	0.92022	0.93914	0.93383	0.93996	0.94992	0.95969	0.58585

B. ETPD	VER: III	PIJ: I	TUX: I	NIC: II	MAN: V	PTO: V	TEC: V	PET: V	CPL: IV	TAP: IV	JOY: I, IV
PIJ: I	0.00000+-0.000	-									
TUX: I	0.00000+-0.000	**0.33640+-0.0073**	-								
NIC: II	0.00005+-0.0001	0.00000+-0.0000	0.00000+-0.0000	-							
MAN: V	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	-						
PTO: V	0.00000+-0.0000	0.00020+-0.0002	0.00005+-0.0000	0.00000+-0.0000	**0.21250+-0.0053**	-					
TEC: V	0.00095+-0.0004	0.00040+-0.0002	0.00000+-0.0000	0.00055+-0.0002	0.03325+-0.0081	**0.09680+-0.0100**	-				
PET: V	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00005+-0.0001	0.00045+-0.0003	0.00120+-0.0008	**0.07455+-0.0104**	-			
CPL: IV	0.00260+-0.0008	0.00050+-0.0004	0.00140+-0.0010	0.00105+-0.0005	0.00050+-0.0002	0.00055+-0.0003	0.01115+-0.0030	0.00005+-0.0001	-		
TAP: IV	0.00145+-0.0006	0.00130+-0.0006	0.00170+-0.0007	0.00250+-0.0007	0.00040+-0.0001	0.00070+-0.0004	0.01150+-0.0035	0.00040+-0.0003	**1.00000+-0.0000**	-	
JOY: I, IV	0.00000+-0.0000	**0.11120+-0.0116**	**0.07320+-0.0071**	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00015+-0.0002	0.00000+-0.0000	-
SAL: II	0.00255+-0.0012	0.00060+-0.0003	0.00025+-0.0003	0.00255+-0.0010	0.00065+-0.0007	0.00085+-0.0005	0.01210+-0.0020	0.00100+-0.0007	0.00820+-0.0015	0.00965+-0.0025	0.00000+-0.0000

Table 2. List of morphotype C voucher specimens that show typical traits from morphotype B populations. Specimens marked with an asterisk (*) were used in our study and are referred in the Appendix 1. Museum acronyms are MZFC: Museo de Zoología "Alfonso L. Herrera" Universidad Nacional Autónoma de Mexico; KU: University of Kansas Natural History Museum and Biodiversity Research; USNM: Smithsonian Institution; AMNH: American Museum of Natural History.

	Heavily marked whiskers and/or undertail coverts						
Mexico	MZFC	*CHIS235	*CHIS237	*17561	*17562		
Guatemala	KU	72498	72497	AMNH	399221	395857	395852
El Salvador	KU	18709	*109650	*109557	*109336	*109366	
Nicaragua	KU	45738	USNM	151436	AMNH	144332	144325
	144324	144330	144327	144333	101345	144329	101343
Costa Rica	USNM	92807	198480	361652	361653		
	Lightly marked whiskers and/or undertail coverts						
Mexico	KU	106936	106935	101681	101683		
Guatemala	AMNH	395838	813605	395837	395839	395847	395862
	395850	395855					
El Salvador	KU	37317	*109649	*109718	93815		
Nicaragua	KU	37672	AMNH	144322	144323	144334	
Honduras	USNM	161683	161684	237642			
Costa Rica	USNM	199380	200168	89697	361655	361650	361651

Appendix 1. List of specimens used in this study. For all outgroups and unique haplotype Genbank accession number, museum[a] where the skin is deposited, specimen tissue identification number (in parenthesis), and their respective localities are given. All specimens marked CHIS were collected for this study. Fields marked with an asterisk (*) denote mtDNA hybrid specimens; cross on tissue numbers (+) means skin sample.

Outgroup	Genbank	Specific locality and specimen catalog numbers
Thryomanes bewickii		MZFC (QRO29). Mexico: Querétaro, El Derramadero.
Campylorhynchus jocosus		MZFC (HMM02-1). Mexico: Puebla, San Juan Raya.
Campylorhynchus chiapensis		MZFC (CHIS-244), Mexico: Chiapas, Tuxtla Chico, Rancho El Porvenir.
Campylorhynchus yucatanicus		MZFC (B603), Mexico: Yucatán, Rancho Sinkhuel, ¿ km E Dzilam de Bravo
Campylorhynchus megalopterus		MZFC (FD65), Mexico: Estado de México, Km 14 carr. Ocuilan-Cuernavaca
Campylorhynchus brunneicapillus		MZFC (CONACYT648), Mexico: Baja California Sur, Rancho Monte alto, 15 km N San Javier.
Campylorhynchus gularis		MZFC (FMNH393977), Mexico, Nayarit, Sierra de Nayarit.
Campylorhynchus zonatus		MZFC (CHIS565) Mexico: Veracruz, Jamapa.
Ingroup		
Morphotype A		
h19		MZFC (MZFC3339+) Mexico: Veracruz, Alvarado, KM 23-25, 180 Highway Veracruz-Alvarado. MZFC (CHIS572, 586, 587, 592, 595) Mexico: Veracruz, Actopan, 3 km La Mancha – Palmas Abajo.
h20		MZFC (MZFC3340+) Mexico: Veracruz, Alvarado, KM 23-25, 180 Highway Veracruz-Alvarado. MZFC (CHIS573, 574, 578) Mexico: Veracruz, Actopan, 3 km La Mancha – Palmas Abajo.
h21		MZFC (MZFC3341+) Mexico: Veracruz, Alvarado, KM 23-25, 180 Highway Veracruz-Alvarado.
Morphotype B		
h22		MZFC (OMVP728, CONACYT04-17, 74, 115) Mexico: Oaxaca, San Carlos Yautepec, Cerro Piedra Larga, Base. MZFC (CHIS379, 387, 397, 398) Mexico: Oaxaca, Tapanatepec, Rancho Las Minas.
h24		MZFC (CONACYT04-18) Mexico: Oaxaca, San Carlos Yautepec, Cerro Piedra Larga, Base.
h25		MZFC (CHIS399, 450) Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río. MBM (DHB5580, 5581, MM105, 107, GMS924, 925) Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido. MZFC (JK04-235) Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido.
h26		MZFC (CONACYT1049, 1050) Mexico: Michoacán, Lázaro Cárdenas, Presa Infiernillo 1 km N Camp CFE. MZFC (CHIS400, 435, 470, 471) Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río. MBM (JK04-75) MZFC (JK04-227) Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido. MZFC (CONACYT946) Mexico: Guerrero, Tecpan, Fracc. Laguna Nuxco. MZFC (CONACYT998) Mexico: Guerrero, San Luis Acatlan, 2 KM NE El Carmen. MZFC (CHIS476,477, 483, 490, 500, 501, 502) Mexico: Guerrero, San Marcos, Tecomate. MZFC (CHIS515,519, 525, 526, 527, 550, 553, 555, 561) Mexico: Guerrero, Petatlán, Los Cirilos.
h27		MZFC (CHIS546) Mexico: Guerrero, Petatlán, Los Cirilos.
h28		MZFC (CHIS444) Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río.
h29		MZFC (CHIS449) Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río.
h30		MZFC (CHIS484) Mexico: Guerrero, San Marcos, Tecomate
h31		MZFC (CHIS491) Mexico: Guerrero, San Marcos, Tecomate
h32		MZFC (CHIS492,499) Mexico: Guerrero, San Marcos, Tecomate
h33		MZFC (CHIS551) Mexico: Guerrero, Petatlán, Los Cirilos.
h34		MZFC (CHIS552) Mexico: Guerrero, Petatlán, Los Cirilos.
h35		MZFC (JK04-241) Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido.
Morphotype C		
h1		MZFC (AMTB15, 16, CONACYT1339) Mexico: Chiapas, Pijijiapan, Rancho Nueva Ensenada. MZFC (CHIS1, 164, 201, 202) Mexico: Chiapas, Pijijiapan, Rancho Lluvia de oro. MZFC (CHIS235, 236, 237, 238, 239, 269, 270, 271, 272) Mexico: Chiapas, Tuxtla Chico, Rancho El Porvenir. MZFC (CHIS156) Mexico: Chiapas, Tonalá, 1.7 Km E Rancho "El Vergel", Laguna La Joya. MZFC (CHIS293, 295, 319) Mexico: Chiapas, Tonalá, Tres Picos, Llano. MZFC (CHIS321) Mexico: Chiapas, Tonalá, La Polka, Rancho Bellavista, Laguna La Joya. MZFC (CHIS358, 377) Mexico: Chiapas ,Tonalá, Rancho La

	Industria. MBM (JK02-23) Guatemala: Retalhuleu, San Felipe Retalhuleu 5km S, Finca El Niño.
h2	MZFC (CHIS163) Mexico: Chiapas, Pijijiapan, Rancho Lluvia de oro.
h3	MZFC (CHIS309) Mexico: Chiapas, Tonalá, Tres Picos, Llano. MZFC (CHIS333) Mexico: Chiapas, Tonalá, La Polka, Rancho Bellavista, Laguna La Joya.
h4	MZFC (CHIS378) Mexico: Chiapas ,Tonalá, Rancho La Industria.
h5	MBM (JK03-7) Guatemala: Retalhuleu, San Felipe Retalhuleu 5km S, Finca El Niño.
h6	MBM (JK03-482) Guatemala: Zacapa, Motagua Valley, 10 KM E Rio Hondo
h7	MZFC (CHIS99, 100) Mexico: Chiapas, Pijijiapan, Rancho Lluvia de oro. MZFC (CHIS308) Mexico: Chiapas, Tonalá, Tres Picos, Llano.
h8	KU (B9378) El Salvador: Usulutlan, 2.6 KM E Boca del Rio Lempa.
h9	KU (B7654, 7803) El Salvador: San Vicente, Volcán San Vicente.
h10	KU (B7690) El Salvador: San Vicente, Volcán San Vicente.
h11	KU (B7655) El Salvador: San Vicente, Volcán San Vicente. KU (B9037, 9039) El Salvador: Chalatenango, La Laguna, La Montañona. KU (B9261, 9262) El Salvador: La Paz, Zacatecoluca.
h12	MBM (DHB4338) Guatemala: Retalhuleu, San Felipe Retalhuleu 5km S, Finca El Niño.
h13	MBM (DHB4337) Guatemala: Retalhuleu, San Felipe Retalhuleu 5km S, Finca El Niño. FMNH (FMNH) El Salvador: Sonsonate, Izalco, Cantón Cruz Verde, Finca Nuevos Horizontes.
h14	MBM (DAB1931) Nicaragua: Granada, Las Plazulas, Laguna Blanca.
h15	MBM (DAB1879, 1905) Nicaragua: Granada, Las Plazulas, Laguna Blanca.
h16	UWBM (1928) Nicaragua: Granada, Las Plazulas, Laguna Blanca. MBM (DAB1855) Nicaragua: Granada, Las Plazulas, Laguna Blanca.
h17	UWBM (DAB1869, 1883, 1904, 1924, 1927) Nicaragua: Granada, Las Plazulas, Laguna Blanca. AMNH (GFB1027) Costa Rica: Puntarenas, 0.8 km NW Quatro Cruces, On Rte.1 (PanAm Hwy).
h18	UWBM (DAB1576) Nicaragua: Chinandega, Casita, Ladera del Volcán Casita.
h23*	MZFC (CHIS113, 114, 137) Mexico: Chiapas, Tonalá, 1.7 Km E Rancho "El Vergel", Laguna La Joya. MZFC (CHIS320) Mexico: Chiapas, Tonalá, Tres Picos, Llano. MZFC (CHIS326) Mexico: Chiapas, Tonalá, La Polka, Rancho Bellavista, Laguna La Joya.

[a] Museum abbreviation are as follows: MZFC, Museo de Zoología “Alfonso L. Herrera” Universidad Nacional Autónoma de Mexico; KU, University of Kansas Natural History Museum and Biodiversity Research; MBM, Marjorie Barrick Museum, University of Nevada Las Vegas; FMNH, Field Museum of Natural History; UWBM, Burke Museum, University of Washington; AMNH, American Museum of Natural History.

CONCLUSIONES

Se presenta un estudio filogeográfico de un complejo taxonómico de matracas. Se recomienda que los tres linajes sean reconocidos como tres especies diferentes. Estos resultados son consistentes con la primer (Ridgway 1904) y la última (Navarro y Peterson 2004) taxonomías propuestas para estas aves. Tanto los árboles filogenéticos como la red de haplotipos muestran dichos linajes independientes profundamente diferenciados. Los valores del índice de fijación Φst sugieren una casi completa diferenciación genética entre las tres formas morfológicas, incluyendo valores significativos de la prueba ETPD. En estudios similares, los altos valores de diferenciación genética han llevado a otros autores a también sugerir varias especies de matracas previamente consideradas una sola especie (Drovetski et al. 2004). De reconocerse la existencia de las tres especies sugeridas aquí, no solo existen importantes implicaciones en estudios ecológicos y conductuales, sino también en la conservación. La ley sólo protege bajo una categoría especial de riesgo a *C. rufinucha*. *C. humilis* y *C. nigricaudatus* siguen sin alguna norma de protección actual (DOF 2002). Debe de prestarse especial atención a *C. humilis,* ya que es endémica a México. Es probable que adicionales complejos taxonómicos de matracas muestren descubrimientos similares.

La especiación dentro de este complejo taxonómico parece estar influenciado del Istmo de Tehuantepec. Esto es un patrón común en Mesoamérica para otras aves (Peterson et al. 1999), lo cual es consistente con los patrones biogeográficos de otros taxones. Es también probable que otros grupos con distribuciones similares muestren los mismos patrones.

Aunado a los eventos de especiación dentro de este grupo de aves, se encontró evidencia genética de hibridización de dos linajes que nos son grupos hermanos. La hibridización ocurre todo el tiempo en la naturaleza (Hewitt 2001), y parece ser común entre especies de aves distantemente relacionadas (Price y Bouvier 2002). Las especies de matracas parecen tener patrones ecológicos y conductuales muy similares (Brewer y MacKay 2001). Tales similitudes abren la posibilidad de que especies distantemente relacionadas del género *Campylorhynchus* (Barker 1999) hibridicen en simpatría (Haffer 1975, Ridgely 2001). Entonces, la comprobada hibridización dentro del complejo *C. rufinucha* no es sorprendente. Adicionalmente, encontramos fuertes evidencias genéticas de una que la zona de hibridización es debida a contacto secundario, previamente sugerido por morfometría (Selander 1964,1965). Los criterios morfométricos usados por Selander para la determinación de híbridos parecen estar bien apoyados en DNAmt. Los resultados de este estudio apoyan la idea de que la hibridización hipotetizada o confirmada es sólo una parte del fenómeno evolutivo y no debe de ser el único u optado criterio para el reconocimiento de especies. Tomar evidencias de múltiples fuentes (de Queiroz 1998, Helbig et al. 2002) ayuda a identificar linajes evolutivos y abre nuevas interrogantes para nuevos estudios.

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