MOLECULAR SYSTEMATICS OF THE RHINOCRYPTID GENUS *PTEROPTOCHOS*[1]

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Abstract. Relationships within the rhinocryptid genus *Pteroptochos* (huet-huets and turca) were investigated using complete sequences of the mitochondrial genes COII and ND3. Phylogenetic analysis of multiple individuals per taxon revealed that *P. castaneus, P. tarnii,* and *P. megapodius* constitute separate lineages, with *P. castaneus* and *P. tarnii* as sister taxa, and *P. megapodius* sister to these. Bootstrap support for these results was strong (79–100%). Sequence divergence between species was high, ranging from 6.1% between *P. castaneus* and *P. tarnii* to 7.6% between *P. castaneus* and *P. megapodius.* High genetic divergence between *P. castaneus* and *P. tarnii* is consistent with plumage and vocal differences between these taxa, and they appear to be separate species under both biological and phylogenetic species concepts. The Bío-Bío River, a proposed dispersal barrier to *P. tarnii,* may be ineffective in limiting gene flow in this species, east of its confluence with the Laja River.

Key words: Chile, huet-huet, Nothofagus forest, phylogenetics, riverine barriers, species limits, turca.

Resumen. Las relaciones filogenéticas dentro del género rhinocrypto *Pteroptochos* (huet-huets y turca) fueron investigadas usando secuencias completas de los genes mitocondriales COII y ND3. Los análisis filogenéticos que incluyeron múltiples individuos por taxón revelaron que *P. castaneus, P. tarnii,* y *P. megapodius* constituyen taxones separados. *Pteroptochos castaneus* y *P. tarnii* son taxones hermanos, y *P. megapodius* es el taxón hermano a estos. El apoyo de "bootstrap" de estos resultados fue fuerte (79–100%). La divergencia entre las secuencias fue alta, de 6.1% entre *P. castaneus* y *P. tarnii* y de 7.6% entre *P. castaneus* y *P. megapodius.* Esta considerable divergencia genética entre *P. castaneus* y *P. tarnii* concuerda con las diferencias que hay entre estas especies en plumaje y vocalizaciones. Por estos resultados se recomienda que estos dos taxa sean considerados especies separadas en términos biologicos y filogenéticos. Es posible que el río Bío-Bío, propuesto como una barrera a la dispersión de *P. tarnii,* no sea efectivo al este de su confluencia con el río Laja en limitar el flujo genético de estas especies.

Pteroptochos is a genus of large, mainly terrestrial tapaculos (Passeriformes: Rhinocryptidae), generally considered to consist of two or three species. The Moustached Turca, *P. megapodius,* is endemic to arid areas of central and northern Chile, and has been con-

sidered a distinctive species since its original description by Kittlitz (1830), whereas the Black-throated Huet-Huet, *P. tarnii,* of southern Chile and adjacent Argentina, and the Chestnut-throated Huet-Huet, *P. castaneus,* of central Chile, have been alternately recognized as distinct biological species (Hellmayr 1932, Goodall et al. 1946, Ridgely and Tudor 1994) or as the subspecies *P. t. tarnii* and *P. t. castaneus,* respectively (Johnson 1967, Fjeldså and Krabbe 1990, Sibley and Monroe 1990). Contributing to this taxonomic uncertainty is the allopatric distribution of *P. tarnii* and *P. castaneus.* Ranges of these taxa, both of which inhabit the understory of *Nothofagus* forest, have generally been considered to be delimited by the Bío-Bío River in south-central Chile, with *P. castaneus* to the north and *P. tarnii* to the south (Goodall et al. 1946, Ridgely and Tudor 1994, Howell and Webb 1995).

Rivers have long been recognized as important barriers to dispersal and gene flow, and thus as potentially important causes of allopatric speciation (Mayr 1963). Riverine barriers are especially important in Amazonia, where the Amazon and its major tributaries prominently separate the ranges of many species of birds and primates (Wallace 1852, Sick 1967, Hershkovitz 1977), and also restrict intraspecific gene flow (Capparella 1988, Peres et al. 1996). This phenomenon is geographically widespread, however, and Chilean rivers, such as the Maipo and the Yeso, have also been shown to limit dispersal and gene flow (Lamborot and Eaton 1992). Riverine barriers should be most effective in their wide lower reaches, and their effectiveness is expected to diminish in their relatively narrow upper reaches and headwaters (Hershkovitz 1977). For instance, the upper half of the Bío-Bío River is much narrower than its lower reaches (Vuilleumier 1985), and Behn's (1944) original description of range delimitation in *Pteroptochos* restricted the distributional barrier to the relatively wide portion of the river below its confluence with the Laja River. The suggestion that the upper Bío-Bío may not be an effective geographical barrier (Vuilleumier 1985) is supported by the recent collection of the first specimen of *P. tarnii* from north of the Bío-Bío (Chesser, unpubl. data).

In this paper I report the results of a molecular systematic investigation of the genus *Pteroptochos* with reference to the following questions: (1) What are the phylogenetic relationships among *Pteroptochos* species? Are *tarnii* and *castaneus* sister taxa, as has been traditionally assumed? (2) How divergent genetically are the species of *Pteroptochos*? Do *tarnii* and *castaneus* show genetic differentiation typical of avian sister species? (3) Does the Bío-Bío River appear to be an effective barrier to gene flow? Is a *tarnii* individual obtained from the north side of the Bío-Bío River genetically similar to individuals from south of this river?

TABLE 1. List of tissue reference numbers, geographical coordinates, and localities for sequenced individuals of *Pteroptochos* species and the outgroups *Scelorchilus rubecola* and *Scytalopus magellanicus*.

| Species and tissue number	Geographical coordinates	Locality
Pteroptochos megapodius (RTC 405)	32°59' S, 71°01' W	Chile: Region Metropolitana, Provincia Chacabuco, ca. 4 km SSW by road from peak of Cerro de El Roble, ca. 1,600 m
P. megapodius (RTC 475)	32°45' S, 70°30' W	Chile: Region V (Valparaíso), Provincia Los Andes, ca. 7 km by road WNW Higueral (road to La Canabina), 1,100 m
P. castaneus (RTC 470, 471)	36°55' S, 71°27' W	Chile: Region VIII (Bío-Bío), Provincia Ñuble, 6 km by road WSW Termas de Chillán, 1,200 m
P. tarnii (RTC 450)	37°57' S, 71°34' W	Chile: Region VIII (Bío-Bío), Provincia Bío-Bío, ca. 15 km by road SE Ralco, north side of Río Bío-Bío, near SE end Embalse El Pangue, 600 m
P. tarnii (RTC 455)	37°48' S, 71°44' W	Chile: Region VIII (Bío-Bío), Provincia Bío-Bío, ca. 7 km by road ESE Loncopangue, south side of Río Bío-Bío, 500 m
P. tarnii (RTC 462)	38°32' S, 73°07' W	Chile: Region IX (de La Araucania), Provincia Cautín, Chacamo, 650 m
Scelorchilus rubecola (RTC 452)	37°57' S, 71°34' W	Chile: Region VIII (Bío-Bío), Provincia Bío-Bío, ca. 15 km by road SE Ralco, north side of Río Bío-Bío, near SE end Embalse El Pangue, 600 m
S. rubecola (PRS 1125)	41°03' S, 71°32' W	Argentina: Provincia Río Negro, Departamento Bariloche, Llao Llao, 800 m
Scytalopus magellanicus (B-8348)	ca. 10°00' S, 75°40' W	Peru: Departamento Pasco, Millpo, E Tambo de Vacas on Pozuzo-Chaglla trail
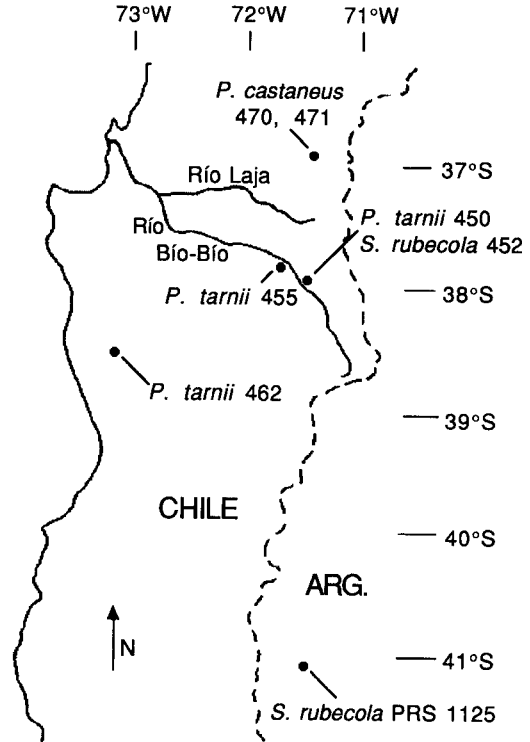
FIGURE 1. Map showing localities for individual *Pteroptochos tarnii, P. castaneus,* and *Scelorchilus rubecola* sequenced for this study. Both specimens of *P. megapodius* were collected north of the area depicted.

METHODS

Two *P. megapodius,* two *P. castaneus,* and three *P. tarnii* were sampled (Table 1, Fig. 1), as well as two Chucao Tapaculo, *Scelorchilus rubecola* (one of two species of the presumed sister genus to *Pteroptochos*), and one Magellanic Tapaculo, *Scytalopus magellanicus,* a relatively distant outgroup within the family Rhinocryptidae (Sibley and Ahlquist 1990). Tissue samples of the *Pteroptochos* species and *Scelorchilus rubecola* were obtained during fieldwork in Chile and Argentina (RTC and PRS reference numbers, for collectors R. T. Chesser and P. R. Sweet, in Table 1; vouchers at the American Museum of Natural History, New York, New York). A sample of *Scytalopus magellanicus* was obtained from the Genetic Resources Collection of the Louisiana State University Museum of Natural Science, Baton Rouge, Louisiana.

DNA was extracted using a 5% Chelex solution (Walsh et al. 1991). Two complete protein-coding mitochondrial genes, cytochrome oxidase II (COII, 684 base pairs) and NADH dehydrogenase subunit 3 (ND3, 351 base pairs), were amplified using the polymerase chain reaction (PCR). Typical PCR conditions were an initial denaturation at 94°C for 4 min, followed by 41 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 45 sec, and extension at 72°C for 1 min 20

sec. These reactions were conducted in a Peltier-effect thermocycler (MJ Research), using 50 µl reaction volumes, including 5 µl of 2 mM dNTPs, 5 µl of 10X reaction buffer, 5 µl of MgCl₂, 2.5 µl of each 10 µM primer, 1.5 µl of Chelex-extracted DNA, and 0.3 µl of *Taq* polymerase, added midway through the initial 94°C denaturation. Primers used for COII were: (1) L8263 (5'-GCCACTCATGCCTCTTTCTTATGGG-3', developed by the author for use with these taxa and other suboscine birds); (2) L8629 (5'-CCAGACTT-GACCCTAAAAGCCATCGG-3', developed by the author); (3) L8740 (5'-GGCCACTTCCGACTACTA-GAAGT-3'; Lee et al. 1997); (4) H8856 (5'-AT-GAAGGAGGTTTGATTTAGTCGTCC-3', courtesy of J. Cracraft and J. Feinstein); and (5) H9085 (5'-CAGGGGTTTGGGTTGAGTTGTGGCAT-3'; Lee et al. 1997). "H" and "L" refer here to the heavy and light strands, respectively, of the mitochondrial genome, and reference numbers are for the 3' base corresponding to the chicken sequence of Desjardins and Morais (1990). Primers used for ND3 were L10755 (5'-GACTTCCAATCTTTAAAATCTGG-3') and H11151 (5'-GATTTGTTGAGCCGAAATCAAC-3'), both of which were developed for use with these taxa and other suboscine birds.

Aliquots from the initial PCRs were purified on gels stained with ethidium bromide, and portions were removed using sterile Pasteur pipets, melted in 270 µl of H₂O, and used as template for an additional PCR. Reamplifications were conducted in an Idaho Technologies air thermocycler, using 42 cycles of denaturation at 94°C for 13 sec, annealing at 50°C for 5 sec, and extension at 71°C for 25 sec. Total reaction volumes were 40 µl, including 8 µl of Turbo-buffer, 4 µl of 2mM dNTPs, 2 µl of each 10 µM primer, 2 µl of template, and 0.2 µl of *Taq* polymerase. PCR products were purified using the GeneClean II system (BIO 101 Inc., San Diego, California) and used as template for cycle sequencing reactions. Sequencing was conducted using dye-terminator chemistry in an ABI 377 automated sequencer (Applied Biotechnologies Inc., Foster City, California), following standard procedures. Both heavy and light strands were sequenced for all PCR products. Sequences were aligned using the computer program Sequencher 3.0 (GeneCodes Corp. 1995). All sequences used in this study have been deposited in GenBank (accession numbers AF111815–AF118824 for COII sequences and AF111827–AF111836 for ND3 sequences).

Because previous research involving rhinocryptids (Arctander 1995) revealed the existence of nuclear copies of the mitochondrial gene cytochrome-*b*, I investigated the possibility that nuclear sequences were amplified in the present study. All atypical sequences, specifically *P. tarnii* 455 (see below), were critically examined for the following characteristics of nuclear copies (Sorensen and Quinn 1998): (1) ambiguous sequence due to co-amplification with the mitochondrial gene, noticeable at sites of divergence between the original and the copy, (2) variation in measures of divergence among different mitochondrial genes, important because nuclear copies tend only to consist of portions of the mitochondrial genome, (3) mismatches in overlapping sequences amplified using different primer pairs, due to only one primer pair amplifying a nuclear copy, (4) slower evolution than in mtDNA, yielding shorter branch lengths, and (5) evolution atypical of functional mtDNA sequences, resulting in protein changes, stop codons, or length variation.

Data analysis was performed using the computer programs PAUP* 4.0d64 (Swofford 1998) and MacClade 3.05 (Maddison and Maddison 1993), with maximum parsimony as the primary method of analysis. *Scytalopus magellanicus* was designated the outgroup in all analyses. Parsimony analyses were performed on both the combined and separate data from the two genes. Because of the potential for saturation of character changes at third positions, parsimony analyses were conducted with both equal weighting of data and 7:1 downweighting of third position transitions (based on the observed transition/transversion ratio in this data set, as estimated from the most parsimonious tree). Downweighting was accomplished by assigning all first and second position sites a weight of seven, then applying a weight of seven to third position transversions and a weight of one to third position transitions via a stepmatrix. Character support for phylogenies was assessed via bootstrapping (Felsenstein 1985) and branch support (Bremer 1994), which was calculated using the program TreeRot (Sorensen 1996).

Because simulations have shown that agreement among phylogenies estimated using more than one method can be an index of the reliability of those phylogenies (Kim 1993), maximum likelihood analyses also were performed on the combined data. Analyses were conducted using heuristic searches and 10 random addition replicates, under three models of increasing complexity, using: (1) empirical base frequencies, equal substitution rates for all types of changes, equal substitution rates for all sites, and no invariable sites, (2) empirical base frequencies, a transition-transversion ratio estimated from a neighbor-joining tree constructed using Kimura 2-parameter distances, equal substitution rates for all sites, and no invariable sites, and (3) empirical base frequencies, with transition-transversion ratio, gamma value for distribution of unequal site changes, and proportion of invariable sites estimated from the neighbor-joining tree.

RESULTS

Of 1,035 base pairs sequenced, 249 sites (24.1%) were variable, and 162 of these were phylogenetically informative. The COII sequence contained 105 (65%) of the informative sites, and the ND3 sequence 57 (35%). Because the COII sequence contained 66% (684 of 1,035) of the base pairs in this study, the ratio of informative sites was very similar to that of the relative size of the genes themselves. First, second, and third positions differed greatly in their variability: 38 first position sites were variable (15.3% of total variable sites), 16 second position sites (6.4%), and 195 third position sites (78.3%). The two genes differed significantly ($\chi^2_2 = 7.5$, $P < 0.05$) in their distribution of site changes, with relatively low first and (especially) second position variability in COII (22, 5, and 127 sites variable in COII, compared to 16, 11, and 68 in ND3). Ratio of synonymous to non-synonymous sub-

TABLE 2. Sequence divergence among individuals of *Pteroptochos* species and the outgroups *Scelorchilus rubecola* and *Scytalopus magellanicus* (uncorrected distance above the diagonal and Kimura 2-parameter distance below).

Species	P.m. 405	P.m. 475	P.c. 470	P.c. 471	P.t. 450	P.t. 455	P.t. 462	S.r. (Ch)	S.r. (Ar)	Scytal.
P. megapodius 405	—	0.005	0.076	0.076	0.068	0.065	0.069	0.102	0.101	0.145
P. megapodius 475	0.005	—	0.077	0.077	0.069	0.066	0.070	0.103	0.102	0.149
P. castaneus 470	0.082	0.083	—	0.000	0.059	0.063	0.060	0.118	0.117	0.164
P. castaneus 471	0.082	0.083	0.000	—	0.059	0.063	0.060	0.118	0.117	0.164
P. tarnii 450	0.072	0.073	0.062	0.062	—	0.014	0.001	0.098	0.097	0.150
P. tarnii 455	0.069	0.070	0.067	0.067	0.015	—	0.015	0.096	0.095	0.146
P. tarnii 462	0.073	0.074	0.063	0.063	0.001	0.016	—	0.099	0.098	0.151
S. rubecola (Chile)	0.113	0.114	0.132	0.132	0.107	0.105	0.109	—	0.003	0.141
S. rubecola (Arg.)	0.112	0.113	0.131	0.131	0.106	0.104	0.107	0.003	—	0.140
Scytalopus magellanicus	0.165	0.170	0.190	0.190	0.171	0.166	0.172	0.159	0.158	—

stitutions was likewise significantly different (Fisher Exact test, $P < 0.05$), with relatively fewer non-synonymous substitutions in COII (13 non-synonymous and 141 synonymous) compared to ND3 (17 and 78, respectively), consistent with greater functional constraint in cytochrome oxidase genes (Nachman et al. 1996).

Uncorrected mean sequence divergence was 6.1% between *P. castaneus* and *P. tarnii*, 6.8% between *P. megapodius* and *P. tarnii*, and 7.6% between *P. castaneus* and *P. megapodius* (Table 2). Divergence was 0.5% between the two individuals of *P. megapodius* and 0.0% between the two individuals of *P. castaneus*. Sequence divergence within *P. tarnii* averaged 1.0%; divergence between individual 455 (from the south bank of the Bío-Bío River) and the other two individuals averaged 1.5%, whereas divergence between in-

dividuals 450 and 462 was 0.1%. Patterns of sequence divergence for the individual genes were generally similar.

No instances of amplification of nuclear copies of ND3 or COII were detected. The most likely candidate for a nuclear origin, based on its sequence divergence of 1.5% relative to its conspecifics, was *P. tarnii* 455. Sequences of ND3 and COII for this individual, however, showed none of the features characteristic of nuclear copies. Specifically, (1) the sequences were clean, and sites of divergence from conspecifics showed no traces of ambiguity, (2) both COII and ND3 showed the same pattern of high divergence of *P. tarnii* 455 from its conspecifics, (3) there were no mismatches in more than 200 bases of overlapping sequence in COII, (4) the branch to *P. tarnii* 455 was the same length as the branch to its conspecifics, both containing six unambiguous nucleotide changes, and (5) all nucleotide changes on the branch to *P. tarnii* 455 were third position transitions, as would be expected of functional mitochondrial genes.

Analysis of the equally weighted data for both genes combined resulted in a single most parsimonious tree (Fig. 2; length 307, CI excluding uninformative characters = 0.82, RI = 0.87). The genus *Pteroptochos* was found to be monophyletic and sequences from each of the three named *Pteroptochos* taxa formed monophyletic groups, with *P. tarnii* and *P. castaneus* as sister taxa, and *P. megapodius* as sister to these. Within *P. tarnii*, individual 455 was sister to the other two individuals. The two individuals of *Scelorchilus rubecola* formed a monophyletic group and were sister to the *Pteroptochos* clade. Analysis of 1,000 branch-and-bound bootstrap replicates produced strong support for all branches (79–100%; Fig. 2). Weighted parsimony analysis resulted in this same topology.

Analysis of the equally weighted COII data also yielded a single most parsimonious tree, identical to the topology for the combined data (Fig. 2; length = 198, CI excluding uninformative characters = 0.79, RI = 0.85). Bootstrap analysis again revealed high support for all branches, ranging from 77% to 100%. Weighted parsimony analysis resulted in the same topology.

Analysis of the equally weighted ND3 data yielded four most parsimonious trees (length = 109, CI ex-
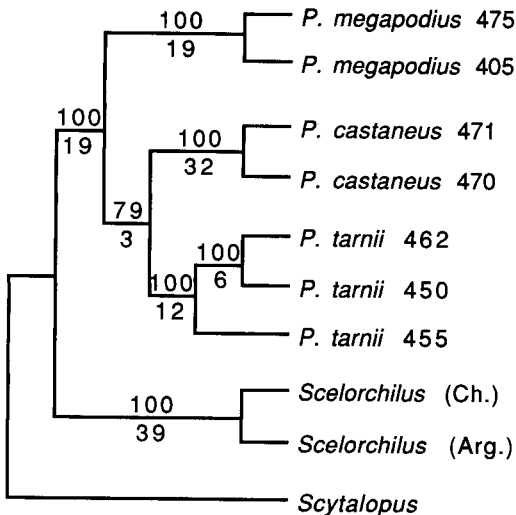

FIGURE 2. Single most parsimonious tree based on combined sequences of COII and ND3. Numbers above branches are percentages of time that branch was recovered in 1,000 bootstrap replicates, and those below branches are Bremer support values.

cluding uninformative characters = 0.87, RI = 0.90), one of which was identical to the combined data topology (Fig. 2). The other three topologies produced several elements at variance with the combined data tree, including lack of monophyly of *P. tarnii* in two trees and the grouping of *P. megapodius* and *P. castaneus* as sister taxa in each of the three alternative trees. A strict consensus tree of the four most parsimonious equally weighted trees was poorly resolved. Analysis using differentially weighted data, however, resulted in a single most parsimonious tree, identical to the combined data tree.

The maximum likelihood analyses yielded trees identical to the combined-data topology obtained using maximum parsimony: the genus *Pteroptochos* and all *Pteroptochos* species were monophyletic, *P. tarnii* and *P. castaneus* were sister species, with *P. megapodius* sister to them, and *P. tarnii* 455 was sister to the other *P. tarnii* individuals.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

Four species were originally described in the genus *Pteroptochos*: *P. albicollis* and *P. rubecola* (Kittlitz 1830), which now constitute the genus *Scelorchilus*, and *P. megapodius* (Kittlitz 1830) and *P. castaneus* (Philippi and Landbeck 1864). *Pteroptochos tarnii* was originally described as the type of the genus *Hylactes* (King 1831). The currently recognized conception of relationships among these large tapaculos stems from Sclater (1874), who grouped *megapodius, tarnii,* and *castaneus* into a single genus (*Hylactes*), and *albicollis* and *rubecola* into a separate genus (*Pteroptochos*). These designations were used until Oberholser (1923) recognized that Gray (1840) had designated *P. megapodius* as the type specimen of *Pteroptochos*; consequently, Oberholser transferred *megapodius, tarnii,* and *castaneus* to *Pteroptochos,* and erected the genus *Scelorchilus* for *albicollis* and *rubecola.* Representation in *Pteroptochos* and *Scelorchilus* has remained constant in subsequent years and a close relationship between the two genera has been implied by subsequent linear classifications (e.g., Peters 1951), in which they have been placed adjacently. Monophyly of *Pteroptochos* and a close relationship between *Pteroptochos* and *Scelorchilus* is supported by the molecular data presented here. The two genera are more closely related to each other than either is to representatives of several other genera of the family Rhinocryptidae (e.g., *Scytalopus, Rhinocrypta* and *Melanopareia,* Chesser, unpubl. data), and are almost certainly sister groups.

Within the genus *Pteroptochos,* it has been commonly accepted that *P. tarnii* and *P. castaneus* are sister taxa. Philippi and Landbeck (1864), for example, in their description of *P. castaneus,* proposed that it was a more northerly representative of *P. tarnii,* and Goodall et al. (1946) expressed little doubt that *P. castaneus* is much closer to *P. tarnii* than to *P. megapodius* or any other rhinocryptid. This sister relationship is likewise supported by the present study.

SPECIES LIMITS

More controversial than phylogenetic relationships within *Pteroptochos* and between *Pteroptochos* and

Scelorchilus has been the precise nature of the relationship between *P. tarnii* and *P. castaneus,* which, as noted above, have been alternately considered species or subspecies.

There would seem to be little doubt concerning the status of *P. tarnii* and *P. castaneus* under the phylogenetic species concept (Nelson and Platnick 1981, Cracraft 1983), owing principally to easily diagnosable differences in plumage, such as patterns of head, throat, and breast coloration. Philippi and Landbeck (1864), for instance, considered it impossible to confuse the two species, and Hellmayr (1932) noted that *P. castaneus* "differs strikingly" from *P. tarnii.* More recently, Howell and Webb (1995) echoed these sentiments and described differences in voice between the two taxa, noting that both songs and alarm calls "are readily distinguishable." Songs of suboscine birds investigated to date are not learned (Kroodsma and Konishi 1991), but are instead innate. Although geographic variation in voice has not been studied extensively in *Pteroptochos,* no evidence of intergradation in plumage has been found (Johnson 1967; Howell and Webb 1995, pers. observ.) despite extensive sampling (more than 200 specimens collected to date; Chesser, unpubl. data). The specimen of *P. tarnii* from north of the Bío-Bío River, perhaps the most likely to show intergradation, is a representative specimen, showing no tendency toward *P. castaneus.*

The biological species concept (Mayr 1963) requires that species be reproductively isolated from each other. In the case of two sympatric or parapatric populations, lack of interbreeding is *prima facie* evidence of species status. Although the range extension of *P. tarnii* (north of the Bío-Bío; Fig. 1) might appear to be evidence for sympatry between *P. tarnii* and *P. castaneus,* this cannot be demonstrated with current data. A thorough investigation of the distribution of *P. castaneus* (Marín and Chesser, unpubl. data) has shown that the southernmost records of this species approach the Bío-Bío River only downstream of its confluence with the Laja River, and that upstream of this confluence *P. castaneus* has been found only north of the Laja River (as in Behn 1944). Thus, it is unknown whether both or any species of *Pteroptochos* occur within most of the area between the Laja River and the upper Bío-Bío. Due to the extreme width of the lower Bío-Bío (more than 1 km in places; Vuilleumier 1985), where the two taxa occur on opposite banks of the river, and the lack of known contact in the vicinity of the upper Bío-Bío, it seems prudent at this time to treat these taxa as allopatric.

Application of the biological species concept to allopatric populations is problematic, and judgments must be made as to whether populations would likely interbreed if sympatric. Mayr and Ashlock (1991) discussed ways of inferring the status of allopatric populations by comparing morphological differences between these populations with morphological differences between: (1) sympatric species within the genus or closely related genera, (2) the most divergent intergrading subspecies among congeneric species, and (3) hybridizing populations of congeneric species. Although two of these comparisons cannot be applied to *Pteroptochos,* because of the lack of intergrading sub-

species or hybridizing populations within the genus, the first comparison can be used to some degree. *Pteroptochos castaneus* and *P. megapodius,* although partially sympatric, occupy different habitats (Ridgely and Tudor 1994, pers. observ.) and are not actually potentially interbreeding taxa; these taxa are therefore of no use in inferring the reproductive status of allopatric populations. However, the rhinocryptid genus *Scytalopus* contains many species that are both sympatric and syntopic. Plumage differences between sympatric, syntopic species of *Scytalopus* range from slight to distinctive; much current taxonomy is based on differences in song (e.g., Krabbe and Schulenberg 1997). *Pteroptochos tarnii* and *castaneus* differ as much in plumage as any sympatric species of *Scytalopus,* and likewise display differences in song. Plumage differences between *P. tarnii* and *P. castaneus* also exceed those among any subspecies and many closely related species of rhinocryptids (Howell and Webb 1995). Thus, it seems likely that *P. tarnii* and *P. castaneus* would not interbreed if in contact, and that they are separate biological species.

The large genetic divergence between *P. tarnii* and *P. castaneus,* although based on limited sampling, likewise would appear to support biological species status for both taxa. If the mitochondrial genes used in this study are evolving at roughly 2% per million years, an estimate converged upon by avian mitochondrial studies involving restriction fragment length polymorphism and cytochrome-*b* sequence data (Shields and Wilson 1987, Tarr and Fleischer 1993, Zink and Blackwell 1998), then these two taxa diverged approximately 3 million years ago. Even if ranges of *P. tarnii* and *P. castaneus* must currently be considered allopatric, as argued above, they are clearly in close geographical proximity and, given the climatic changes associated with the Pleistocene, have in all likelihood been in geographical contact sometime within their history. The upper Bío-Bío River does not provide effective geographical isolation, and the Laja River is almost certainly a weak dispersal barrier, as well. Of significance, then, is the lack of evidence, phenotypic or genetic, of past interbreeding between *P. tarnii* and *P. castaneus.*

Finally, although degree of genetic differentiation should not be used arbitrarily to delineate species limits, it is noteworthy that the 6.1% divergence between *P. tarnii* and *P. castaneus* is well in excess of published mitochondrial divergences between true subspecies of birds (Seutin et al. 1993) and is toward the high end of divergences between avian sister species (Avise and Zink 1988, Seutin et al. 1993).

GEOGRAPHY AND GEOGRAPHIC DIFFERENTIATION

Intraspecific divergences within *P. tarnii* and *S. rubecola* provide some intriguing (although extremely preliminary) results concerning geographic barriers and differentiation in the south temperate forests of South America. Because the *P. tarnii* individual collected from north of the river differed from an individual collected well south of the river (Fig. 1) by only a single base pair out of 1,035 sequenced, it appears likely that the Bío-Bío River does not provide an effective barrier to gene flow in *P. tarnii,* and that populations from

north and south of the river are not genetically differentiated. A variant individual (roughly 1.5% divergent from the other *P. tarnii*) was collected nearby on the south bank of the river (Fig. 1), but its significance is unclear without additional sampling. At this point there is no evidence of population structure based on geography.

It also appears that the south temperate Andes may constitute an ineffective barrier to gene flow in *S. rubecola:* divergence between the two individuals of this species, one from the Chilean side of the Andes, the other from 350 km south on the Argentine side, is only 0.3% (Table 2). This may appear surprising, given the large divergences within avian forest species on opposite sides of the Andes farther north (Brumfield and Capparella 1996), but the Andes south of 38–39°S do not form a continuous range topped by treeless habitat, but are instead breached by numerous forested passes (Hueck and Seibert 1972, Vuilleumier 1985). Elevational ranges of such forest species as *S. rubecola* are large enough (from sea level to 1,500 m; Fjeldså and Krabbe 1990) that these passes provide corridors of continuous appropriate habitat for populations on the eastern and western sides of the Andes, and opportunities for gene flow.

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PHYLOGENETIC PATTERNS IN MONTANE *TROGLODYTES* WRENS[1]

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Abstract. Phylogenetic studies based on mitochondrial DNA sequences of 10 species of wrens in *Troglodytes* and related genera suggest a new hypothesis of relationships for the group. The Winter Wren (*T. troglodytes*) and the anomalous Timberline Wren (*Thryorchilus browni*) are distantly related to the remainder of *Troglodytes*. The latter group divides into a tropical montane group and a northern/lowland group that includes the northernmost two montane taxa (*T. rufociliatus, T. brunneicollis*). Erection of the genus *Nannus* for the Winter Wren is proposed. Song evolution in the complex has involved either convergent derivation or retention of primitive song types in distant lineages.

Key words: mtDNA sequences, Nannus, phylogeny, Troglodytes, *wrens*.

North American ornithologists are well-acquainted with the relatively simple song of the House Wren (*Troglodytes aedon*), in contrast to the long and complex song of the Winter Wren (*T. troglodytes*). Recent field work in the mountains of southern Mexico and El Salvador brought two of us into contact with the Rufous-browed Wren (*T. rufociliatus*); we were struck by the extreme similarity of its song with that of Winter Wrens. Further examination of *Troglodytes* song variation, in which two major song types were noted

(Fig. 1), motivated the study reported herein. Songs of Northern and Southern House Wrens (*T. aedon* and *T. musculus,* respectively) and Brown-throated Wrens (*T. brunneicollis*) have long trills, whereas Winter, Rufous-browed, Ochraceus (*T. ochraceus*), and Mountain (*T. solstitialis*) Wrens all have longer, more varied songs largely lacking trills.

Our working hypothesis was that Winter Wrens might share a close phylogenetic relationship with the montane tropical forms of *Troglodytes,* representing an early lineage separate from the lowland forms. The only previous phylogenetic study of the genus did not include taxa critical to testing this hypothesis (Brumfield and Capparella 1996). Furthermore, morphometric studies of the entire genus by one of us (Escalona-Segura 1995) did not lead to firm conclusions regarding the evolutionary history of *Troglodytes.* For these reasons, we undertook a test of our hypothesis based on studies of mitochondrial DNA sequences.

METHODS

Tissue samples are listed in Table 1. One or two representatives of each mainland taxon that has at some point been considered as a species were included for analysis, as well as the enigmatic Timberline Wren (*Thryorchilus browni,* at times placed in *Troglodytes*) and outgroup taxa (White-breasted Wood-Wren *Henicorhina leucosticta,* Pinyon Jay *Gymnorhinus cyanocephalus*). Use of single or few individuals to represent taxa in phylogenetic analyses based on mitochondrial DNA sequence data follows Moore and DeFilippis