The Auk 126(4):765–778, 2009

Printed in USA.

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

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Abstract.—The Rufous-naped Wren (*Campylorhynchus rufinucha*) is a sedentary, morphologically variable species 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 forms on the Pacific slope intergrade in Chiapas, Mexico, apparently as a result of secondary contact. We sequenced a mitochondrial DNA (mtDNA) gene to explore phylogeographic patterns and hybridization. We found three divergent lineages, two geographically spanning the Isthmus of Tehuantepec and a disjunct Veracruz population. Analyses of molecular variation and Φ statistics are consistent with genetically distinct populations. Morphological and behavioral evidence from other studies is consistent with the existence of these three independent evolutionary lineages. However, the geographic distribution of haplotypes suggests mtDNA introgression east of the isthmus. Our data suggest that this secondary contact could be explained by population expansions. We recommend recognizing three species, two of which hybridize in a narrow contact zone. *Received 12 March 2007, accepted 19 April 2009.*

Key words: *Campylorhynchus rufinucha,* Central America, hybridization, Mesoamerica, Mexico, phylogeography, Rufous-naped Wren, species limits.

Filogeografía de *Campylorhynchus rufinucha*: Especiación e Hibridación en Mesoamérica

Resumen.—*Campylorhynchus rufinucha* es una especie con variación morfológica marcada, sedentaria y asociada 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 formas se traslapan en el extremo oeste de la costa de Chiapas, México, aparentemente producto de un contacto secundario. Secuenciamos un gen del DNA mitocondrial para explorar patrones filogeográficos y la hibridación. Encontramos tres linajes divergentes, dos asociados geográficamente al Istmo de Tehuantepec y la población disyunta de Veracruz. Los análisis de varianza molecular y los estadísticos Φ son consistentes con poblaciones genéticamente distintas. Evidencias de otros estudios, tanto morfológicas como conductuales, también apoyan esos tres linajes evolutivamente independientes. Sin embargo, la distribución geográfica de los haplotipos sugiere introgresión de DNA mitocondrial en el este del istmo. Nuestros datos sugieren que dicho contacto secundario puede ser explicado por expansiones poblacionales. Recomendamos reconocer tres especies, dos de las cuales hibridan en una estrecha zona de contacto.

Southern Mexico and Central America (known collectively as Mesoamerica) contain a wealth of geographic barriers, such as isthmuses and extensive mountain chains (Ferrusquía-Villafranca 1993), which give this region strong altitudinal gradients and fragmented lowland habitats. One result of this topographic complexity is an impressive number of species and endemic taxa (Campbell 1999) in a variety of taxonomic groups, including salamanders, toads, lizards, and mice (García-Paris et al. 2000, Mulcahy and Mendelson 2000, Sullivan et al. 2000, Castoe et al. 2003, Hasbún et al. 2005 and references therein, Mulcahy et al. 2006).

Mesoamerican birds also show high levels of endemism (Navarro-Sigüenza and Sánchez-González 2003; García-Moreno et al. 2004, 2006), and the wrens in particular (family Troglodytidae) are a good example. The species in the genus *Campylorhynchus* are the largest members of the family and are among the best-known cooperatively breeding birds (Rabenold 1990). A molecular phylogeny of this genus revealed two lineages (Barker 2007): a group with brownish plumage generally found in dry habitats (the Heleodytes group), and a group with grayish plumage usually found in more humid areas (the Campylorhynchus group).

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The Auk, Vol. 126, Number 4, pages 765–778. ISSN 0004-8038, electronic ISSN 1938-4254. DOI: 10.1525/auk.2009.07048

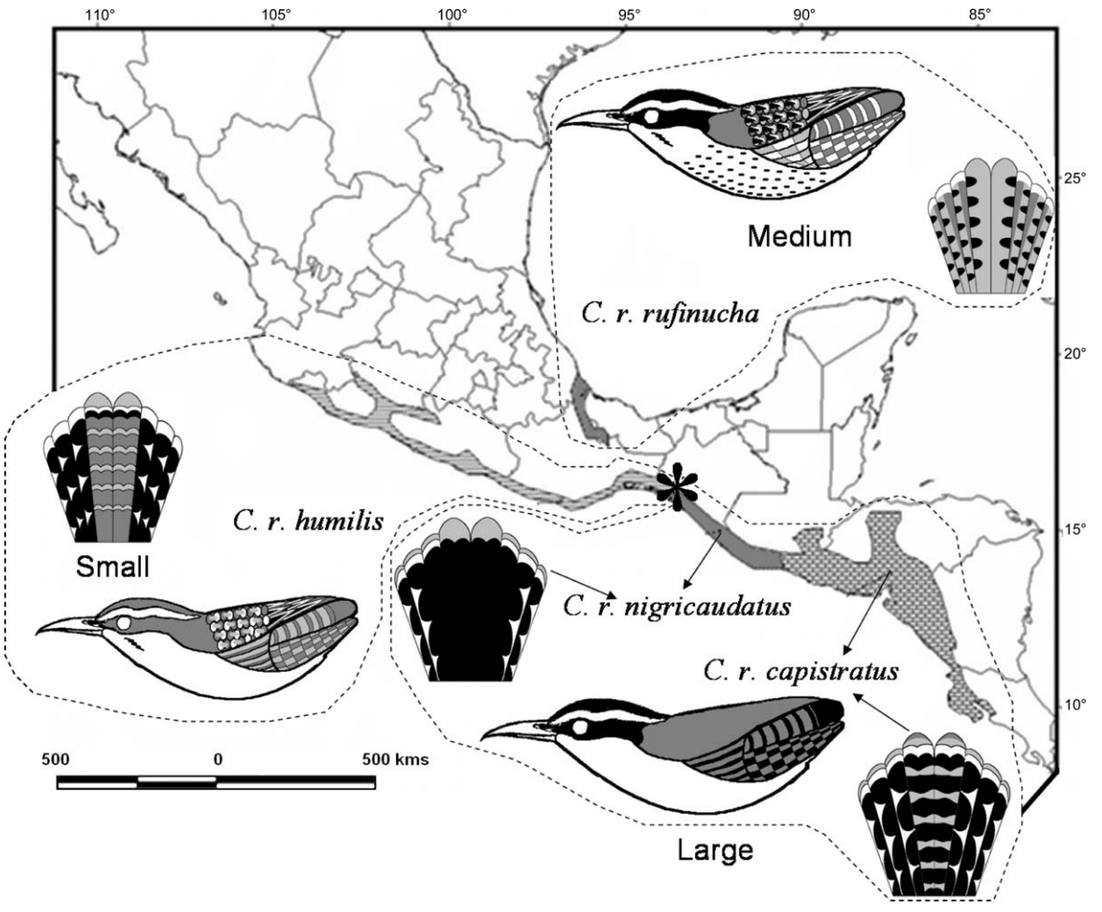

Fig. 1. Distribution of the Rufous-naped Wren, with currently recognized groups (American Ornithologists' Union [AOU] 1998) and subspecies (Selander 1964). Major morphological differences in plumage patterns are depicted for AOU groups (medium: rufinucha, small: humilis, large: capistratus) and tail patterns for subspecies, delimited by dashed lines. The putative hybrid zone discovered by Selander (1964) is marked with an asterisk.

One representative species in the Heleodytes group is the Rufous-naped Wren (*C. rufinucha*), a tropical dry-forest resident, distributed continuously from western Mexico to northwestern Costa Rica on the Pacific slope, with an isolated population on the plains of central Veracruz near the Gulf of Mexico (Fig. 1). Some authors recognize the Rufous-naped Wren as three species (Ridgway 1904, Navarro-Sigüenza and Peterson 2004). Others recognize a single species with five (Hellmayr 1934, Peters 1960) or nine (Phillips 1986, Dickinson 2003) subspecies. The American Ornithologists' Union (1998) *Check-list* considers it a species complex.

The Rufous-naped Wren shows marked morphological (Fig. 1; Selander 1964) and song variation (Selander 1965, Sosa 2007) throughout its range. There are three major morphological divisions (Selander 1964), hereafter referred to as "small," "medium," and "large" forms (Fig. 1). The small form, *C. r. humilis* (♂ weight = 28.20 ± 0.60 g; $n = 15$), has rufous coloration on the head and nape, patterned upperparts, light grayish underparts, "whiskers," and barred undertail coverts and rectrices (Selander 1964). The medium form, *C. r. rufinucha* (♂ weight = 30.42 ± 0.27 g; $n = 12$), has a black crown, rufous nape, patterned upperparts, light grayish underparts heavily covered by small spots, "whiskers," and barred undertail coverts and rectrices (Selander 1964). The large form, *C. r. nigricaudatus* (♂ weight = 33.5 ± 0.00 g; $n = 9$) and *C. r. capistratus* (♂ weight = 35.7 ± 0.00 g; $n = 2$), has a black crown, rufous nape and upperparts, whitish underparts, and generally unpatterned undertail coverts; the back presents irregular variation (see Selander 1964). Tail pattern in the large form ranges in a cline from individuals with full black central tail feathers to those with heavily barred tail feathers (Fig. 1). There is no clear distributional gap between the latter two subspecies, and, therefore, subspecific determination is done by geographic distribution (black = *C. r. nigricaudatus* from Chiapas, Mexico, to Guatemala; barred = *C. r. capistratus* from El Salvador to Costa Rica; Selander 1964).

Selander (1964, 1965) discovered individuals intermediate in morphology between the small and large forms in the vicinity of Laguna La Joya (Fig. 1). He considered these hybrids on the basis of their average intermediate size and plumage traits. Lastly, pairs of the small form sing simple duets, those of the medium form sing

antiphonal duets, and pairs from the large form sing simultaneous duets (Sosa 2007); it is not clear whether these are mated pairs.

Our main goals were to (1) determine whether the forms defined by morphology were genetically coherent and, if so, assess their phylogenetic relationships; (2) assess the degree of population differentiation; and (3) determine whether there is genetic evidence of introgression among forms, as suggested by morphology

METHODS

Specimens.—We collected specimens of Rufous-naped Wrens from across the species' range in Mexico. These specimens are housed at the Museo de Zoología (MZFC) "Alfonso L. Herrera," Universidad Nacional Autónoma de México (see Appendix). Muscle, heart, and liver samples were preserved either in pure ethanol or frozen in liquid nitrogen. We borrowed additional tissues from Mexican and U.S. bird collections that represent Central American populations. To increase the sample size for the Veracruz population, we used skin samples from three museum specimens, giving a total of 128 individual Rufous-naped Wrens; eight outgroups were used (see Appendix for voucher specimen data and GenBank accession numbers).

Lab procedures and protocols.—We extracted genomic DNA from muscle and liver using a phenol-chloroform protocol and DNeasy kits (Qiagen, Valencia, California) following manufacturer instructions. Polymerase chain reaction (PCR) products from the first half of the mitochondrial DNA (mtDNA) gene Nicotidamide Adenine Dinucleotide Hydrogenase 2 (ND2) were amplified using the forward primer L5215 (Hackett 1996) and a specific reverse primer 5′-GGAGATKGAGGAGAAGGCTA-3′ (designed in PRIMER3; see Acknowledgments). We amplified DNA using the following PCR protocol: initial phase at 92°C for 3 min, denaturing at 92°C for 1 min, annealing at 50°C for 1 min, elongation at 72°C for 1 min, with a final extension at 72°C for 3 min for 38 cycles. The skin samples were processed in a different molecular genetics lab than the tissue processing (see Acknowledgments), using new reagents to avoid contamination. The 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, Foster City, California) following recommended guidelines. Excess sequencing-reaction reagents were eliminated using ethanol–EDTA precipitation according to the manufacturer's instructions. Automated sequencing was done in an ABI 3100 sequencer, with assembly carried out using SEQUENCHER, version 4.5 (Genecodes, Ann Arbor, Michigan). The ND2 protein coding gene had no indels; therefore, it was easy to align. We translated the ND2 sequences to detect stop codons and to check for the possibility of nuclear copies. Additionally, we compared the 35 unique haplotypes with a previously published Rufous-naped Wren haplotype in GenBank BLAST (accession number AY460230).

Phylogenetic analyses.—We used NETWORKS, version 4.1.1.1 (see Acknowledgments; Bandelt et al. 1999), to construct a parsimony network (PN) using the median-joining algorithm. Parsimony trees with 1,000 bootstrap pseudoreplicates were constructed in NONA, version 2.0 (Goloboff 1993), with WINCLADA, version 0.9.99 (Nixon 2000). We used the TBR algorithm with additive characters and 100 replicates, keeping 10 initial trees. We considered nodes with bootstrap values ≥70% to be well supported (Hillis and Bull 1993). In PAUP*, version 4.0b10 (Swofford 2002), we constructed a maximum-likelihood tree. We discarded the maximum-likelihood tree because it had the same topology as our Bayesian trees. To estimate Bayesian trees and posterior probabilities, we used partitioned and nonpartitioned data sets in MRBAYES, version 3.1 (Huelsenbeck and Ronquist 2001), with flat prior probabilities, two runs, and four chains each for 2×10^6 generations sampling every 100th generation. We then constructed a majority-rule consensus tree and its associated –LnL value by using arithmetic means. We considered nodes with posterior probabilities ≥0.95 to be well supported (Larget and Simon 1999). We tested whether we should partition our data set by using harmonic means and the Bayes empirical factor (Nylander et al. 2004) as indicated in Kass and Raftery (1995). Parameters for the nonpartitioned data set used a GTR+I+G model (rate matrix = 0.4582, 17.3782, 0.00, 0.4948, 5.3094; $\alpha = 2.5657$; I = 0.5789) as selected by Akaike's information criterion in MODELTEST, version 3.7 (Posada and Crandall 1998). We partitioned the data set by codon, analyzing the first codon position by means of an HKY+G model (transitions:transversions ratio = 7.748; $\alpha = 0.1716$), the second position using an HKY+I model (transitions:transversions ratio = 4.1838; I = 0.7562), and the third codon positions using a TrN+G model (rate matrix = 1.00, 50.6385, 1.00, 1.00, 31.8769; $\alpha = 2.7196$). We checked for significant differences among our resulting trees using the SH test for likelihood (Shimodaira and Hasegawa 1999) and the KH test for parsimony (Kishino and Hasegawa 1989). We tested for a molecular clock in all our trees in PAUP using the likelihood ratio test (LRT; Felsenstein 1981).

Population delimitation, genetic diversity parameters, and demographic history.—Analyses of intra- and interpopulation variability were performed for individual populations containing four or more individuals. To increase sample sizes, we pooled localities within a 20-km linear distance (considered by Selander [1965] as the area of a reproductive group), yielding 13 populations. We also grouped individuals by mitochondrial–haplotype group. We computed haplotype (h) and nucleotide diversities (π) (Nei 1987) using ARLEQUIN, version 3.01 (Excoffier et al. 2005). For genetic differences among populations, we calculated pairwise Φ statistics and the exact test of population differentiation (ETPD; Raymond and Rousset 1995) in ARLEQUIN. We also employed ARLEQUIN to test for population expansions with several tests. We computed mismatch distributions (MM; Rogers and Harpending 1992) and tested them against expectations of a sudden-expansion model (Rogers 1995) and used 1,000 bootstrap replicates to evaluate statistical significance. In many populations, the least-squares fit of the model MM and the observed distribution did not converge. Thus, we calculated the R2 statistic (Ramos-Onsins and Rozas 2002) in DNASP, version 4.01 (Rozas et al. 2003). We used 10,000 coalescent replicates to assess R2 significance. We also searched for past changes in population size using Fu's (Fu 1997) and Tajima's D (Tajima 1989) tests of neutrality in ARLEQUIN with 1,000 simulated samples to evaluate significance levels. We calculated pairwise gene-flow estimates (Nm; equation 4 in Hudson et al. 1992) in DNASP. To assess population structure and its relation to morphology, we used the analysis of molecular variance (AMOVA; Excoffier et al. 1992) in ARLEQUIN. We defined three groups of populations based on the three main forms (Fig. 1)

to assess whether there is correspondence between genetics and morphology. Then we calculated two AMOVAs, one excluding putative intermixed populations and a second including all populations. Differences in the amount of variation explained by locality between designs would signify that introgression is influencing the genetic structure of this species. We tested for isolation by distance (IBD; Slatkin 1993) in ARLEQUIN using Mantel's test correlations between Φst and pairwise straight-line geographic distance. Significance levels were assessed using 1,000 bootstrap replicates. We ran several variants: all populations, only those on the Pacific slope, and populations within their haplotype group. All statistics were calculated with a significance cutoff at $P \leq 0.05$.

Determination of hybrid individuals.—We reasoned that individuals with mismatched mtDNA and morphology were "hybrids" by comparing mtDNA and specimen morphology (Fig. 1 and Appendix). Because mitochondria are maternally inherited and their flow in hybridization is limited (Haldane's rule; Orr 1997), we assumed that general morphological traits are controlled by nuclear loci (Selander 1964, 1965) and, thus, make better markers to assess gene flow across the hybrid zone. Therefore, a mismatch between morphology and mtDNA haplotype group is an indication of hybridization (we refer to these as "hybrids"; for similar reasoning, see Zink 1994, Brumfield 2005).

Selander (1964) considered the presence of whiskers and undertail barring, which are typical of small and medium forms, an indicator of hybridization. We scored the presence of these traits in large-form birds using specimens from the new series collected for the present study and previous series available at MZFC, KU, USNM, UNLV, and AMNH (see Appendix for museum names) to determine whether those two traits occur only at Laguna La Joya or elsewhere as well (which would suggest a mechanism other than hybridization for their geographic distribution).

Results

Phylogenetic analyses.—We resolved a 547-base-pair product of ND2 for all 136 individuals. We detected 35 unique haplotypes from the 128 Rufous-naped Wrens. Between forms, there were large levels of uncorrected sequence divergence. Between the small and medium forms, the average was 2.9%; medium and large averaged 4.6% divergence; and small and large averaged 3.6%. There was a 4.1% average sequence divergence and a minimum of 2.4% sequence divergence (excluding putative hybrids) between large and small or medium forms.

The haplotype network (Fig. 2) showed five main haplotype groupings (M, S1, S2, L1, L2) generally matched to a specific form

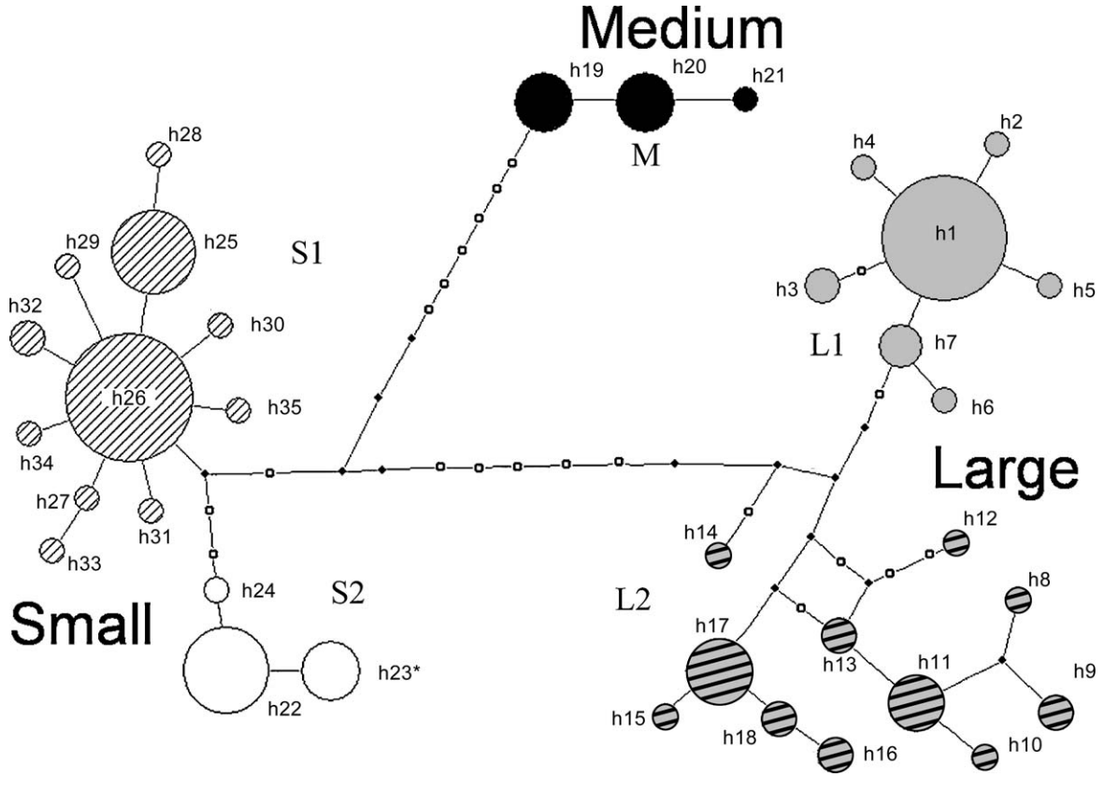

Fig. 2. Minimum spanning network (PN) of all Rufous-naped Wren haplotypes. Small capital letters depict different haplotype groups. M: specimens from Veracruz, Mexico. S1: specimens from Michoacán, Guerrero, and Oaxaca, Mexico. S2: specimens from Oaxaca and Chiapas, Mexico. L1: specimens from Chiapas and Guatemala. L2: specimens from Guatemala, El Salvador, Nicaragua, and Costa Rica. Letters and numbers on circles correspond to haplotypes and follow the Appendix. "Small," "medium," and "large" labels link haplotype groups with forms (with the exception of h23, marked with an asterisk; see text). Open circles with thick borders depict unsampled haplotypes, and closed circles depict median-joining vectors.

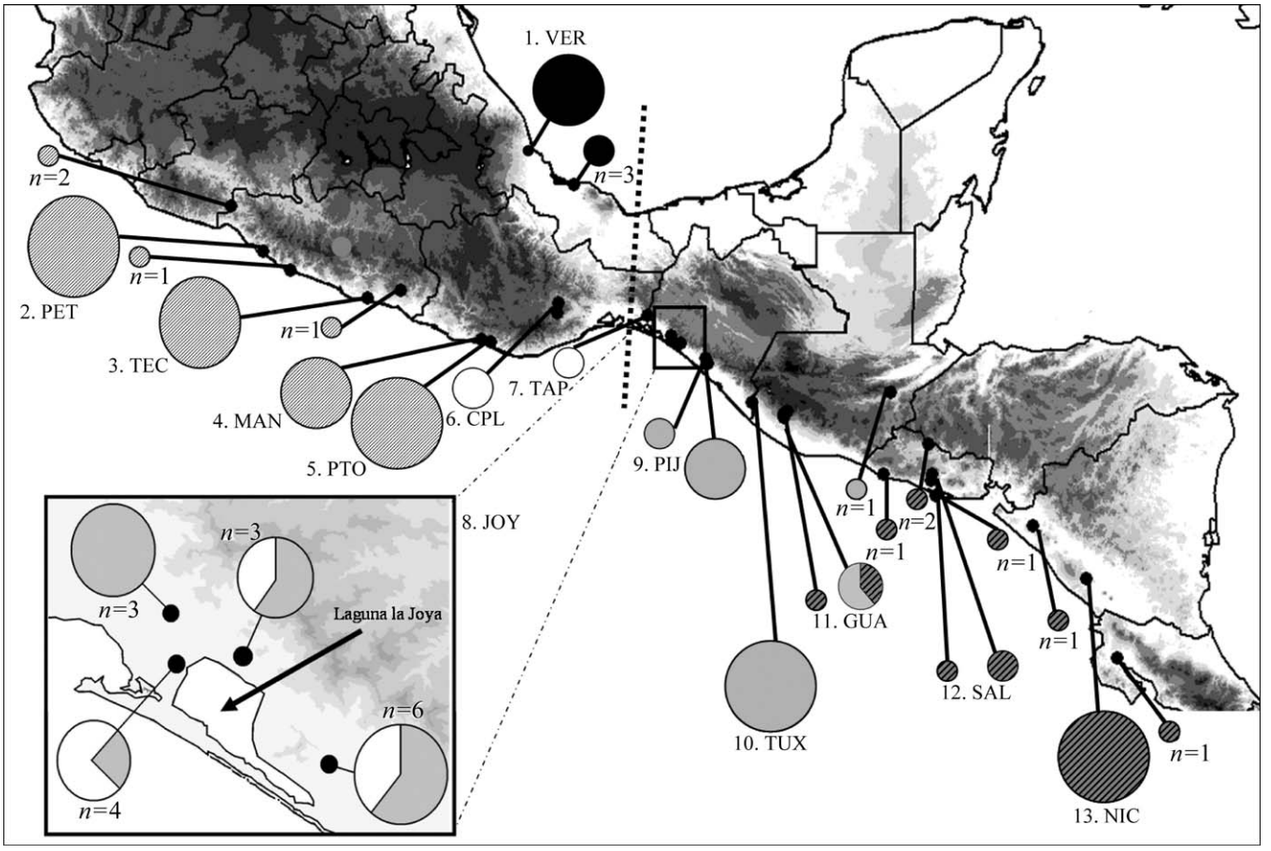

FIG. 3. Geographic distribution of the main five Rufous-naped Wren haplotype groupings found on the minimum spanning network (Fig. 2). Also shown are sampled localities and the 13 populations or operational geographical units (OGUs) used in the present study. A three-letter code identifies each population as follows: (1) VER = central Veracruz, Mexico; (2) PET = Petatlán, Guerrero, Mexico; (3) TEC = Laguna Tecomate, Guerrero; (4) MAN = Río Manialtepec, Oaxaca, Mexico; (5) PTO = Puerto Escondido, Oaxaca; (6) CPL = Cerro Piedra Larga, Oaxaca; (7) TAP = Tapanatepec, Oaxaca; (8) JOY = Laguna La Joya, Chiapas, Mexico (detailed in the square); (9) PIJ = Pijijiapan, Chiapas; (10) TUX = Tuxtla Chico, Chiapas; (11) GUA = Retalhuleu, Guatemala; (12) SAL = La Paz, El Salvador; and (13) NIC = Las Plazulas, Granada, Nicaragua. Dotted line represents the Isthmus of Tehuantepec.

(Fig. 1 and Appendix). Haplotype group M (haplotypes h19–21) included samples restricted to the isolated Veracruz population (Fig. 3), matching the medium form. Haplotype group S1 (haplotypes h25–35) included samples restricted to the western Pacific coast of Mexico (Fig. 3), matching the small form. Haplotype group S2 (haplotypes h22 and h24) included samples from the center of the Isthmus of Tehuantepec, Mexico, matching the small form, and five individuals from Laguna La Joya populations in Chiapas (haplotype h23) matched the large form. Group S2 intergrades with group L1 in Laguna La Joya (Fig. 3). L1 (haplotypes h1–7) included samples from the eastern part of the Isthmus of Tehuantepec, and three from Guatemala, matching the large form. Group L1 also intergrades with group L2 in Retalhuleu, Guatemala (Fig. 3). Haplotype group L2 (haplotypes h8–18) included two samples from Guatemala and all samples from Nicaragua, El Salvador, and Costa Rica (Fig. 3), matching the large form.

All trees (Fig. 4) recovered the Rufous-naped Wren as monophyletic in relation to the eight outgroup species. The Bayesian majority-rule consensus tree from the partitioned data set (Fig. 4A) had −LnL = 2,173.72, and the nonpartitioned data set had −LnL = 2,313.98 (arithmetic means). The Bayes factor (Kass and Raftery 1995) indicated that the tree from the partitioned data set was superior ($2\log B_{10} = 354.66$), and we chose it as our Bayesian tree. The maximum-likelihood tree (not shown) had −LnL = 2,429.9004, with the same topology as the partitioned-data Bayesian tree. The strict consensus (Fig. 4B) of 24 equally parsimonious cladograms had a length of 612 steps. There were no significant differences between trees using likelihood or parsimony criteria (not shown; SH and KH tests).The molecular-clock hypothesis was rejected for all trees obtained by the two phylogenetic methods (Bayesian: $\chi^2 = 140.08$, df = 41, $P = 9.18 \times 10^{-13}$; maximum parsimony: $\chi^2 = 89.51$, df = 41, $P = 1.81 \times 10^{-5}$).

There are well-supported similarities in all trees. The Bayesian (Fig. 4A) and maximum-parsimony (Fig. 4B) trees generally resemble the haplotype network (Fig. 2). In both trees, haplotype groups M, S1, and S2 are a well-supported clade. Haplotypes in

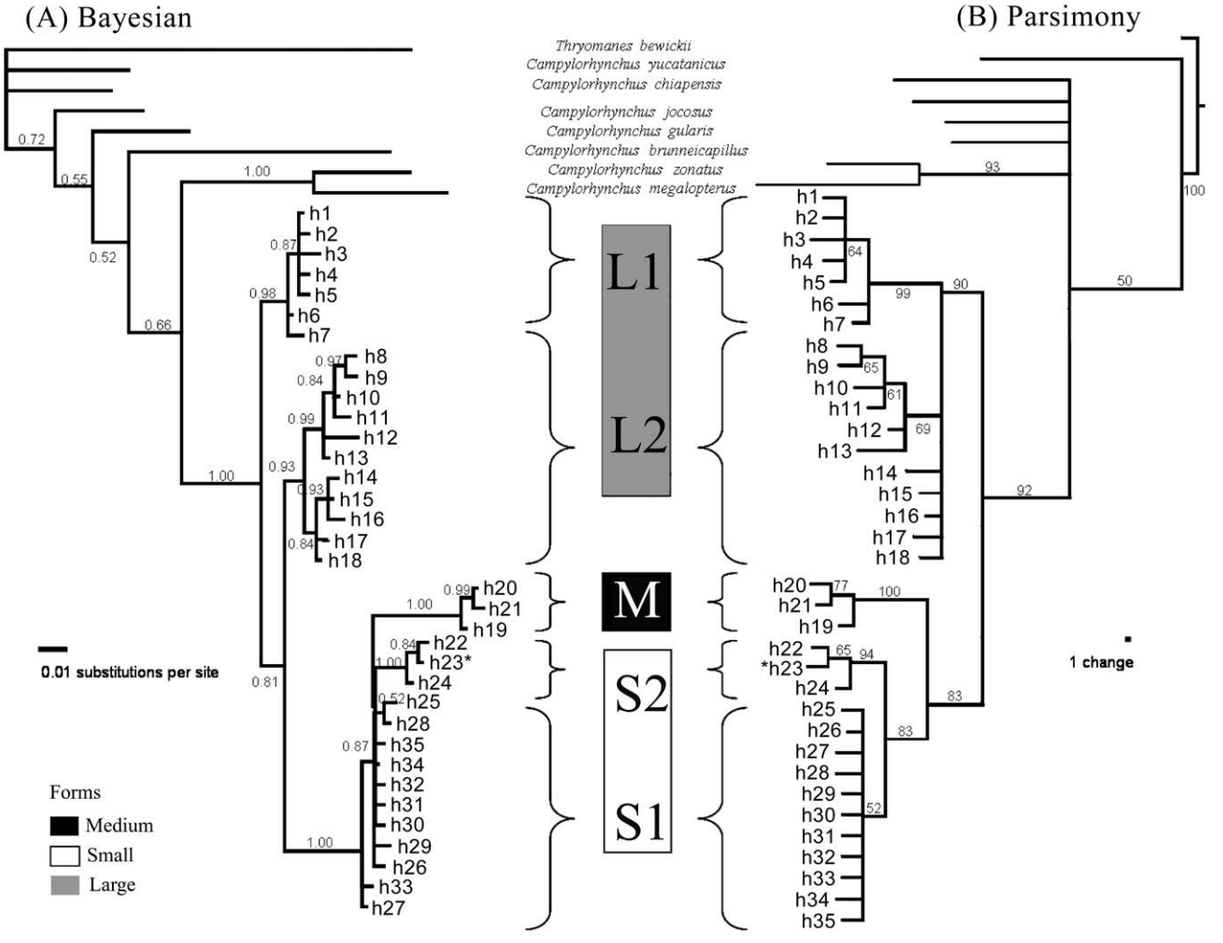

Fig. 4. Phylogenetic relationships of the unique haplotypes of Rufous-naped Wren. Haplotypes marked with an asterisk have a distinct morphological assignment. (A) Bayesian tree branch-support, depicting posterior probabilities of the clades. (B) Parsimony tree with length = 600, consistency index = 0.57, and retention index = 0.75. Capital letters refer to haplotype groupings found on the network (Fig. 2). Branch support depicts >50% bootstrap values for each clade; branches <50% are shown collapsed. Haplotypes belonging to a particular haplotype grouping are delimited by brackets.

M, S2, and L1 are monophyletic in both trees with high posterior probabilities and bootstrap support. The parsimony tree shows small, medium, and large forms to be reciprocally monophyletic and well supported by bootstrap values >70%.

Conflicts among trees are not well supported. In the Bayesian tree (Fig. 4A), S1 haplotypes are a paraphyletic grade in relation to M and S2. Groups M and S2 form a polytomy with low posterior probabilities. By contrast, in the parsimony tree (Fig. 4B), haplotype group S1 individuals are monophyletic but have a low bootstrap value. However, parsimony shows that haplotypes in L2 are paraphyletic in relation to haplotypes in L1.

Genetic Diversity, Differentiation, and Demography

Descriptive statistics and demographic history.—The population with the highest haplotype diversity is GUA, followed by MAN, JOY, NIC, and SAL (Table 1). The intermixed population JOY, despite having haplotypes from different groups, shared haplotypes with only the PIJ and TUX populations (L1 group). Population GUA shared the most common haplotype of L1 (h1) with JOY, GUA, and TUX. In individual populations, nucleotide diversity (×100) ranged from 0.00 to 0.32. Nucleotide diversity in the GUA and JOY populations, which include hybrids, is 10× higher (Table 1). Considering individual populations, different tests of demographic expansions were inconsistent (Table 1). However, considering haplotype groups, all tests of population growth in M and S2 failed to reject stasis. Groups S1 and L1 showed significant growth in three out of four tests (Fs, D, and R2; Table 1). Also, group L2 showed evidence of population expansion in just one test (MM; Table 1). This indicates strong evidence of expansion on the western Pacific slope and Chiapas, and moderate evidence in Central America.

TABLE 1. Genetic diversity and demographic patterns in haplotype groups and populations of Rufous-naped Wren. For population codes, see Figure 4. Sample sizes (*n*) are given with unique haplotypes in parentheses. Haplotype (*h*) and nucleotide diversity (π) values are shown ± SD. *P* values are given to the right of Fu's *Fs*, Tajima's *D*, and *R*2 statistics (*MM* = mismatch distribution; NS = $P > 0.05$; NC = nonconvergence in the bootstrap replications or simulation samples). Values marked with an asterisk indicate population expansions. Populations that show introgression are in bold.

Group or population	*n*	*h*	π (×100)	F_s	*P*	*D*	*P*	*MM*	*R*2	*P*
M	11 (3)	0.62 ± 0.10	0.1330 ± 0.121	−0.02	NS	0.20	NS	NC	0.198	NS
(1) VER	8 (2)	0.54 ± 0.12	0.0979 ± 0.103	0.87	NS	1.17	NS	NC	0.268	NS
S1	46 (11)	0.64 ± 0.07	0.1588 ± 0.126	−8.07	0.00*	−1.79	0.02*	NC	0.050	0.02*
(2) PET	12 (3)	0.32 ± 0.16	0.0914 ± 0.094	−0.61	NS	−1.63	0.04*	NS*	0.198	NS
(3) TEC	11 (4)	0.60 ± 0.15	0.1263 ± 0.117	−1.52	0.02*	−1.11	NS	NC	0.140	0.02*
(4) MAN	8 (5)	0.86 ± 0.11	0.2416 ± 0.191	−2.10	0.02*	−0.62	NS	NC	0.141	0.06
(5) PTO	11 (3)	0.47 ± 0.16	0.1130 ± 0.108	−0.31	NS	−0.29	NS	NS*	0.180	NS
S2	14 (4)	0.58 ± 0.09	0.1165 ± 0.108	−0.04	NS	0.04	NS	NC	0.178	0.43
(6) CPL	5 (2)	0.40 ± 0.23	0.0731 ± 0.093	0.09	NS	−0.82	NS	NC	0.400	NS
(7) TAP	4 (1)	0.00 ± 0.00	0.0000 ± 0.000	NC	NC	0.00	NS	NC	NC	NC
(8) JOY	**16 (5)**	**0.73 ± 0.08**	**2.0018 ± 1.076**	**7.17**	**NS**	**1.63**	**−0.06**	**NS***	**0.208**	**NS**
L1	33 (7)	0.47 ± 0.10	0.1600 ± 0.128	−3.07	0.01*	−1.84	0.02*	NS*	0.058	0.00*
(9) PIJ	10 (3)	0.51 ± 0.16	0.1016 ± 0.102	−0.59	NS	−0.69	NS	NC	0.174	NS
(10) TUX	9 (1)	0.00 ± 0.00	0.0000 ± 0.000	NC	NC	0.00	NS	NC	NC	NC
(11) GUA	**4 (4)**	**1.00 ± 0.18**	**1.7063 ± 1.189**	**0.25**	**NS**	**1.43**	**NS**	**NS***	**0.218**	**NS**
L2	24 (11)	0.89 ± 0.04	0.7061 ± 0.410	−1.98	NS	−0.87	NS	NS*	0.097	NS
(12) SAL	6 (3)	0.73 ± 0.16	0.3169 ± 0.246	0.76	NS	−0.06	NS	0.03	0.229	NS
(13) NIC	10 (4)	0.73 ± 0.12	0.2397 ± 0.184	−0.31	NS	−0.28	NS	NS*	0.188	NS

Population structure and differentiation.—Both AMOVAs showed that more than half of the variation is explained by differences among forms. The second-largest source of variation is differences among populations within forms, and the third source is within-population variation. The relative levels of variation are similar in both AMOVAs. The fixation index excluding intermixed populations from Laguna La Joya and Guatemala (Φ_{st} = 0.96 [among forms: 74%; among populations within forms: 22%], $P < 0.001$) shows almost complete genetic differentiation among forms. Even including intermixed populations, AMOVA still shows high genetic differentiation (Φ_{st} = 0.87 [among forms: 67%; among populations within forms: 19%], $P < 0.001$).

Pairwise ETPD results demonstrate that pairwise Φ_{st} values between populations of distinct haplotype groups are significant ($P < 0.001$; Table 2). Gene flow among haplotype groups is limited (Nm = 0.01–0.88; Hudson et al. 1992; Table 2). However, comparisons between intermixed and L1 populations (PIJ, TUX, JOY, and GUA) showed higher gene flow and no population differentiation (Nm = 1.47–6.25). Thus, intermixed populations JOY and GUA apparently correspond to L1 populations. Within the same haplotype group, there were low levels of population differentiation and high gene flow (Nm = 2.16–∞; Table 2). Even so, gene flow between populations on each side of the range of S1 is low (Nm = 0.55). All IBD test variants were significant (data not shown), indicating dispersal among adjacent populations. Central American populations (SAL and NIC) also showed population differentiation (Table 2). Those two populations were even well differentiated from population GUA. This suggests that haplotype groups are genetically separated, along with five population partitions on the Pacific coast: western coast of Mexico, center of the Isthmus, Chiapas coast including Guatemala, El Salvador coast, and Nicaragua coast.

Hybrid individuals.—Only five specimens from three localities near Laguna La Joya (Fig. 3) in Chiapas did not match morphology (*C. r. nigricaudatus*; based on plumage) and haplotype group (S2: haplotype h23). All other specimens from La Joya display a clear genetic correspondence to the large form (Fig. 1). From this comparison, we considered the five mismatched specimens to be hybrids (see samples marked with an asterisk in Appendix). In addition, two specimens from Retalhuleu, Guatemala, correspond to group L2 and two to group L1. On the basis of photographic evidence (not shown), those four specimens have mtDNA haplotypes that are discordant with their subspecific morphology (L2 birds have full black tails and L1 birds have barred tails; see Fig. 1). Because of the similarities between all large-form birds and the clinal variation in tail pattern, we were unable to determine whether Guatemalan specimens were hybrids by mere morphological comparison (see below).

Presence of whiskers and barred undertail feathers.—We found these two traits in several specimens from throughout Central America, considerably south of Selander's (1964) hybrid zone (Table 3). Some individuals showed those two traits clearly, whereas others showed only hints of them. Thus, these two traits occur in some large-form birds outside of the hybrid zone.

DISCUSSION

Phylogenetic relationship among forms.—The geographic structure found in the mtDNA haplotype network suggests three divergent groups. The small, medium, and large forms all differ substantially from each other in sequence divergence, revealing strong evidence for their evolutionary distinctiveness.

Table 2. Genetic differentiation and gene flow in populations of Rufous-naped Wren. Data below the diagonal are pairwise Φ_{st} values among populations, with migrants per generation (*Nm*) in parentheses. Data above the diagonal are pairwise ETPD *P* values among populations. Populations are arranged from west to east. Each population is labeled with its correspondent haplotype group. Values in bold correspond to comparisons between the populations with introgression (JOY and GUA). Asterisks indicate significance at $P < 0.01$(*) and $P < 0.001$(**).

	VER: M	PET: S1	TEC: S1	MAN: S1	PTO: S1	CPL: S2	TAP: S2	**JOY**	PIJ: L1	TUX: L1	**GUA**	SAL: L2	**NIC: L2**
(1) VER	—	**	**	**	**	**	**	**	**	**	*	**	**
(2) PET	0.96 (0.02)	—	NS	*	**	**	**	**	**	**	*	**	**
(3) TEC	0.96 (0.02)	0.03 (16.37)	—	NS	**	**	**	**	**	**	**	**	**
(4) MAN	0.93 (0.04)	0.21 (2.35)	0.20 (2.16)	—	NS	*	*	**	**	**	NS	*	**
(5) PTO	0.96 (0.02)	0.48 (0.55)	0.45 (0.62)	0.01 (−124.46)	—	**	**	**	**	**	*	**	**
(6) CPL	0.97 (0.01)	0.91 (0.05)	0.88 (0.06)	0.83 (0.09)	0.90 (0.05)	—	NS	**	**	**	*	**	**
(7) TAP	0.98 (0.01)	0.93 (0.03)	0.90 (0.03)	0.85 (0.06)	0.92 (0.03)	−0.05 (∞)	—	**	**	**	*	**	**
(8) JOY	**0.68 (0.18)**	**0.61 (0.28)**	**0.60 (0.28)**	**0.55 (0.31)**	**0.59 (0.29)**	**0.53 (0.26)**	**0.52 (0.25)**	—	**NS**	**NS**	**NS**	**	**
(9) PIJ	0.98 (0.01)	0.97 (0.01)	0.97 (0.02)	0.95 (0.02)	0.97 (0.02)	0.98 (0.01)	0.98 (0.01)	**0.18 (1.62)**	—	NS	NS	**	**
(10) TUX	0.99 (0.01)	0.99 (0.01)	0.98 (0.01)	0.97 (0.02)	0.98 (0.01)	0.99 (0.00)	1.00 (0.00)	**0.19 (1.44)**	0.06 (6.25)	—	*	**	**
(11) GUA	**0.87 (0.12)**	**0.87 (0.17)**	**0.85 (0.17)**	**0.80 (0.19)**	**0.85 (0.17)**	**0.81 (0.14)**	**0.79 (0.13)**	**0.10 (3.87)**	**0.44 (1.76)**	**0.47 (1.47)**	—	**NS**	*
(12) SAL	0.96 (0.02)	0.95 (0.03)	0.94 (0.04)	0.92 (0.04)	0.95 (0.03)	0.95 (0.03)	0.95 (0.02)	**0.50 (0.35)**	0.92 (0.05)	0.95 (0.04)	0.43 (0.88)	—	**
(13) NIC	0.96 (0.02)	0.95 (0.03)	0.94 (0.03)	0.92 (0.04)	0.95 (0.03)	0.95 (0.02)	0.95 (0.02)	**0.50 (0.42)**	0.91 (0.05)	0.94 (0.03)	0.55 (0.77)	0.75 (0.17)	—

Analyses of mtDNA (Fig. 4) show that the medium and small forms are sister taxa, which is corroborated by their sharing patterned backs, tails, dorsal parts, and relatively small body size (Fig. 1). Even some immature small-form specimens show hints of spots on the chest, similar to birds of the medium form (H. Vázquez-Miranda pers. obs.). However, paraphyly is evident in the Bayesian tree. Paraphyly is usually attributed to incomplete lineage sorting, hybridization, or incorrect taxonomy (Funk and Omland 2003). Either of the two former causes would seem plausible here, because lineages appear to have diverged recently and are geographically adjacent. We did not find haplotypes from M, S1, and L2 groups at La Joya (only S2 and L1 groups, which are the closest geographically and not sister taxa), which suggests secondary contact. This indicates that the mtDNA paraphyly is likely caused by recent introgression.

Geographic patterns.—We found strong spatial structuring across the Isthmus of Tehuantepec. This isthmus apparently began forming in the Middle to Late Pliocene (Barrier et al 1998), and it is possible that a marine barrier separated the east and west lowlands during the Late Pliocene (Mulcahy et al. 2006; but see Campbell 1999). If an ancestral population existed in the Mesoamerican lowlands, a Late Pliocene seaway could have caused population isolation for this highly sedentary taxon. Although our data do not fit a molecular clock, the 4.1% average sequence divergence between the large and small or medium forms suggests a Late Pliocene divergence, using generally accepted rates for birds (1.6–2.0% Ma^{-1}; Fleischer et al. 1998). Divergence estimates from other taxa on the isthmus are also consistent with a Late Pliocene division (Sullivan et al. 2000, Mulcahy et al. 2006). Two cytochrome-*b* estimates placed the divergence of Rufous-naped Wren either in the Early Pleistocene (0.8–1.7 mya; Barker 2007) or within the Pliocene–Pleistocene boundary (1.4–2.4 mya; Barker 1999). Using a more recent ND2 rate (2.7% Ma^{-1}; Arbogast et al. 2006), the divergence would be closer to the Early Pleistocene (1.5 mya). All these time estimates predate a Late Pleistocene divergence, as suggested for several North American bird taxa (Klicka and Zink 1997). The confidence-interval overlap for all estimates make it difficult to reject a correlation between the Isthmus split and clade divergence in the absence of a specific rate for our data.

A seemingly plausible alternative to a marine isolating barrier is habitat diversification. However, diversification and expansion of some members of the dry forest (*Bursera* trees) predate the isthmus formation by 19–22 million years, with insignificant increases in diversity for the past 5 million years (Becerra 2005). This is far outside the margin of error of any available molecular estimates of divergence, making it unlikely that Rufous-naped Wren speciation was correlated with diversification of dry forest.

Wrens of the genus *Campylorhynchus* are highly territorial, with tight social groups (Rabenold 1990). The social structure of the Rufous-naped Wren leaves detectable signatures on genetic variation. Gene flow exists only between neighboring populations of the same haplotype group (*Nm*; Table 2). Even between the extremes of the S1 group, there is limited gene flow (Table 2). This indicates that long-distance dispersal is unlikely, allowing for genetic divergence along the Pacific coast. The levels of genetic diversity of each population (π; Table 1) are also consistent with this wren's social structure. Birds that breed with members of the same or neighboring social groups are likely to have small

TABLE 3. List of voucher specimens of individuals included in the large form of Rufous-naped Wren that have traits typical of the small and medium forms. Specimens marked with an asterisk were used in the present study and are included in the Appendix.

Heavily marked whiskers, heavily marked undertail coverts, or both							
Mexico	MZFC	*CHIS235	*CHIS237	*AMTB15	*AMTB16		
Guatemala	KU	72498	72497	AMNH	399221	395857	395852
El Salvador	KU	18709	109650	*B9037	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, lightly marked undertail coverts, or both							
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

Museum acronyms: MZFC = Museo de Zoología "Alfonso L. Herrera," Universidad Nacional Autónoma de Mexico; KU = University of Kansas Natural History Museum and Biodiversity Research; USNM = National Museum of Natural History, Smithsonian Institution; and AMNH = American Museum of Natural History.

effective population sizes that do not allow accumulation of as much genetic diversity as is found in nonsocial species (Spellman et al. 2007). Other species with similar behaviors show IBD resulting from restricted natal dispersal and gene flow (Spellman et al. 2007).

We detected population separation along the Pacific slope (Table 2). Even within the same haplotype group, there were significant differences, especially between Central American populations. It is likely that these population partitions are evolutionarily significant units (ESUs; Moritz 1994), though not all of them are reciprocally monophyletic. Conservation in Mesoamerica is difficult because present-day anthropogenic disturbance reduces natural habitats in favor of cultivation and grazing (Challenger 1998). Mexican dry forests represent one of the largest gaps of protected areas in the world (Brooks et al. 2004). Detailed population studies should be conducted to ensure protection of these ESUs, given that only the population from Veracruz is currently under a conservation regime (Diario Oficial de la Federación 2001).

Contact zones.—We found evidence of population expansion in three haplotype groups (S1, L1, and L2; Table 1). The Chiapas group (L1) is placed at the center of the two contact points (Fig. 3). We detected that the mtDNA hybrids in La Joya had a distinctive haplotype (h23), which differed by one mutational step from the S2 group. Selander (1964, 1965) also suggested that the hybrid zone was formed by secondary contact, perhaps promoted by habitat disturbance near the Laguna La Joya, perhaps <100 years ago. By increasing the sampling, it may be possible to find haplotype h23 in S2 populations, which would support Selander's (1964, 1965) hypothesis. However, if we sampled extensively and found it to be restricted to the hybrid zone, we would conclude that introgression occurred earlier. At the moment, our data are more consistent with the latter proposal. Gene flow and population differentiation tests indicate significant population isolation of Laguna La Joya from S2 populations (Table 2). The most parsimonious explanation suggests a brief period of hybridization sometime in the past, with little or no current gene flow (Table 2).

Selander (1964) concluded that nearly all of his 125 specimens from the hybrid zone showed evidence of mixed ancestry in size and plumage. He also mentioned that he observed breeding pairs of birds with different forms. However, none of our new series of specimens from the hybrid zone shows such intermediate plumage (all resemble *C. r. nigricaudatus*; vouchers MZFC CHIS113, 114, 137, 320, and 326 in Appendix). Song characters of the small form are not found in the hybrid zone (Sosa 2007), and all are typical of the large form. Only song frequency, correlated to body size, is intermediate in hybrid-zone birds (Sosa 2007). These observations suggest that the presence of whiskers and barring on the undertail coverts do not necessarily indicate the level of hybridization. In addition, it is possible that the characteristics of birds in the hybrid zone have changed over time or that the zone is moving.

The population in Guatemala shows a mixing of haplotype groups L1 and L2. Those groups would seem to correspond to *C. r. nigricaudatus* and *C. r. capistratus*, respectively. There are several plausible explanations for the intermixing: retained ancestral polymorphisms, sympatric lineages, or a secondary contact zone. These two groups diverged recently; therefore, incomplete lineage sorting is a plausible explanation. Alternatively, secondary contact of these two groups cannot be rejected, given the population expansions of the L1 and L2 groups. Either process could have caused the large form to occur at both ends of the distribution with intermixing in the middle (Fig. 1), but we need coalescent estimates to distinguish incomplete lineage-sorting from secondary contact (Knowles and Maddison 2002). Both lineages living in sympatry is unlikely, because the vouchers we used from Guatemala show a disparity between their haplotype group and subspecific morphology; *C. r. capistratus* replaces *C. r. nigricaudatus* east of Escuintla (eastern Guatemala; Selander 1964).

There is also a genetically unsampled population of *C. rufinucha* in the San Pedro Sula Valley of Honduras. Specimens from this

population morphologically resemble birds from Chiapas and Guatemala (Monroe 1968). Additional specimens from this Honduras population are required to test for genetic differentiation and phylogeographic structure on the Central American Atlantic slope.

Taxonomic implications.—Our AMOVA results suggest that even though hybridization has occurred, it does not seem to have diminished the morphological (Selander 1964) and genetic divergence of the three groups of Rufous-naped Wren. This has particularly interesting taxonomic implications, because application of different species concepts leads to contrasting taxonomic decisions (Lovette 2004b). Our genetic data revealed three phylogenetically distinct lineages (Figs. 2 and 4). We delimited species on the basis of multiple criteria: distinct lineages generally with strong bootstrap support; high levels of divergence among lineages, similar to other pairwise values between well-recognized avian species (Lovette 2004a); and morphometric differentiation (Selander 1964). On the basis of this evidence, we propose the following taxonomic recommendations for this group. (1) *Campylorhynchus rufinucha* (Lesson 1838), the medium form, including individuals from Veracruz (M). (2) *Campylorhynchus humilis* (Sclater 1856), the small form, including individuals from the western Pacific Coast (S1) and the populations from the center of the Isthmus of Tehuantepec (S2). There is not enough support to consider S1 phylogenetically independent from S2. (3) *Campylorhynchus capistratus* (Lesson 1842), the large form, including individuals from Chiapas (L1) and Central America (L2). There is significant population differentiation between the Chiapas (most individuals correspond to *C. r. nigricaudatus*) and the Central American (most individuals correspond to *C. r. capistratus*) populations, but they are not reciprocally monophyletic in the parsimony tree, and L2 has low posterior probability support in the Bayesian tree (Fig. 4). There is no clear differentiation in their morphometrics (Selander 1964) or song (Sosa 2007). It is possible that the L1 populations constitute a separate evolutionary lineage; however, at this point we do not have enough evidence to separate L1 from L2.

Employing multiple criteria (de Queiroz 1998, Helbig et al. 2002) helps identify evolutionary lineages and provides new insights for future research. In our case, multiple criteria suggest that deep lineage distinction exists, and the fact that there is or was limited hybridization should not negate these distinctions. In the Rufous-naped Wren, multiple criteria support the recognition of three separate species. Our results provide a case study of hybridization as a part of the evolutionary process that should not be the sole criterion for species recognition.

Acknowledgments

This work received funding from Semarnat–Consejo Nacional de Ciencia y Tecnología [CONACYT] Sectorial Fund (C01-0265). H.V.-M. was supported by CONACYT and Dirección General de Estudios de Posgrado, Universidad Nacional Autónoma de México [UNAM] graduate scholarships and a graduate travel grant from Posgrado en Ciencias Biológicas, UNAM. K.E.O. was supported by National Science Foundation grant DEB-0347083. The Omland laboratory is a participant in the Smithsonian Ornithology group. R. Sosa, V. Rodríguez, S. López, and A. Calderón participated intensively in field collecting in Mexico. N. Cortés (UNAM) and J. Peters and B. Kondo (University of Maryland, Baltimore County [UMBC]) greatly helped in all lab procedures. L. Kiere proofread the manuscript. J. Leips and the UMBC *Drosophila* lab crew allowed us to process skin samples. M. Mika provided photographs of specimens from the University of Nevada, Las Vegas [UNLV]. We received tissue loans from J. Klicka (UNLV), M. Robbins and A. T. Peterson (University of Kansas [KU]), D. Willard (Field Museum of Natural History), S. Birks (University of Washington Burke Museum), and P. Sweet (American Museum of Natural History [AMNH]). A. T. Peterson, J. Dean, and P. Sweet allowed us to inspect their specimen holdings at KU, the National Museum of Natural History (USNM), and AMNH, respectively. M. Okada, C. Blake, and M. LeCroy assisted us greatly at the AMNH. T. Chesser helped us get literature from USNM libraries. We thank J. Klicka, R. Zink, and four anonymous reviewers for their comments and thoughtful suggestions. We appreciate the efforts and fellowship of R. Sosa, V. Rodríguez, S. López, H. Moya, L. Sánchez, A. Calderón, J. Klicka, G. Spellman, M. Mika, M. Robbins, A. T. Peterson, N. Herrera, C. Zaldaña, Á. Nyári, F. Delgado, G. F. Barrowclough, and F. J. Sahagún. PRIMER3 is available at frodo.wi.mit.edu/primer3. NETWORKS is available at www.fluxus-technology.com.

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Associate Editor: J. Klicka

APPENDIX. List of specimens used in the present study. Given for every outgroup and unique haplotype are GenBank accession number, museum[a] where the skin is deposited, specimen tissue identification number (in parentheses), and locality where collected. All specimens marked "CHIS" were collected for the present study. Asterisk denotes mtDNA hybrid specimen, and plus sign denotes skin sample.

	GenBank accession	Specimen catalogue numbers and localities
		Outgroup
Thryomanes bewickii	GQ241988	MZFC (QRO29), Mexico: Querétaro, El Derramadero
Campylorhynchus jocosus	GQ241986	MZFC (HMM02-1), Mexico: Puebla, San Juan Raya
C. chiapensis	GQ241982	MZFC (CHIS-244), Mexico: Chiapas, Tuxtla Chico, Rancho El Porvenir
C. yucatanicus	GQ241981	MZFC (B603), Mexico: Yucatán, Rancho Sinkhuel, 18 km E Dzilam de Bravo
C. megalopterus	GQ241985	MZFC (FD65), Mexico: Estado de México, km 14 carr. Ocuilan-Cuernavaca
C. brunneicapillus	GQ241984	MZFC (CONACYT648), Mexico: Baja California Sur, Rancho Monte alto, 15 km N San Javier
C. gularis	GQ241987	MZFC (FMNH393977), Mexico, Nayarit, Sierra de Nayarit
C. zonatus	GQ241983	MZFC (CHIS565), Mexico: Veracruz, Jamapa
		Ingroup
Medium form		
h19	GQ241946	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	GQ241947	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	GQ241948	MZFC (MZFC3341+), Mexico: Veracruz, Alvarado, km 23–25, 180 Highway Veracruz-Alvarado
Small form		
h22	GQ241958	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	GQ241970	MZFC (CONACYT04-18), Mexico: Oaxaca, San Carlos Yautepec, Cerro Piedra Larga, Base
h25	GQ241959	MZFC (CHIS399, 450), Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río; UNLV (DHB5580, 5581, MM105, 107, GMS924, 925), Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido; MZFC (JK04-76, 235), Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido
h26	GQ241960	MZFC (CONACYT1049, 1050), Mexico: Michoacán, Lázaro Cárdenas, Presa Infiernillo 1 km N Camp CFE; MZFC (CHIS400, 470, 471), Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río. UNLV (JK04-75) and 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, 553,546, 555, 561), Mexico: Guerrero, Petatlán, Los Cirilos
h27	GQ241961	MZFC (CHIS435), Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río
h28	GQ241962	MZFC (CHIS444), Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río
h29	GQ241963	MZFC (CHIS449), Mexico: Oaxaca, San Pedro Mixtepec, Manialtepec, Río
h30	GQ241964	MZFC (CHIS484), Mexico: Guerrero, San Marcos, Tecomate
h31	GQ241965	MZFC (CHIS491), Mexico: Guerrero, San Marcos, Tecomate
h32	GQ241966	MZFC (CHIS492,499), Mexico: Guerrero, San Marcos, Tecomate
h33	GQ241967	MZFC (CHIS551), Mexico: Guerrero, Petatlán, Los Cirilos
h34	GQ241968	MZFC (CHIS552), Mexico: Guerrero, Petatlán, Los Cirilos
h35	GQ241980	MZFC (JK04-241), Mexico: Oaxaca, San Gabriel Mixtepec, 5 km N Puerto Escondido
Large form		
h1	GQ241949	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; UNLV (JK02-23), Guatemala: Retalhuleu, San Felipe Retalhuleu 5 km S, Finca El Niño
h2	GQ241955	MZFC (CHIS163), Mexico: Chiapas, Pijijiapan, Rancho Lluvia de oro
h3	GQ241956	MZFC (CHIS309), Mexico: Chiapas, Tonalá, Tres Picos, Llano; MZFC (CHIS333), Mexico: Chia pas, Tonalá, La Polka, Rancho Bellavista, Laguna La Joya
h4	GQ241957	MZFC (CHIS378), Mexico: Chiapas, Tonalá, Rancho La Industria

(*Continued*)

Appendix. Continued.

	GenBank accession	Specimen catalogue numbers and localities
h5	GQ241979	UNLV (JK03-7), Guatemala: Retalhuleu, San Felipe Retalhuleu 5 km S, Finca El Niño
h6	GQ241978	UNLV (JK03-482), Guatemala: Zacapa, Motagua Valley, 10 km E Rio Hondo
h7	GQ241969	MZFC (CHIS99, 100), Mexico: Chiapas, Pijijiapan, Rancho Lluvia de oro; MZFC (CHIS308), Mexico: Chiapas, Tonalá, Tres Picos, Llano
h8	GQ241953	KU (B9378), El Salvador: Usulutlan, 2.6 km E Boca del Rio Lempa
h9	GQ241950	KU (B7654, 7803), El Salvador: San Vicente, Volcán San Vicente
h10	GQ241952	KU (B7690), El Salvador: San Vicente, Volcán San Vicente
h11	GQ241951	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	GQ241976	UNLV (DHB4438), Guatemala: Quetzaltenango, Santa María de Jesús, 5 km SSW, Finca de Santa María
h13	GQ241977	UNLV (DHB4337), Guatemala: Retalhuleu, San Felipe Retalhuleu 5 km S, Finca El Niño; FMNH (FMNH434224), El Salvador: Sonsonate, Izalco, Cantón Cruz Verde, Finca Nuevos Horizontes
h14	GQ241975	UNLV (DAB1931), Nicaragua: Granada, Las Plazulas, Laguna Blanca
h15	GQ241974	UNLV (DAB1870, 1905), Nicaragua: Granada, Las Plazulas, Laguna Blanca
h16	GQ241972	UWBM (DAB1928), Nicaragua: Granada, Las Plazulas, Laguna Blanca; UNLV (DAB1855), Nicaragua: Granada, Las Plazulas, Laguna Blanca
h17	GQ241973	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	GQ241971	UWBM (DAB1576), Nicaragua: Chinandega, Casita, Ladera del Volcán Casita
h23*	GQ241954	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 abbreviations: MZFC = Museo de Zoología "Alfonso L. Herrera," Universidad Nacional Autónoma de Mexico; KU = University of Kansas Natural History Museum and Biodiversity Research; UNLV = 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.