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PHYLOGENETIC RELATIONSHIPS OF THE WHITE-THROATED BARBTAIL, *PREMNOPLEX TATEI* (FURNARIIDAE), AN ENDEMIC OF THE NORTHEASTERN MOUNTAIN RANGE OF VENEZUELA

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Abstract. The White-throated Barbtail, *Premnoplex tatei*, is a poorly known furnariid endemic to the northeastern mountain ranges of Venezuela. Although currently considered a distinct species, it has often been treated as a subspecies of its widespread congener, the Spotted Barbtail, *P. brunnescens*. Here we use mitochondrial and nuclear DNA sequences to assess the taxonomic status of *P. tatei* and its phylogenetic relationships. We found *P. tatei* to be the sister species of *P. brunnescens*, but the genetic divergence between the two species is large in comparison to that within other genera of the Furnariidae. This result is consistent with the plumage, vocal, and ecological differences used to support the recognition of *P. tatei* as a distinct species. Our results also corroborate previous studies in finding *Premnoplex* and *Margarornis* as sister genera. A close relationship between *P. tatei* and *Roraimia adusta*, a scenario hypothesized from the systematic affinities of taxa distributed in the northeastern mountain region of Venezuela and the Pantepui region, was not supported by this study.

Key words: biogeography, Furnariidae, molecular systematics, Premnoplex tatei, *Pantepui,* Roraimia, *taxonomy.*

Relaciones Filogenéticas de *Premnoplex tatei* (Furnariidae), una Especie Endémica de la Región Montana Nororiental de Venezuela

Resumen. *Premnoplex tatei* es una especie endémica de la región montana nororiental de Venezuela. Algunos autores consideran que esta especie representa una subespecie de *P. brunnescens*. En este estudio utilizamos secuencias de ADN mitocondrial y nuclear para evaluar el estado taxonómico de *P. tatei* y sus relaciones filogenéticas. Encontramos que *P. tatei* es la especie hermana de *P. brunnescens*, pero con una divergencia genética que supera niveles observados para otras especies de furnáridos del mismo género. Este resultado es consistente con las diferencias ecológicas, de plumaje y de vocalizaciones que son utilizadas para apoyar el reconocimiento de *P. tatei* como una especie válida. Nuestros resultados corroboran, igualmente, la relación hermana de los géneros *Premnoplex* y *Margarornis*. Una relación cercana entre *P. tatei* y *Roraimia adusta*, basada en la distribución de especies filogenéticamente relacionadas en las regiones montanas del noreste de Venezuela y el Pantepui, no fue validada por este estudio.

INTRODUCTION

The White-throated Barbtail, *Premnoplex tatei*, is a furnariid restricted to the montane areas of northeastern Venezuela. It is known from both the Serranía of Turimiquire and the Paria Peninsula, where it is isolated from the only other species in the genus, the Spotted Barbtail, *P. brunnescens*, which is distributed from Costa Rica to Bolivia, including the Coastal Cordillera in northern Venezuela (Fig. 1). *Premnoplex tatei* is a poorly known species considered threatened by conversion of forest to agricultural land, a fate shared by several species endemic to the northeastern montane region of Venezuela (BirdLife International 2000, Sharpe and Lentino 2008, IUCN 2009). Data that improve our knowledge of this species are clearly needed. In particular, studies aimed at understanding the species status of *P. tatei,* which has long been a source of debate, as well as its phylogenetic relationships, are of conservation and evolutionary significance.

Premnoplex tatei was described by Chapman (1925) on the basis of specimens collected in the Serranía of Turimiquire

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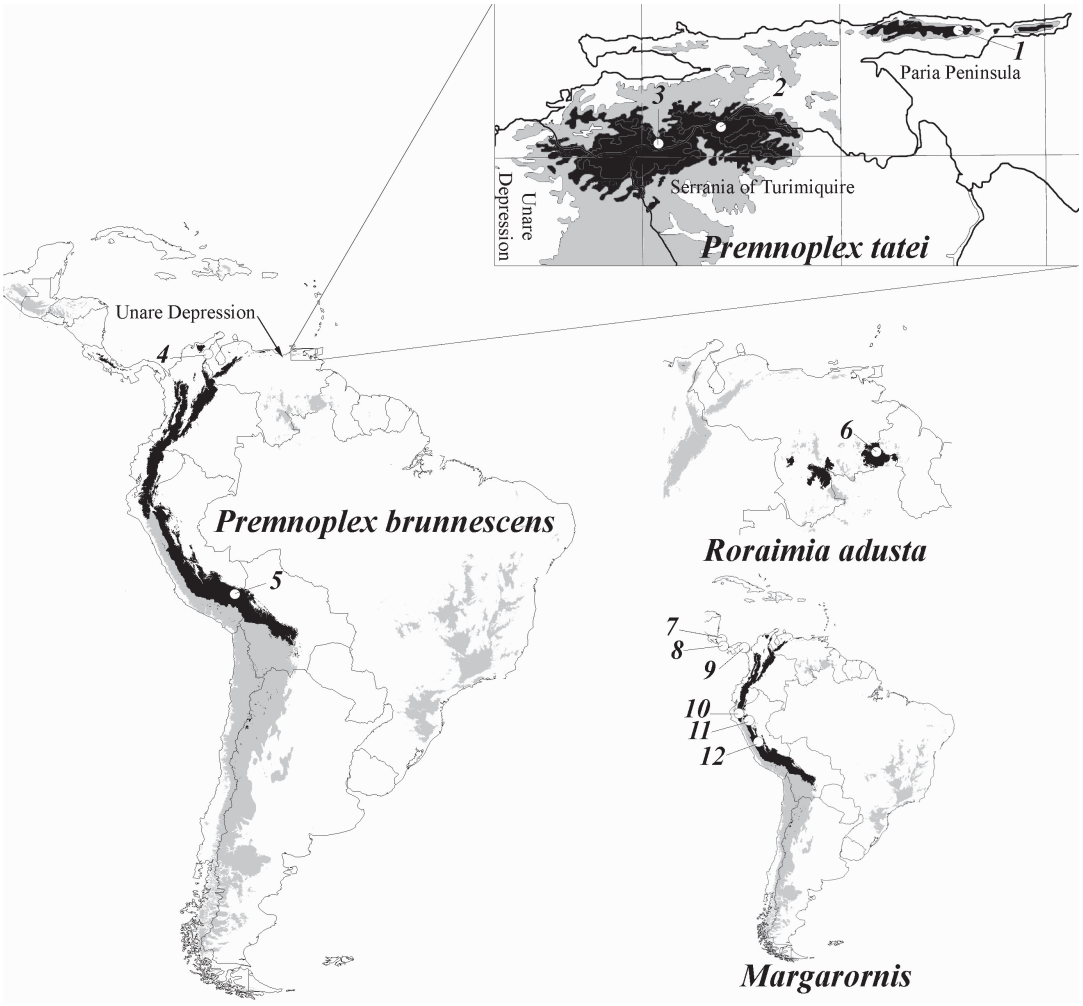

FIGURE 1. Distribution of *Premnoplex*, *Roraimia*, and *Margarornis*. White circles indicate localities at which samples used in this study were collected. Numbers correspond to collection localities as presented in Table 1.

in northeastern Venezuela. Although it is generally similar morphologically to *P. brunnescens* (Cherrie 1891), Chapman justified its status as a species on differences in plumage coloration, most notably the pronounced white streaking of the nape. Cory and Hellmayr (1925) did not consider these differences indicative of species status and so reclassified *P. tatei* as a subspecies of *P. brunnescens*. Following Chapman (1925), Meyer de Schauensee (1966) reelevated *P. tatei* to species rank. Two morphologically differentiated populations are recognized as subspecies, *P. t. tatei* from the Serranía of Turimiquire and *P. t. pariae* from the Paria Peninsula (Hilty 2003; Fig. 1). Recently, Areta (2007) documented significant differences in habitat use and vocal behavior that corroborated maintaining *P. tatei* as a species distinct from *P. brunnescens*. However, in terms of plumage and morphology, the differences between *P. tatei* and *P. brunnescens* are less than between many furnariid taxa currently ranked as subspecies (Vaurie 1980, Remsen 2003).

Relationships of the genus *Premnoplex* within the Furnariidae have also been controversial. Cory and Hellmayr (1925) placed *Premnoplex*, together with *Margarornis* and *Premnornis*, in the subfamily Margarornithinae on the basis of similarities in plumage and the presence of barbs on the rectrices. Vaurie (1980) later merged *Premnoplex*, *Margarornis*, *Premnornis*, and *Roraimia* into *Margarornis*, as did Sclater (1890) but without including *Roraimia*. Monophyly of this group (the "*Margarornis* assemblage") was supported by analyses of the hindlimb musculature (Rudge and Raikow 1992a, b), analyses that also identified *Premnornis* as the sister genus of *Premnoplex*. Recent molecular studies (Irestedt et al. 2006, Moyle et al. 2009), however, have shown these genera to represent a polyphyletic group, a finding that agrees with behavioral data (Dobbs et al. 2003, Areta 2007). The sister relationship between *Premnoplex* and *Margarornis* is the only remaining vestige of the former "*Margarornis* assemblage."

Phelps (1966) suggested an alternative hypothesis, that *P. tatei* and *Roraimia adusta*, a species endemic to the Guayana Highlands (Pantepui; Fig. 1), are closely related. Close phylogenetic relationships between taxa distributed in the northeastern mountain region of Venezuela and the Pantepui Region have been suggested in plants, reptiles, and birds (Mayr and Phelps 1967, Steyermark 1979, Miralles et al. 2005, Pérez-Emán 2005), a pattern that would support Phelps' hypothesis. These two species differ both in morphology and vocal behavior (Areta 2007), but the phylogenetic relationship of *P. tatei* to *Roraimia* has yet to be evaluated with molecular data. Given the increase of information showing that plumage pattern is not a reliable source of phylogenetic information in furnariids, a molecular study seems warranted (Irestedt et al. 2006, Fjeldså et al. 2007, Chesser et al. 2007, Moyle et al. 2009). Here, we use mitochondrial and nuclear DNA sequences to assess both the taxonomic status and phylogenetic relationships of *P. tatei*.

METHODS

We obtained vouchered samples during field work in Venezuela or from the genetic-resource collections of the Colección Ornitológica Phelps (COP), the LSU Museum of Natural Science (LSUMNS), the American Museum of Natural History (AMNH), the Field Museum of Natural History (FMNH) and the Smithsonian Tropical Research Institute (STRI) (Table 1). We included samples of geographically isolated populations of *P. tatei* from the Serranía of Turimiquire and the Paria Peninsula. Because the status of the population in the Serranía of Turimiquire is unclear, our samples from this locality consisted of unvouchered plucked feathers only. To assess intraspecific genetic variation in *P. brunnescens* coarsely, we included individuals from Peru and the Sierra de Perijá in Venezuela (Table 1). The selection of the remainder of tissue samples was designed to include a broad representation of furnariid lineages (Moyle et al. 2009). We also included one

TABLE 1. Samples used in this study with associated museum, voucher number, and localities.

Species and sample	Museum[a]	Voucher no.	Locality[b]
Premnoplex brunnescens 1	LSUMNS	B600	Abra de Maruncunca, Puno Department, Peru (5)
Premnoplex brunnescens 2	COP	IC811	Sierra de Perijá, Zulia, Venezuela (4)
Premnoplex tatei pariae	COP	JP251	Cerro Humo, Península de Paria, Sucre, Venezuela (1)
Premnoplex tatei tatei 1	IZET-UCV	PT-4	Cerro Turimiquire, Sucre, Venezuela (3)
Premnoplex tatei tatei 2	IZET-UCV	PT-1	Cerro Negro, Caripe, Monagas, Venezuela (2)
Margarornis rubiginosus 1	LSUMNS	B19795	Villa Mills, Cartago Province, Costa Rica (7)
Margarornis rubiginosus 2	LSUMNS	B28224	Cerro Punta Boquete, Chiriqui Province, Panama (8)
Margarornis bellulus 1	STRI	GA7	Darien Province, Panama (9)
Margarornis bellulus 2	STRI	GA24	Darien Province, Panama (9)
Margarornis squamiger 1	LSUMNS	B44474	Florida, San Martin Department, Peru (11)
Margarornis squamiger 2	LSUMNS	B8211	Millpo, Pasco Department, Peru (12)
Margarornis squamiger 3	LSUMNS	B31812	Quebrada Lanchal, Cajamarca Department, Peru (10)
Premnornis guttuligera 1	LSUMNS	B611	Abra de Maruncunca, Puno Department, Peru
Premnornis guttuligera 2	LSUMNS	B33864	Cordillera del Condor, Cajamarca Department, Peru
Roraimia adusta 1	FMNH	339646	La Escalera, Bolivar, Venezuela (6)
Roraimia adusta 2	FMNH	339647	La Escalera, Bolivar, Venezuela (6)
Lochmias nematura	LSUMNS	B7481	Cerro La Neblina, Amazonas, Venezuela
Lochmias nematura	LSUMNS	B7459	Cerro La Neblina, Amazonas, Venezuela
Synallaxis candei 1	COP	ML783	El Palmar, Zulia, Venezuela
Synallaxis candei 2	COP	ML800	El Palmar, Zulia, Venezuela
Synallaxis cinnamomea 1	COP	JP352	Cerro El Guamal, Anzoategui, Venezuela
Synallaxis cinnamomea 2	COP	JP237	Las Melenas, Peninsula de Paria, Sucre, Venezuela
Cranioleuca demissa	COP	JP295	Cerro Guaiquinima, Bolivar, Venezuela
Cranioleuca subcristata 1	COP	JP356	Cerro El Guamal, Anzoategui, Venezuela
Cranioleuca subcristata 2	COP	IC1064	Calderas, Barinas, Venezuela
Phleocryptes melanops	LSUMNS	B48218	Puerto Viejo, Lima Department, Peru
Pseudocolaptes boissonneautii	LSUMNS	B406	Cruz Blanca; 33 rd km SW Huancabamba, Piura Department, Peru
Microxenops milleri	LSUMNS	B4828	S Rio Amazonas, ca 10 km SSW mouth Rio Napo, Loreto Department, Peru
Dendrocincla fuliginosa	AMNH	SC771	Cerro La Neblina, base camp, Amazonas, Venezuela
Geositta cunicularia	LSUMNS	B103846	Cerro Cos Cantire, Arequipa Deparment, Peru

[a]LSUMNS, Louisiana State University Museum of Natural Science; COP, Colección Ornitológica Phelps; IZET-UCV, Instituto de Zoología y Ecología Tropical, Universidad Central de Venezuela; STRI, Smithsonian Tropical Research Institute; FMNH, Field Museum of Natural History; AMNH, American Museum of Natural History.
[b]Numbers following locality names correspond to those shown in Fig. 1.

sample of *Dendrocincla fuliginosa* and, as an outgroup, of *Geositta cunicularia* (Chesser 2004, Irestedt et al. 2006).

We used the DNeasy Tissue Kit (Qiagen) to isolate total genomic DNA from pectoral tissue or feathers. For feather samples, we added 30 µL of 10% (100 mg mL^{-1}) dithiothreitol to the digestion buffer. Three complete mitochondrial protein-coding genes were amplified by PCR: subunits 2 and 3 of the NADH dehydrogenase gene (ND2, 1041 base pairs [bp], and ND3, 351 bp) and subunit 2 of the cytochrome oxidase gene (COII, 684 bp). Additionally, we sequenced a portion of the mitochondrial cytochrome *b* gene (cyt *b*, 1045 bp) and intron 7 of the nuclear gene β-fibrinogen (βFib7, variable length). PCR conditions included an initial cycle of denaturating at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 sec, 50 °C (for ND2), 46 °C (for ND3), 45 °C (for cyt *b*), and 55 °C (for COII and βFib7) for 30 sec, then extension phases of 72 °C for 60 sec and finally 72 °C for 7 min. All amplifications were performed in a PTC-200 thermocycler (MJ Research). Each 50-µL reaction contained 5 µL of DNA template (~20 ng μL^{-1}), 1 unit of *AmpliTaq* polymerase (Applied Biosystems, Inc., Foster City, CA), 1.5 mM $MgCl_2$, 0.5 µM of each primer, and 0.2 mM dNTPs. Initial amplification of the complete genes with external primers was followed by amplification with internal primers for ND2 and cyt *b*. For amplification and sequencing of ND2 we used primers L5215, H5766, L5758, and H6313 (Hackett 1996, Johnson and Sorenson 1998), for cyt *b* primers L14990, H658, P5L, and H16065 (Kocher et al. 1989, Helm-Bychowski and Cracraft 1993, Johansson et al. 2002), for ND3 primers L10755 and H11151 (Chesser 1999), for COII primers L8263 and H9085 (Lee et al. 1997, Chesser 1999), and for βFib7 primers FIBI7U and FIBI7L (Prychitko and Moore 1997). Amplification products were purified by PEG precipitation before being cycle-sequenced with BigDye v3.1 (ABI), cleaned with Sephadex, and visualized with an ABI 3100 Genetic analyzer. We aligned sequences by eye, using the program SeqMan II (DNASTAR). All sequences were checked for stop codons, ambiguities in base calls, and mismatches in overlapping sequences produced by different sets of primers. We used IUPAC ambiguity codes to represent apparent heterozygosities. All new sequences generated in this study have been deposited in GenBank (accession numbers HM 125584–HM 125673). Sequences of *Dendrocincla fuliginosa* (AMNH SC771, ND3, COII, Fib7) were downloaded from Genbank (ND3 AY489537, 351 bp; COII AY489489, 684 bp; βFib7 AY489439, 864 bp). Camila Ribas provided ND2 and cyt *b* sequences from the same individual of *D. fuliginosa*. Because of amplification difficulties we built a chimera of *Lochmias nematura* from mitochondrial sequences from individual B7481 and a βFib7 sequence from B7459. These two individuals were collected from the same locality, and their COII and ND3 sequences were identical. Coding the nuclear-sequence positions as missing data would not change the results.

To examine the phylogenetic position of *Premnoplex tatei* we analyzed data from the ND2 gene of all species previously considered in the *Margarornis* assemblage (with the lone exception of *M. stellatus*), as well as representatives of all relevant furnariid lineages (Table 1). To increase the resolution of basal nodes, we followed this with an analysis encompassing all four mitochondrial genes plus the nuclear βFib7. This second analysis included fewer taxa, with a single representative from each of the lineages previously defined on the basis of ND2 analysis.

For the phylogenetic analyses, we used maximum likelihood in PAUP* 4.0b10 (Swofford 2002). We treated gaps in the alignment of nuclear introns as missing data. Using PAUP*, we calculated log likelihoods for a set of nested finite-sites-substitution models on a neighbor-joining tree reconstructed from an uncorrected percent-divergence matrix. We selected the best-fit maximum-likelihood models with the likelihood-ratio test in the program ModelTest (version 3.7, Posada and Crandall 1998). Using the best-fit model, we performed maximum-likelihood analyses with heuristic searches that included 10 random stepwise additions of taxa and the branch-swapping algorithm with tree-bisection reconnection. Nodal support was estimated with 100 bootstrap pseudo-replicates (Felsenstein 1985) with the same search criteria. For the five-gene dataset, we ran separate analyses with either the mitochondrial or the nuclear data, as well as one run that included all five genes together. We used the ILD test (Farris et al. 1995) for two (mitochondrial vs. nuclear) and five partitions (each gene region) to evaluate incongruence in phylogenetic signal among gene regions. We also conducted independent analyses with each gene to check for congruence in strongly supported nodes.

We used the program MrBayes (version 3.1; Ronquist et al. 2003) for a Bayesian analysis with all sequences. The DNA-substitution models used in the analysis were based on results from the Akaike information criterion (AIC) implemented in Mr. ModelTest (version 2.2, distributed by J. A. A. Nylander, Evolutionary Biology Center, Uppsala University). The Bayesian search had four chains running simultaneously for 2 000 000 generations, with trees sampled every 100 generations for a total of 20 000 saved trees. We conducted four independent runs from different random trees. Using the program TRACER (version 1.4; http://tree.bio.ed.ac.uk/software/tracer/), we plotted log-likelihood scores against generation time to identify the point at which log-likelihood values reached stationarity. Using AWTY (Wilgenbusch et al. 2004), we also examined convergence of the clades' posterior probabilities as a function of generation number, as well as the correlation of clade frequencies for pairs of different independent runs. We discarded, conservatively, the first five million generations as burn-in and, on the basis of convergence of the different runs, used the 15 000 saved trees from each dataset to produce a majority-rule consensus tree. As in the maximum-likelihood analysis, we analyzed the mitochondrial, nuclear,

and pooled data sets independently. We analyzed the pooled data with either two (mitochondrial and nuclear) or five partitions (each of the genes sequenced in this study). We unlinked all parameters across partitions and allowed the overall rate, varying under a flat Dirichlet prior, to be different across partitions. We compared the fit of these models with Bayes factors (Kass and Raftery 1995, Nylander et al. 2004).

We contrasted the hypothesis of monophyly and nonmonophyly of sets of species by calculating the likelihood of each hypothesis and statistically comparing them by means of the Shimodaira–Hasegawa test, with RELL optimization and 1000 bootstrap replicates (Shimodaira and Hasegawa 1999). We also calculated site-likelihoods under different hypotheses for each tree and compared those with Shimodaira`s approximately unbiased test (Shimodaira 2002) with the program Consel (Shimodaira and Hasegawa 2001).

Inferences of absolute ages of lineage divergence assume a molecular clock. In birds, divergence levels of 1.6% (Hawaiian honeycreepers; Fleischer et al. 1998) to 2.0% (*Branta*; Shields and Wilson 1987) in mitochondrial sequences are thought to reflect approximately one million years of evolution. For comparison, as a rough benchmark of divergence time for comparative purposes, we used the 2.0% sequence divergence calibration (Lovette 2004, Weir and Schluter 2008). To be consistent with these calibrations, we based our estimates on the cyt *b* sequences only and tested for significant departures from clocklike sequence evolution by likelihood-ratio tests. We calculated divergence time on the basis of genetic distances corrected with the finite-sites GTR + INV + G model.

RESULTS

We obtained complete ND2 sequences from all taxa and, from single individuals of each lineage defined by ND2 sequences, complete ND3, COII and βFib7 sequences and partial cyt *b* sequences. The level of variation (assessed only in individuals from which all five genes were sequenced) in each gene differed. ND2 contained the highest proportion of parsimony-informative sites (43.1% variable sites, 29.3% parsimony informative), more than that of ND3 (39.9% and 27.6%), cyt *b* (36.0% and 27.6%), and COII (33.5% and 24.6%). The length of βFib7 varied from 784 bp in *Phleocryptes melanops* to 881 bp in both *Lochmias nematura* and *Pseudocolaptes boissonneautii*. Among the gene regions we examined, this intron was the least variable, with 25.1% variable sites and 11.3% parsimony-informative sites. Most indels were autapomorphic, the largest being a 96-bp deletion in *Phleocryptes melanops*. *Synallaxis candei*, *Cranioleuca demissa*, *C. subcristata*, and *Roraimia adusta* shared a 27-bp deletion, and both species of *Synallaxis* (*candei* and *cinnamomea*) shared a 4-bp deletion. The alignment was straightforward to construct; the few ambiguities did not change the tree's topology significantly.

The finite-sites substitution model selected for each of the mitochondrial genes was GTR + Γ + I, and the optimal model for βFib7 was GTR + Γ. The phylogenetic signal of the mitochondrial and nuclear partitions differed significantly ($P < 0.01$) but was marginally significant when only mitochondrial gene regions were used ($P = 0.05$). This was a consequence of the ND3 region because excluding this region resulted in a nonsignificant difference in phylogenetic signal. The phylogenetic analyses of independent gene regions yielded no conflicting topologies (results not shown) except for the placement of *Dendrocincla fuliginosa* and *Microxenops milleri* (deleting these two species from the dataset resolved the significant difference in phylogenetic signal between mitochondrial and nuclear gene regions). Therefore, for subsequent phylogenetic analyses, we combined gene regions under the null hypothesis of shared history (Chesser et al. 2007).

The mitochondrial ND2 data supported a monophyletic *Premnoplex* by both maximum likelihood (87% bootstrap) and Bayesian inference (0.99 posterior probability) (Fig. 2). Pairwise distances between the two species of *Premnoplex* averaged 16% (ND2, GTR + Γ + I; uncorrected = 10%). Haplotypes of *P. brunnescens* from Sierra de Perijá (Venezuela) and Department of Puno (Peru) were highly divergent (11.5%; uncorrected = 8.6%), whereas those of the two subspecies of *P. tatei* differed by only 2.5% (uncorrected = 2%). Support for the monophyly of *Margarornis* was also high (100%, 1.00), with *M. squamiger* and *M. bellulus* as closely related sisters (97%, 1.00), and these two as sister to the Central American species *M. rubiginosus* (100%, 1.00). *Roraimia adusta* was included in a well-supported clade with *Cranioleuca* spp. (99%, 1.00). Basal nodes were weakly supported (lower than 50% bootstrap and 0.95 or lower posterior probabilities), precluding identification of relationships among major supported lineages (Fig. 2).

We found no major differences in topology by analyzing either the mitochondrial or nuclear datasets independently, and most basal nodes were weakly supported (results not shown). The sister relationship between *Margarornis* and *Premnoplex* was more strongly supported by the nuclear data than by the mitochondrial data (76% vs. 64% bootstrap values; 1.00 vs. 0.86 posterior probabilities). The topology based on the combined dataset was generally congruent with previously described results but was better supported (Fig. 3). With the exception of a strongly supported sister relationship of *Margarornis* and *Premnoplex* (94%, 1.00), there were no close relationships among the other putative representatives of the *Margarornis* assemblage.

Partitioned Bayesian analyses with a five-partition model (posterior probabilities shown in Fig. 3) fit better than those with a two-partition model (mitochondrial vs. nuclear data; Bayes factor B_{10} of approximately 40.0), and both models fit significantly better than did nonpartitioned analyses (B_{10} of 659.4). Monophyly of both *Premnoplex* and the *Margarornis–Premnoplex* clade was supported by posterior probabilities

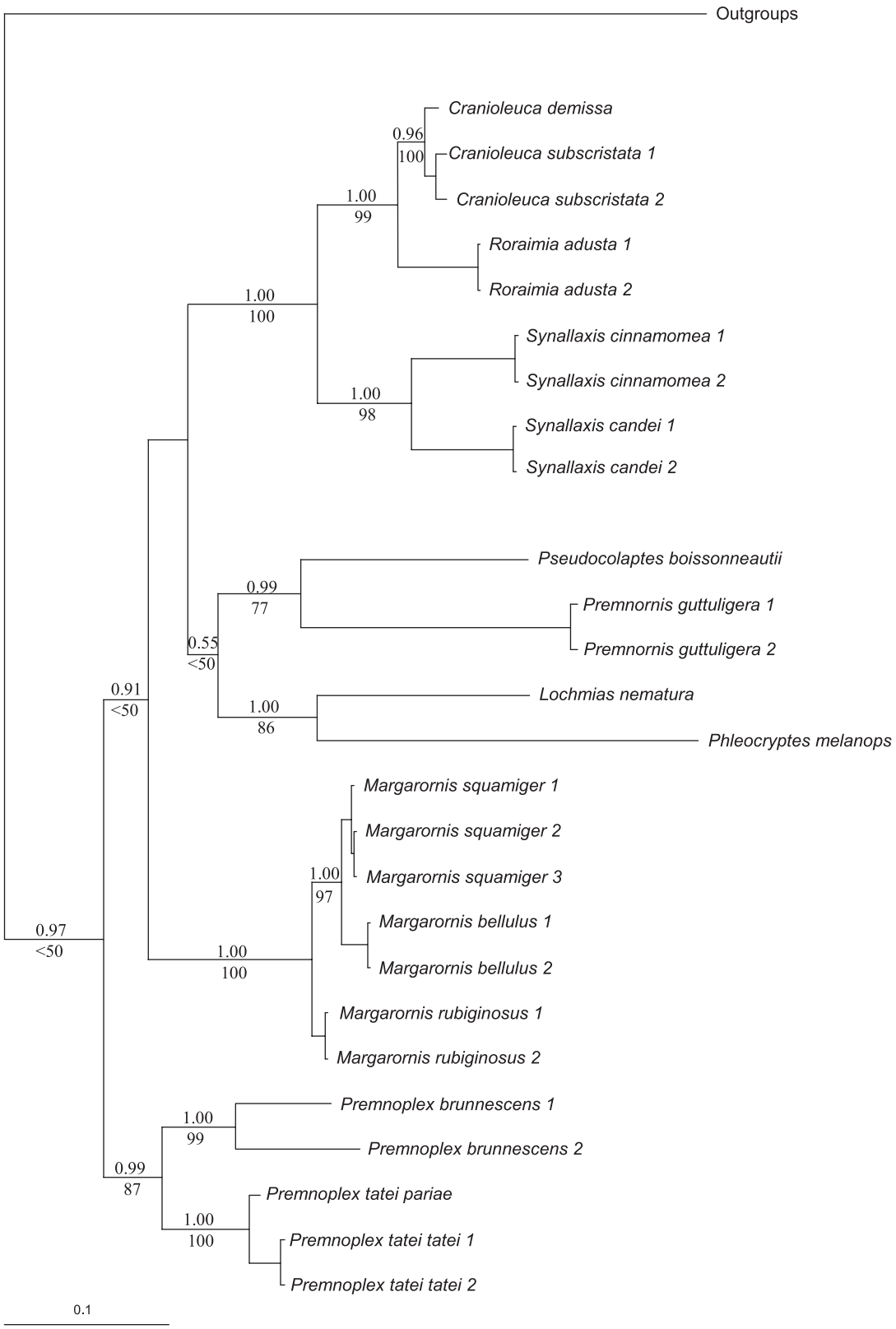

FIGURE 2. Maximum-likelihood tree based on heuristic searches of ND2 sequences. Bayesian posterior probabilities and bootstrap support values are indicated above and below the nodes, respectively. *Geositta cunicularia*, *Dendrocincla fuliginosa*, and *Microxenops milleri* are represented in the tree as outgroups. Numbers after species' names identify samples listed in Table 1.

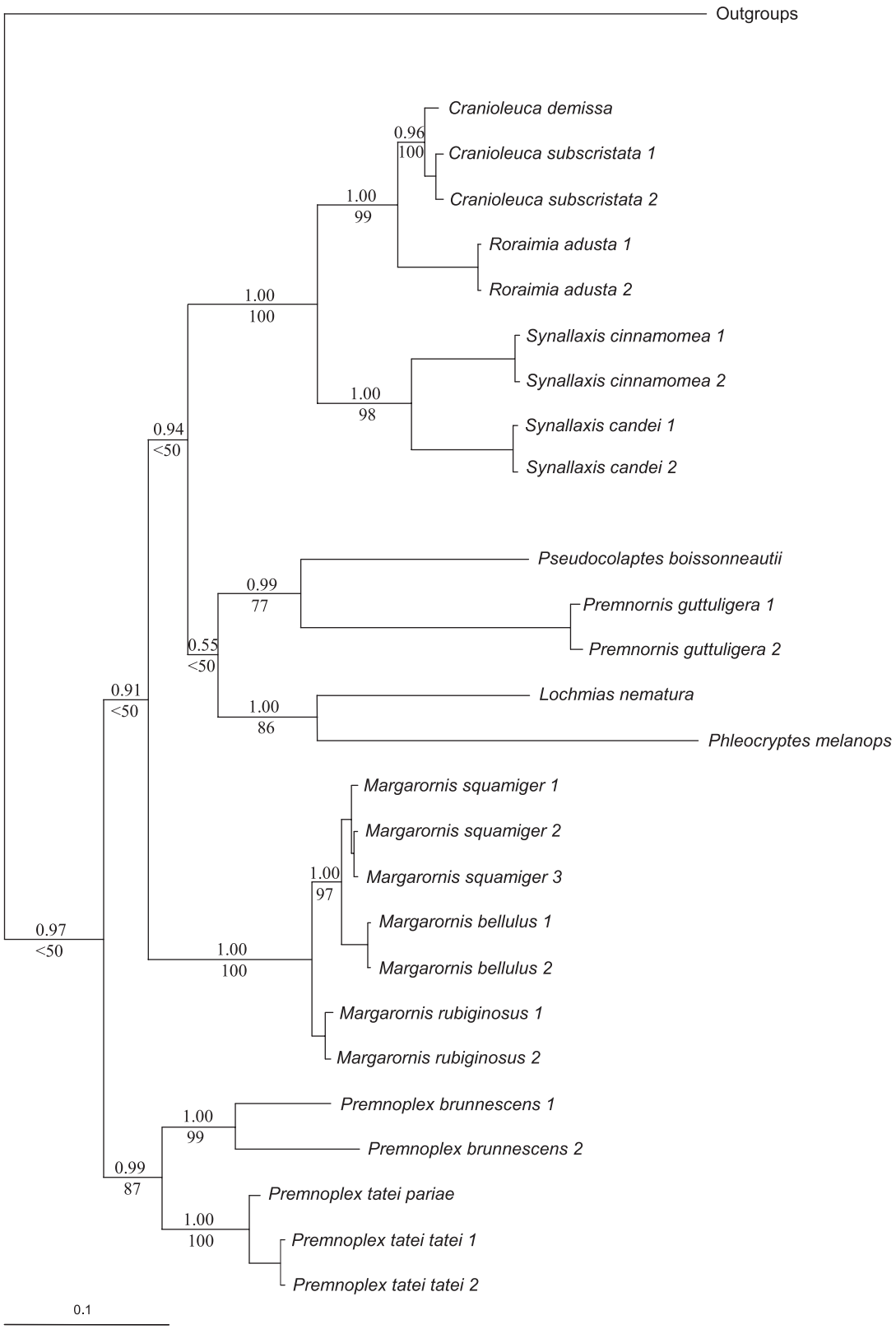

FIGURE 3. Maximum-likelihood estimate of phylogenetic relationships based on data from all five genes combined. Bayesian posterior probabilities and bootstrap support values are indicated above and below the nodes, respectively. *Geositta cunicularia*, *Dendrocincla fuliginosa*, and *Microxenops milleri* are represented in the tree as outgroups. Numbers after species' names identify samples listed in Table 1.

of 1.0. Similarly, the likelihoods of constrained trees that assumed nonmonophyly of *Premnoplex* or the *Margarornis–Premnoplex* clade were significantly worse, according to both the Shimodaira–Hasegawa test (although marginally nonsignificant for *Margarornis–Premnoplex*, $P = 0.06$) and Shimodaira's approximately unbiased test.

A likelihood-ratio test failed to reject a molecular clock for cyt *b* evolution. On the basis of 2% sequence divergence per one million years of evolution, *Margarornis* and *Premnoplex* diverged from each other approximately 12 to 16 Mya (middle Miocene; 24–31% genetic divergence), whereas the two species of *Premnoplex* diverged approximately 7 Mya (late Miocene; 14% genetic divergence).

DISCUSSION

Our results support a sister relationship of *Premnoplex tatei* and *P. brunnescens*. We found a relatively high level of genetic divergence between the two taxa (in ND2, 16% corrected pairwise distance; 10% uncorrected distance), each of them forming a reciprocally monophyletic group according to both mitochondrial and nuclear markers, a pattern suggesting long independent evolutionary histories. Although the amount of genetic divergence between taxa is often only weakly associated with their degree of reproductive isolation and should not be used as a surrogate for it (Ferguson 2002), the level of divergence between *P. brunnescens* and *P. tatei* surpasses that found between many syntopic congeneric furnariids (Brumfield, unpubl. data). Given that Areta (2007) found significant differences between the two taxa in the frequency, spacing, and speed of vocalizations, and the apparent differences in behavior and habitat use (*P. tatei* is more of a ground forager, whereas *P. brunnescens* is more scansorial; Hilty 1999, Areta 2007), we suggest recognition of these taxa as species.

Estimates of temporal divergence between the two species of *Premnoplex* suggest an old split, with independent evolution since the late Miocene. Uplift of the northeastern mountain ranges of Venezuela has been hypothesized to have occurred during the Miocene and Pliocene (Ceballos 1997, Hung 2005). Differentiation of *P. tatei* from *P. brunnescens* of the Central Coastal Cordillera in the Pleistocene, as Vuilleumier (1969) suggested for differentiation of *D. venezuelensis* (another species endemic to the northeastern mountain range) from *Diglossa albilatera*, is not supported by our data. Although recent palynological data on the Cariaco Basin seem to indicate late Pleistocene changes reflecting more mesic conditions in the plant assemblages of the Unare depression (a lowland area that represents a natural separation between both montane ranges, González et al. 2008; Fig. 1), these changes apparently did not result in recent gene flow between the populations of barbtails.

Our study supports a sister relationship between *Premnoplex* and *Margarornis*. Such a relationship has been suggested by previous molecular studies, though the support varied with the gene region used (Irestedt et al. 2006, Fjeldså et al. 2007, Moyle et al. 2009). Our combined analysis of the nuclear and mitochondrial genes supports the relationship strongly, the nuclear data more so than the mitochondrial data (76 vs 64% bootstrap, 1.00 vs. 0.86 posterior probabilities). Irestedt et al. (2006) also found higher support for the sister relationship in the two nuclear markers they examined than in mitochondrial cyt *b* sequences. In addition to morphological, behavioral, and ecological similarities that originally suggested a close evolutionary relationship between *Premnoplex* and *Margarornis*, nest architecture in the two genera is also similar, as both build globular nests made of plant material (Skutch 1996, Zyskowski and Prum 1999, Mennill and Doucet 2005, Irestedt et al. 2006, Areta 2007).

We found that the Roraimian Barbtail, *Roraimia adusta*, is not closely related to *Premnoplex tatei*, contrary to the suggestions of Phelps (1966) and Mayr and Phelps (1967). The Roraimian Barbtail was described originally as *Synallaxis adusta* by Salvin and Godman (1884), but Chapman (1929) transferred it to the old subfamily Margarornithinae (Cory and Hellmayr 1925), based on plumage coloration (a similarity shown to be a superficial by Areta 2007), the length of the middle toe and claw (a character described as rather variable by Chapman in the original description), and the shape of the rectrices (the barbed tail, which is barely evident in specimens in fresh plumage; Areta 2007). Our results, as well as data from broader systematic studies of the Furnariidae (Moyle et al. 2009), support the inclusion of *Roraimia* in the synallaxine radiation, but understanding of the exact evolutionary relationships of this monotypic genus await broader phylogenetic studies of the Furnariidae.

Genetic differentiation between samples of the two subspecies of *Premnoplex tatei*, from the Serranía of Turimiquire (nominate *tatei*) and the Paria Peninsula (*pariae*), is congruent with morphological differences in plumage pattern and coloration (Phelps and Phelps 1949, Areta 2007). However, the wide genetic divergence we observed within *P. brunnescens* suggests that additional sampling within that species will be needed for assessment of the geographic structuring and speciational history of the genus and their taxonomic implications.

Deep divergence between the species of *Premnoplex* suggests that other species endemic to the mountain ranges of northeastern Venezuela may be potential old splits from closely related taxa either in the Coastal Cordillera or the Pantepui Region (as in the case of *Myioborus pariae*; Pérez-Emán 2005). Additional studies are required for the generality of these findings to be assessed, but if the pattern is more general it would highlight the distinctiveness of this region's endemic species, especially in the Serranía of Turimiquire, where human pressures are reducing the remaining natural habitat.

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