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PHYLOGENY OF TITMICE (PARIDAE): II. SPECIES RELATIONSHIPS BASED ON SEQUENCES OF THE MITOCHONDRIAL CYTOCHROME-*B* GENE

Frank B. Gill,[1,4] Beth Slikas,[2] and Frederick H. Sheldon[3]

[1]*The Academy of Natural Sciences, Philadelphia, Pennsylvania 19103, USA;*
[2]*Museum of Vertebrate Zoology, University of California, Berkeley, California 94720, USA; and*
[3]*Museum of Natural Science, 119 Foster Hall, Louisiana State University, Baton Rouge, Louisiana 70803, USA*

Abstract.—We present a phylogenetic hypothesis for 40 species in the bird family Paridae, based on comparisons of nucleotide sequences of the mitochondrial cytochrome-*b* gene. Parids, including tits and chickadees, are an older group than their morphological stereotypy suggests. The longest cytochrome-*b* distances between species reach 12% in uncorrected divergence. With the exception of one thrasher-like terrestrial tit species of the Tibetan plateau (*Pseudopodoces humilis*), morphological and ecological stasis have prevailed since the initial parid radiation in the Old World during the mid-Tertiary.

All trees support monophyly of the family Paridae, which includes *Parus* (*sensu lato*) and the monotypic Oriental genera *Sylviparus*, *Melanochlora,* and *Pseudopodoces*. Within the clade of chickadees and gray tits (*Parus*, subgenus *Poecile*), three Old World species, *Parus lugubris* of the eastern Mediterranean and Balkan regions, *P. superciliosus* of high elevations in the Himalayas of western China, and *P. varius* of the Orient are sisters to all other species. The Eurasian crested titmice (subgenus *Lophophanes*) and North American crested titmice (subgenus *Baeolophus*) are sister groups. Our data suggest two colonizations of the New World by parids in the late Tertiary. The ancestor of modern *Baeolophus* colonized North America ~4 mya, and the ancestor of all North American chickadees colonized North America ~3.5 mya.
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Key words: biogeography, cytochrome *b*, DNA sequences, morphological stasis, Paridae, phylogeny, titmice.

Phylogénie chez la mésange (Paridés): II. Relations entre les espèces basées sur des séquences du gène mitochondrial cytochrome-*b*

Résumé.—Nous présentons une hypothèse phylogénétique pour 40 espèces de la famille des Paridés basée sur des comparaisons de séquences de nucléotides du gène mitocochodrial cytochrome-*b*. Les Paridés constituent un groupe plus vieux que ce qui semble être suggéré par leur stéréotype morphologique. Les plus grandes distances de cytochrome-*b* entre les espèces atteignent 12% en divergence non corrigée. À l'exception d'une espèce terrestre de mésange provenant des plateaux tibétains (*Pseudopodoces humilis*), les stases morphologiques et écologiques ont prévalu depuis la radiation initiale des Paridés dans l'ancien monde au cours du mi-tertiaire.

Tous les arbres phylogénétiques appuient la monophylie de la famille des Paridés, qui inclut les *Parus* (*sensu lato*), les genres orientaux monotypiques *Sylviparus*,

[4]Present address: P.O. Box 428, Rushland, Pennsylvania 18956, USA. E-mail: fbgill@earthlink.net

Melanochlora et *Pseudopodoces*. Dans le clade des mésanges et des mésanges «grises» (*Parus*, sous-genre *Poecile*), trois espèces de l'ancien monde, *Parus lugubris*, de l'est des régions méditerranéennes et balkaniques, *P. superciliosus*, vivant en haute altitude dans la chaîne himalayenne, et *P. varius*, de l'orient, sont apparentées avec toutes les autres espèces. Les mésanges «huppées» eurasiennes (*Lophophanes*) et les mésanges «huppées» de l'Amérique du Nord (*Baeolophus*) sont des groupes apparentés. Nos données suggèrent l'existence de deux colonisations de l'ancien monde par les Paridés à la fin du tertiaire. L'ancêtre moderne de *Baeolophus* a colonisé l'Amérique du Nord il y a environ 4 millions d'années et l'ancêtre de toutes les mésanges d'Amérique du Nord a colonisé l'Amérique du Nord il y a environ 3.5 millions d'années.

MEMBERS OF THE family Paridae exhibit substantial molecular divergences between species (Slikas et al. 1996), which is consistent with a hypothesis of Tertiary speciation. The family consists of ~51 species of familiar, small, arboreal songbirds, called "tits" in Europe and "chickadees" or "titmice" in North America. Because of their overall morphological similarity, all but three species have been assigned to the genus *Parus*. Despite the outward similarity of parid species, however, the molecular divergences among many species within *Parus* are comparable to the molecular divergences among genera in other songbird families (Sheldon and Gill 1996, Slikas et al. 1996). Similarly, the divergences among some conspecific parid populations are comparable to those among full species in other songbird families (Gill et al. 1993). As a consequence of both morphological similarity among many species and the complex patterns of genetic divergence, parid taxonomy is confused, and we have a poor understanding of phylogenetic relationships. Our poor phylogenetic perspective prevents us from exploiting copious volumes of published data on parid ecology and behavior that otherwise could be used in comparative studies of their evolutionary history (e.g. Sheldon and Whittingham 1997).

Here we present a phylogenetic hypothesis for 40 parid species, including 39 traditional species of the family Paridae, plus *Pseudopodoces humilis*, previously misclassified as a corvid (James et al. 2003). Our phylogenetic estimates are based on comparisons of nucleotide sequences of the mitochondrial cytochrome-*b* gene. Our data complement and extend previous studies based on alternative molecular data sets, specifically allozyme comparisons (Gill et al. 1989), mitochondrial restriction fragment-length polymorphism (RFLP) data (Gill and Slikas 1992, Gill et al. 1993), cytochrome-*b* sequences (Kvist et al. 1996), and DNA–DNA hybridization distances (Sheldon et al. 1992, Slikas et al. 1996). In addition, comparison of parid cytochrome-*b* data with earlier molecular data sets permits an assessment of the evolutionary patterns of the cytochrome-*b* gene (e.g. as done by Slikas 1997; Sheldon et al. 1999, 2000).

MATERIALS AND METHODS

Taxa.—Our data set includes sequences from 44 species: 39 species commonly placed in the family Paridae (subfamily Parinae of Sibley and Monroe 1990); *Ps. humilis*, an aberrant species recently revealed to be a member of the Paridae (James et al. 2003); and 4 species from other songbird families (Table 1). Within the traditional Paridae, we have sequences from 37 species in the genus *Parus* and two species representing monotypic genera, *Melanochlora sultanea* and *Sylviparus modestus*. The outgroup taxa include three species representing the penduline tits (*Remiz pendulinus*, *Auriparus flaviceps*, *Anthoscopus minutus*), the parid sister group (Sheldon and Gill 1996), and a more distantly related sylvioid songbird (*Sylvia atricapilla*).

Table 1 includes subgeneric group names used by Thielcke (1968) and Harrap and Quinn (1995), corresponding to genera of Hellmayr (1903). Here, we employ those names as subgenera only, always preceded by the word "subgenus" or "subgenera," to refer to groups of parid species. We address the validity of those groupings below. The American Ornithologists' Union (AOU 1998) elevated the subgeneric names *Poecile* and *Baeolophus* to generic names for North American parid species. For clarity, however, we here use the generic name *Parus sensu lato* to include all species in the Paridae, except *Sylviparus modestus*, *M. sultanea*, and *Ps. humilis*. We do so to establish a comprehensive

TABLE 1. Study taxa, DNA preparations, and collection localities.

Taxonomic information		Sample information[a]			Collection locality		
Species[b]	Subspecies	Source	Tissue type	Accession number	Region	Locality	GenBank accession number
***Parus* (*Poecile*)**							
atricapillus[c]	*atricapillus*	ANSP	froz	5426, 5427	North America	Alaska	AF347937
gambeli	*gambeli*	ANSP	froz	5428, 5429	North America	Arizona	AF347938
gambeli	*baileyi*	ANSP	froz	978	North America	California	AF347939
carolinensis (*E*)[c]	*extimus*	ANSP	froz	5430, 5431	North America	New Jersey	AF347940
carolinensis (*W*)	*carolinensis*	ANSP	froz	865, 869	North America	Louisiana	AF347941
palustris	*brevirostris*	ANSP	froz	5432	Eurasia	Siberia	AF347942
palustris	*palustris*	ANSP	froz	5433	Eurasia	Sweden	AF347943
montanus	*songarus*	ANSP	froz	5435	China	Szechuan	AF347946
montanus	*borealis*	ANSP	froz	5436	Eurasia	Sweden	AF347944
sclateri	*rayi*	ANSP	froz	5437–5439	Mexico	Michoacan	AF347947
rufescens[c]	*rufescens*	ANSP	froz	5440, 5441	North America	California, Washington	AF347948
hudsonicus[c]	*littoralis*	ANSP	froz	5442, 5443	North America	Alaska	AF347949
cinctus	*cinctus*	ANSP	froz	5444	Eurasia	Siberia	AF347950
lugubris[c]	*lugens*	ANSP	froz	5364	Eurasia	Greece	AF347951
superciliosus		ANSP	froz	5445	China	Szechuan	AF347952
davidi		ANSP	froz	5446	China	Szechuan	AF347953
***Parus* (*Sittiparus*)**							
varius	*varius*	MCZ	ss	166231	Asia	Korea	AY308718
***Parus* (*Lophophanes*)**							
cristatus	*cristatus*	ANSP	froz	5447	Eurasia	Sweden	AF347954
dichrous[c]	*dichroides*	ANSP	froz	5448	China	Szechuan	AF347955
***Parus* (*Baeolophus*)**							
wollweberi[c]	*phillipsi*	ANSP	froz	5449	North America	Mexico	AF347956
inornatus[c]	*transpositus*	GenBank		X60944	North America	California	X60944
bicolor[c]	*bicolor*	ANSP	froz	5450	North America	Pennsylvania	AF347957
***Parus* (*Periparus*)**							
ater[c]	*aemodius*	ANSP	froz	5451	China	Szechwan	AF347958
ater	*ater*	ANSP	froz	5309	Eurasia	Greece	AF347959
melanolophus[c]		ANSP	froz	5452	Asia	Nepal	AF347960
rubidiventris[c]		AMNH	froz	JGG1048	Asia	Nepal	AY308725
rufonuchalis		FMNH	froz	395843	Asia		AY308729
***Parus* (*Pardaliparus*)**							
elegans	*elegans*	ZMUC	blood	O1431	Philippines	Luzon	AF347964
elegans	*mindanensis*	FMNH	froz	357570	Philippines	Mindanao	AY308719
amabilis		MCZ	ss	94997	Philippines	Palawan	AY308730

TABLE 1. Continued.

Taxonomic information		Sample information[a]			Collection locality		
Species[b]	Subspecies	Source	Tissue type	Accession number	Region	Locality	GenBank accession number
***Parus* (*Cyanistes*)**							
caeruleus[c]	*caeruleus*	ANSP	froz	5459	Eurasia	Greece	AF347961
cyanus	*tianschanicus*	UWBM	froz	CSW5839	Asia	Mongolia	AF347966
***Parus* (*Parus*)**							
major[c]	*afrodite*	ANSP	froz	5453	Eurasia	Greece	
major	*tibetanus*	ANSP	froz	5454	China	Szechwan	AF347962
monticolus[c]	*yunnanensis*	ANSP	froz	5455	China	Szechwan	AF347963
xanthogenys		MCZ	ss	185468	Asia	Nepal	AY308734
spilonotus		AMNH	froz	PRS2463	Asia	Vietnam	AY308726
Parus (*Melaniparus*)							
niger		LSU	froz	B34244	Africa	South Africa	AF347967
niger		FMNH	froz	390158	Africa	South Africa	AY308720
albiventris		ZMUC	blood	O1397	Africa	Kenya	AF347965
afer	*thruppi*	MCZ	ss	79073	Africa	Kenya	AY308731
funereus		MCZ	ss	270881	Africa	Uganda	AY308732
rufiventris	*pallidiventris*	MCZ	ss	279710	Africa	Malawi	AY308723
fasciiventer		FMNH	froz	355987	Africa	Uganda	AY308728
fasciiventer		FMNH	froz	355988	Africa	Uganda	AY308727
Parus (*Machlolophus*)							
holsti		AMNH	froz	NNSM4911	Asia	Taiwan	AY308724
Pseudopodoces humilis		GenBank					AF377281
Melanochlora sultanea		AMNH	froz	PRS2247	Asia	Vietnam	AY308722
Melanochlora sultanea		MCZ	ss	268318	Asia	India	AY308721
Sylviparus modestus		MCZ	ss	185445	Asia	India	AY308733
Outgroups							
Remiz pendulinus[c]		ANSP	froz	5457	Eurasia	Greece	AF347968
Auriparus flaviceps		LSU	froz	14366	North America	California	AF347969
Anthoscopus minutus		ANSP	froz	5456	Africa	South Africa	AF347970
Sylvia atricapilla[c]		ANSP	froz	5371	Eurasia	Greece	AY308735

[a] Sources of samples are abbreviated as follows: AMNH = American Museum of Natural History; ANSP = Academy of Natural Sciences of Philadelphia; FMNH = Field Museum of Natural History; MCZ = Museum of Comparative Zoology (Harvard); LSU = Louisiana State University Museum of Natural Science; UWBM = University of Washington Burke Museum; ZMUC = Zoological Museum of the University of Copenhagen. Tissue types are abbreviated as follows: froz = frozen tissue, ss = study skin (toe pad).

[b] Historical subgenera are in parentheses. We recommend the elevation of those in bold italic to genera but do not recommend elevation of those in regular italic.

[c] Indicates species for which we have complementary DNA–DNA hybridization distances (Sheldon et al. 1992, Slikas et al. 1996).

taxonomic perspective consistent with recent world checklists (e.g. Dickinson 2003) and to avoid prejudging or misrepresenting relations among species in the formal phylogentic analysis. In the end, we recommend adoption of a total of nine genera of parids, including *Poecile* and *Baeolophus*. Some subgenera included in Table 1 are in bold font, and others are not. We recommend elevation of those in bold to genera, but we do not recommend, at this time, the elevation of those in normal (italic) font.

We have sequences from most parid species of North America and Europe; the sampling of species from Africa and Asia is less complete. In particular, we have sequenced all known species of chickadees or "gray tits" (subgenus *Poecile*) and all but two species of New World parids. Not included are (1) the Juniper Titmouse (*Parus ridgwayi*), a species recently split from the Oak Titmouse (*P. inornatus*; Cicero 1996, AOU 1998) with correction of specific name from *P. griseus* to *P. ridgwayi* (AOU 2000); and (2) the Black-crested Titmouse (*P. atricristatus*), a species recently split from the Tufted Titmouse (*P. bicolor*; AOU 2002). For many taxa in the data matrix, we obtained at least partial sequences from additional individuals to control for identification or sequencing errors (Table 1). For some species, we included individuals from different populations to check for monophyly of the species.

Cytochrome-b *DNA sequencing*.—We extracted DNA from tissue and blood samples using standard protocols. Samples were incubated overnight at 55°C in a buffer including STE (sucrose/Tris/EDTA), 1% sodium lauryl sulfate, and 1 mg mL^{-1} proteinase K. Following overnight digestion, samples were purified by phenol-chloroform extraction. We precipitated DNA by adding to each sample 0.10 volume of 3M sodium acetate and 2 volumes of cold 95% ethanol. The DNA was pelleted by centrifugation, washed with 70% ethanol, air-dried, and resuspended in 0.1X TE to a concentration of ~1 mg mL^{-1}. For each sample, concentration and 260:280 ratio were read on a spectrophotometer. We diluted an aliquot of each sample with H_2O to a concentration of 25 ng μL^{-1} to serve as template for polymerase chain reaction (PCR) amplification. For eight individuals, we extracted DNA from a sliver (1 × 3 mm) of toepad from a museum study skin following protocols described in Joseph et al. (1999) and Slikas et al. (2000). We did not attempt to read the concentration of the latter extracts.

We amplified a 1,068-base-pair (bp) fragment of the mitochondrial genome, including most of the cytochrome-*b* gene, via PCR. The reaction mix included 1× buffer (Promega, Madison, Wisconsin), 200 μm of each dNTP, 0.8 μm of each primer, 1.5–3.0 mm $MgCl_2$, 0.25 units of *Taq* polymerase (Promega), and 25 ng of whole-genomic DNA as template, in a 50-μL reaction volume. We amplified most samples with the primer pair: L14990 (5′-CCATCCAACATCTCA GCATGATGAAA-3′) and H16065 (5′-GGAGTC TTCAGTCTCTGGTTTACAAGAC-3′), modified from primers in Kocher et al. (1989). The PCR cycle included a denaturation step at 93°C, an annealing step at 45–52°C, and an extension step at 72°C. For each reaction, 35–38 cycles were run in an automated thermocycler (MJ Research, Waltham, Massachusetts). A 2-min denaturation at 94°C preceded the first cycle, and an 8-min extension at 72°C followed the last cycle.

Following amplification, we purified the double-stranded PCR product of excess nucleotides and primers by electrophoresis on a 1% agarose gel. We stained gel with ethidium bromide to visualize the PCR product. The product band was excised, and the amplified DNA was extracted using glassmilk (Geneclean, Bio 101, Vista, California). The double-stranded product was sequenced manually, using the dideoxy chain termination method (Sanger et al. 1977), with T7 DNA polymerase (SEQUENASE 2.0. United States Biochemical, Cleveland, Ohio) and P^{33}-labeled nucleotides. Sequences were read manually from the autoradiographs and entered into the SEQED editing program (GCG Wisconsin Package; Accelrys, San Diego, California).

We obtained some sequences using automated sequencing methods. We cleaned double-stranded PCR products using a QIAquick PCR purification kit (Qiangen, Valencia, California). Sequences were generated by cycle sequencing with either dRhodamine or BigDye fluorescent Ready Reaction terminator mix (Applied Biosystems, Foster City, California). Sequences were visualized on an ABI 377 or 373 automated sequencer. We used SEQUENCHER 4.1 (Gene Codes Corporation, Ann Arbor, Michigan) to edit chromatograms, align sequences, and create NEXUS files.

DNA–DNA hybridization comparisons.—In previous work, we used DNA–DNA hybridization of single-copy nuclear DNA to estimate phylogenetic relationships among selected

parid species (Sheldon et al. 1999, 2000). Here, we use those distances as a measure of time-since-divergence to examine patterns and rates in the evolution of the cytochrome-*b* gene (Slikas 1997). The single-copy nuclear genome evolves much more slowly than the mitochondrial genome. As a result, DNA–DNA hybridization distances are not compressed by saturation over the range of divergence among parids (Sheldon and Bledsoe 1989), making them useful as a proxy for time. In addition, DNA–DNA hybridization distances are based on comparisons of single-copy nuclear genes, thus providing a measure of time that is independent of the mitochondrial cytochrome-*b* data. For 14 parid species and 2 outgroup species sequenced here (footnote c in Table 1), we have complementary DNA–DNA hybridization distances (Sheldon et al. 1992, Slikas et al. 1996). We plotted uncorrected percentage of difference between cytochrome-*b* sequences (*y*-axis) against uncorrected DNA–DNA hybridization distances (*x*-axis). For the cytochrome-*b* data, percentage differences were calculated and plotted for the following data partitions: (1) complete sequences; (2) transitions and transversions, each partitioned by codon position; and (3) transitions and transversions partitioned by codon position and by position along the cytochrome-*b* gene, as defined by Zhang et al. (1998). We also plotted transition-to-transversion ratios.

Phylogenetic analysis.—The final data matrix included 1,068 base pairs of sequences from cytochrome *b* for each of 54 individuals representing 44 species (Table 1). Sequences for *Ps. humilis* and *Parus inornatus* were retrieved from GenBank; all other sequences were generated for the present study and deposited in GenBank (accession numbers AF347937–AF347970, AY308717–AY308735). We were able to align all sequences with each other and the *Gallus gallus* cytochrome-*b* sequence with no insertions or deletions; our sequences begin at position 14,894 in the *Gallus gallus* mitochondrial genome (Desjardins and Morais 1990). Amino acid translation was checked in MACCLADE 4.0 (Maddison and Maddison 2000); we also used MACCLADE to assign codon positions and to create step matrices. We used PAUP* (Swofford 2002) to calculate genetic distances, to search for optimal trees using maximum-parsimony (MP) and maximum-likelihood (ML) criteria, and to perform bootstrap analyses (MP only, 1,000 reps). All PAUP* searches were heuristic, with tree bisection reconnection (TBR) branch-swapping and the multiple parsimony (MULPARS) option in effect. Maximum-parsimony searches were run with a random addition-sequence of taxa (10 replicates), and ML searches were run with a simple addition sequence of taxa. All MP analyses imposed a step-matrix weighting transversions (Tv) over transitions (Ti) by 5:1 at first- and third-codon positions, to discount the more saturated transitional changes. This weighting falls within the range of observed Ti/Tv ratios between parid taxa.

We used MODELTEST 3.0 (Posada and Crandall 1998) to determine the optimal model of sequence evolution and parameter values for the ML analysis. The optimal model was general time-reversible, with gamma-distributed rate variation across sites and invariant sites (GTR + G + I). The parameter values for that model include a symmetric rate matrix specifying relative probabilities for all possible nucleotide changes (Rmatrix = 1.085700 [A–C], 13.511800 [A–G], 0.314600, [A–T], 1.336000 [C–G], 10.341600 [C–T], 1.000000 [G–T]), the proportion of invariant sites (pinvar = 0.5125), and the shape parameter for the gamma distribution of rate variation (shape = 0.8614). The base frequencies were set as follows: A = 0.30410, C = 0.40820, G = 0.10080, T = 0.18690.

We used MRBAYES (Huelsenbeck and Ronquist 2001) to search for the best tree using Bayesian likelihood and to generate a posterior probability distribution of trees. The model selected for the MRBAYES search was a general time-reversible model with gamma-distributed rate variation across sites (GTR + G), with the following starting parameters: Rmatrix = 1.40881 [A-C], 13.00650 [A-G], 1.64341 [A-T], 0.33936 [C-G], 12.48968 [C-T], 1.00 [G-T], shape = 0.213875, and empirical base frequencies. A neighbor-joining tree, based on uncorrected sequence divergence, was generated in PAUP* and used as the starting tree in the MRBAYES search. The search was run with 4 chains for 500,000 generations, with a sampling frequency of 100 generations.

To test for rate heterogeneity among lineages, we employed a likelihood-ratio test. The test compares the log-likelihood for the ML tree to the log-likelihood for the same tree topology,

but with a molecular clock enforced. We used a six-parameter substitution model with gamma-distributed rate variation across sites; all parameters were optimized separately for clock and nonclock analyses. Twice the difference in log-likelihood is expected to have a chi-square distribution with df = $n - 2$, where n = number of taxa in the data matrix (Felsenstein 1988). With all taxa included, no significant rate heterogeneity was found with this test ($P > 0.1$).

Results

Sequence data characteristics.—The sequence data matrix shows a skewed frequency distribution of nucleotides. Averaged across all taxa and codon positions, base frequencies are 27% for adenine, 36% for cytosine, 13% for guanine, and 23% for thymidine. Base frequencies do not differ significantly across taxa (chi-squared test in PAUP*, $P = 1.00$). The skew in base frequencies is not uniform across codon positions. At first-codon positions, base frequencies are relatively uniform; at second-codon positions, guanine (13%) and adenine (20%) are relatively uncommon, whereas thymine is over-represented (41%). At third-codon positions, guanine (2.6%) and thymine (8.4%) are markedly uncommon, and adenine (36%) and cytosine (53%) are over-represented. Across taxa, base frequency composition is most variable at third-codon positions. These patterns are typical for avian cytochrome-*b* sequences (e.g. Edwards et al. 1991, Helm-Bychowski and Cracraft 1993, Kornegay et al. 1993, Hackett 1996, Bloomer and Crowe 1999, Voelker 1999).

Among parid species, 405 (40%) of the 1,008 sites in the sequence data matrix are variable (19% at first-codon positions, 6% at second-codon positions, and 75% at third-codon positions) and 326 (32%) are potentially parsimony-informative (15% at first-codon positions, 3% at second-codon positions, and 82% at third-codon positions). With outgroups included, 437 (43%) of the nucleotide sites are variable (21% at first-codon positions, 8% at second-codon positions, 71% at third-codon positions) and 360 (36%) are parsimony-informative (17% at first-codon positions, 4% at second-codon positions, and 79% at third-codon positions). Among parids, 19% of the 336 amino acids in the data matrix are variable, and 10% of those are phylogenetically informative. With outgroups included, 24% of the amino acids are variable, and 13% are phylogenetically informative.

Uncorrected sequence divergences among species in the genus *Parus* range from 2.0% (between *P. ater aemodius* and *P. melanolophus)* to 12.0% (between *P. fasciiventer* and *P. inornatus*), with a median divergence of 8.73% (Table 2). Between taxa in *Parus* and the other three species in the Paridae, average pairwise divergences are as follows: 9.4% to *Ps. humilis* (SD = 0.7%), 10.4% to *M. sultanea* (SD = 0.7%), and 11.3% to *S. modestus* (SD = 0.8%). The average pairwise sequence divergence (uncorrected) from taxa in the family Paridae to the remizid outgroups is 13.8% (*Remiz, Auriparus, Anthoscopus*; SD = 1.0%) and to *Sylvia atricapilla* is 14.8% (SD = 0.7%).

Within-taxa sequence divergences.—Our sampling within species was not extensive. Within populations, divergence in cytochrome *b* was near zero. Complete sequences of two individuals of *P. sclateri rayi* from Michoacan (Mexico) were identical; the two differed by 0.6% from a third individual from the same locality. Two individuals of *P. rufescens rufescens* from California yielded identical sequences, and two individuals of *P. hudsonicus littoralis* from Alaska differed by 0.3%. Two individuals of *P. niger niger* from South Africa differed by 0.1%, and two individuals of *P. fasciiventer* from Uganda differed by 0.8%. For some species, we also found little divergence between populations. An individual of *P. palustris* from Siberia differed from an individual from Sweden by 0.60%. Two individuals of *M. sultanea,* one from India and one from Vietnam, differed by 0.9%.

Other parids displayed more substantial divergence between populations. An individual of *P. montanus* from Sweden (*borealis*) differed by 4.9% from an individual from China (*songarus*). *Parus ater aemodius* (China) differed by 3.3% from an individual of *P. ater ater* (Greece), and an individual of *P. elegans* from Luzon differed from an individual from Mindanao by 3.4%. For both *P. ater* and *P. elegans,* the individuals representing different subspecies are not sister taxa in the optimal trees (Fig. 1). Individuals of *P. carolinensis* from the east (*P. c. extimus*) differed by 2.7% from two individuals (*P. c. carolinensis*) from the west (Louisiana). Two individuals of *P. gambeli* from Arizona (*P. g. gambeli*) were identical in sequence, but differed by 4.5% from an individual from California (*P. g. baileyi*). The

TABLE 2. Paridae cytochrome-*b* sequence divergence matrix. Upper = *P* distance; lower = maximum-likelihood distance.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*atricapillus*		0.055	0.052	0.056	0.059	0.064	0.062	0.066	0.063	0.062	0.059	0.052	0.047	0.079	0.085	0.062	0.088	0.084
2	*gambeli gambeli*	0.066		0.045	0.068	0.070	0.076	0.077	0.088	0.079	0.069	0.071	0.072	0.066	0.079	0.086	0.073	0.089	0.082
3	*gambeli baileyi*	0.061	0.051		0.064	0.067	0.067	0.067	0.085	0.070	0.068	0.069	0.069	0.064	0.077	0.087	0.069	0.084	0.084
4	*carolinensis* (east)	0.067	0.087	0.080		0.027	0.067	0.066	0.068	0.065	0.055	0.057	0.048	0.051	0.073	0.072	0.057	0.082	0.082
5	*carolinensis* (west)	0.071	0.090	0.084	0.028		0.066	0.065	0.071	0.068	0.063	0.060	0.051	0.049	0.070	0.074	0.056	0.085	0.077
6	*palustris brevirostris*	0.085	0.108	0.089	0.087	0.085		0.006	0.063	0.064	0.073	0.064	0.061	0.059	0.065	0.070	0.059	0.077	0.072
7	*palustris palustris*	0.082	0.111	0.090	0.086	0.084	0.006		0.061	0.062	0.073	0.065	0.060	0.058	0.064	0.070	0.059	0.079	0.074
8	*montanus songarus*	0.087	0.127	0.122	0.089	0.094	0.083	0.079		0.049	0.076	0.066	0.066	0.062	0.078	0.079	0.068	0.085	0.093
9	*montanus borealis*	0.083	0.110	0.096	0.084	0.088	0.081	0.078	0.056		0.072	0.065	0.056	0.054	0.077	0.084	0.061	0.083	0.088
10	*sclateri*	0.077	0.090	0.091	0.065	0.076	0.102	0.101	0.106	0.098		0.063	0.055	0.058	0.085	0.081	0.071	0.087	0.084
11	*rufescens*	0.073	0.094	0.091	0.068	0.072	0.086	0.087	0.085	0.083	0.078		0.033	0.038	0.069	0.074	0.063	0.083	0.085
12	*hudsonicus*	0.065	0.096	0.092	0.056	0.061	0.081	0.080	0.085	0.071	0.066	0.036		0.030	0.071	0.073	0.061	0.082	0.076
13	*cinctus*	0.057	0.088	0.083	0.061	0.057	0.078	0.076	0.078	0.068	0.071	0.042	0.033		0.068	0.073	0.056	0.083	0.076
14	*lugubris*	0.111	0.115	0.111	0.099	0.093	0.086	0.084	0.109	0.106	0.121	0.093	0.097	0.093		0.072	0.070	0.082	0.085
15	*superciliosus*	0.127	0.127	0.133	0.098	0.103	0.095	0.096	0.112	0.120	0.118	0.102	0.101	0.101	0.100		0.071	0.083	0.086
16	*davidi*	0.082	0.102	0.096	0.070	0.071	0.077	0.077	0.087	0.077	0.094	0.080	0.079	0.071	0.095	0.100		0.079	0.064
17	*varius* (Taiwan)	0.136	0.136	0.128	0.118	0.123	0.113	0.118	0.127	0.124	0.131	0.121	0.121	0.123	0.120	0.126	0.117		0.060
18	*varius* (Korea)	0.128	0.121	0.128	0.119	0.105	0.103	0.108	0.142	0.133	0.121	0.120	0.104	0.105	0.126	0.133	0.089	0.077	
19	*cristatus*	0.139	0.149	0.145	0.144	0.127	0.126	0.116	0.136	0.129	0.128	0.115	0.112	0.110	0.123	0.139	0.144	0.149	0.128
20	*dichrous*	0.152	0.149	0.155	0.136	0.128	0.138	0.131	0.127	0.130	0.143	0.125	0.122	0.124	0.125	0.133	0.123	0.158	0.147
21	*wollweberi*	0.162	0.165	0.168	0.134	0.121	0.134	0.130	0.124	0.122	0.157	0.139	0.116	0.120	0.136	0.133	0.151	0.154	0.151
22	*inornatus*	0.184	0.204	0.203	0.166	0.139	0.162	0.160	0.167	0.162	0.182	0.146	0.143	0.148	0.165	0.140	0.156	0.185	0.149
23	*bicolor*	0.185	0.177	0.174	0.154	0.139	0.146	0.148	0.153	0.155	0.173	0.148	0.134	0.122	0.139	0.148	0.134	0.182	0.134
24	*ater aemodius*	0.123	0.138	0.130	0.115	0.119	0.123	0.115	0.150	0.135	0.134	0.121	0.117	0.108	0.121	0.149	0.121	0.141	0.130
25	*ater ater*	0.125	0.136	0.149	0.123	0.123	0.121	0.115	0.126	0.126	0.130	0.121	0.117	0.108	0.110	0.126	0.117	0.140	0.128
26	*melanolophus*	0.121	0.126	0.126	0.117	0.122	0.121	0.119	0.142	0.124	0.123	0.117	0.109	0.099	0.114	0.135	0.118	0.134	0.131
27	*rubidiventris*	0.141	0.150	0.155	0.124	0.129	0.142	0.138	0.133	0.131	0.137	0.136	0.141	0.136	0.138	0.145	0.127	0.145	0.143
28	*rufonuchalis*	0.191	0.193	0.198	0.170	0.161	0.166	0.157	0.187	0.161	0.173	0.145	0.148	0.149	0.161	0.162	0.178	0.174	0.137
29	*elegans* (Luzon)	0.138	0.139	0.127	0.114	0.115	0.120	0.117	0.125	0.108	0.134	0.117	0.108	0.111	0.120	0.113	0.109	0.148	0.131
30	*elegans* (Mindanao)	0.121	0.135	0.131	0.111	0.106	0.110	0.108	0.111	0.104	0.126	0.106	0.103	0.096	0.115	0.123	0.112	0.146	0.133
31	*amabilis*	0.130	0.127	0.136	0.116	0.117	0.107	0.109	0.135	0.117	0.121	0.105	0.109	0.111	0.121	0.115	0.116	0.143	0.133
32	*caeruleus*	0.158	0.175	0.191	0.158	0.157	0.154	0.152	0.145	0.144	0.158	0.140	0.130	0.123	0.144	0.135	0.135	0.180	0.138
33	*cyanus*	0.159	0.174	0.176	0.171	0.158	0.150	0.154	0.155	0.150	0.164	0.152	0.141	0.123	0.143	0.135	0.150	0.184	0.131
34	*major*	0.159	0.168	0.160	0.158	0.151	0.168	0.161	0.153	0.147	0.158	0.141	0.132	0.135	0.162	0.139	0.139	0.187	0.154
35	*monticolus*	0.172	0.182	0.187	0.168	0.169	0.179	0.175	0.162	0.162	0.153	0.139	0.140	0.138	0.171	0.162	0.155	0.194	0.178
36	*xanthogenys*	0.189	0.192	0.189	0.190	0.190	0.166	0.168	0.188	0.201	0.210	0.187	0.171	0.152	0.176	0.179	0.189	0.187	0.160
37	*spilonotus*	0.208	0.213	0.218	0.185	0.174	0.177	0.171	0.191	0.186	0.219	0.200	0.180	0.161	0.181	0.173	0.180	0.189	0.188
38	*niger* (AF347967)	0.136	0.155	0.151	0.126	0.126	0.123	0.117	0.141	0.135	0.137	0.130	0.126	0.119	0.121	0.136	0.119	0.163	0.118
39	*niger* (AY308720)	0.132	0.149	0.144	0.120	0.118	0.120	0.113	0.139	0.131	0.135	0.126	0.122	0.113	0.117	0.134	0.118	0.156	0.113
40	*albiventris*	0.156	0.157	0.160	0.143	0.135	0.151	0.149	0.157	0.145	0.148	0.145	0.139	0.132	0.149	0.165	0.136	0.182	0.144
41	*afer*	0.156	0.155	0.171	0.128	0.113	0.148	0.150	0.175	0.151	0.163	0.154	0.141	0.139	0.147	0.133	0.139	0.158	0.128

Table 2. Continued.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
42	*funereus*	0.162	0.159	0.177	0.146	0.129	0.150	0.151	0.160	0.160	0.167	0.149	0.147	0.141	0.154	0.165	0.150	0.199	0.144
43	*rufiventris*	0.170	0.177	0.173	0.159	0.165	0.160	0.156	0.162	0.169	0.163	0.166	0.159	0.150	0.137	0.159	0.157	0.179	0.155
44	*fasciiventer*	0.191	0.179	0.193	0.177	0.159	0.170	0.166	0.178	0.159	0.171	0.159	0.158	0.152	0.146	0.168	0.163	0.171	0.178
45	*holsti*	0.160	0.177	0.167	0.169	0.148	0.143	0.139	0.148	0.152	0.165	0.148	0.154	0.125	0.129	0.161	0.145	0.190	0.183
46	*Pseudopodoces*	0.169	0.187	0.171	0.159	0.155	0.167	0.162	0.156	0.150	0.184	0.169	0.149	0.156	0.154	0.159	0.165	0.199	0.171
47	*Melanochlora* (India)	0.161	0.184	0.192	0.182	0.204	0.198	0.189	0.169	0.162	0.187	0.185	0.165	0.162	0.201	0.200	0.204	0.243	0.180
48	*Melanochlora* (Vietnam)	0.167	0.192	0.199	0.195	0.215	0.205	0.196	0.176	0.165	0.199	0.194	0.174	0.171	0.203	0.205	0.212	0.248	0.205
49	*Sylviparus*	0.216	0.234	0.261	0.238	0.230	0.234	0.230	0.176	0.203	0.257	0.213	0.199	0.189	0.195	0.218	0.227	0.312	0.240
50	*Remiz*	0.352	0.390	0.344	0.411	0.396	0.387	0.369	0.315	0.341	0.390	0.370	0.366	0.362	0.413	0.321	0.345	0.393	0.396
51	*Auriparus*	0.353	0.377	0.431	0.385	0.412	0.371	0.356	0.396	0.364	0.392	0.376	0.341	0.353	0.414	0.423	0.375	0.432	0.431
52	*Anthoscopus*	0.331	0.353	0.351	0.389	0.361	0.395	0.387	0.380	0.351	0.419	0.354	0.338	0.365	0.376	0.376	0.424	0.399	0.367
53	*Sylvia*	0.416	0.409	0.433	0.438	0.434	0.424	0.416	0.451	0.422	0.461	0.414	0.432	0.386	0.418	0.474	0.372	0.430	0.325

		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	*atricapillus*	0.088	0.095	0.098	0.107	0.108	0.081	0.082	0.081	0.090	0.102	0.089	0.081	0.086	0.092	0.094	0.093	0.100	0.098
2	*gambeli gambeli*	0.092	0.095	0.099	0.116	0.104	0.088	0.088	0.083	0.094	0.106	0.089	0.088	0.085	0.100	0.100	0.099	0.103	0.102
3	*gambeli baileyi*	0.090	0.095	0.099	0.115	0.101	0.083	0.092	0.082	0.095	0.106	0.082	0.083	0.086	0.104	0.100	0.094	0.104	0.100
4	*carolinensis* (east)	0.092	0.090	0.087	0.104	0.099	0.079	0.083	0.081	0.084	0.096	0.079	0.076	0.080	0.094	0.100	0.093	0.099	0.102
5	*carolinensis* (west)	0.085	0.087	0.081	0.092	0.092	0.081	0.084	0.084	0.087	0.094	0.080	0.074	0.081	0.095	0.096	0.091	0.100	0.101
6	*palustris brevirostris*	0.082	0.087	0.086	0.098	0.091	0.080	0.079	0.079	0.089	0.090	0.079	0.072	0.072	0.090	0.090	0.095	0.100	0.092
7	*palustris palustris*	0.077	0.084	0.084	0.098	0.092	0.076	0.076	0.078	0.087	0.088	0.078	0.072	0.073	0.091	0.092	0.092	0.098	0.092
8	*montanus songarus*	0.088	0.084	0.082	0.103	0.098	0.093	0.083	0.089	0.086	0.101	0.085	0.076	0.090	0.087	0.092	0.090	0.096	0.099
9	*montanus borealis*	0.084	0.085	0.080	0.100	0.096	0.087	0.083	0.082	0.086	0.091	0.076	0.072	0.080	0.087	0.092	0.088	0.097	0.104
10	*sclateri*	0.083	0.092	0.096	0.109	0.104	0.087	0.086	0.083	0.090	0.096	0.088	0.083	0.081	0.094	0.098	0.093	0.092	0.107
11	*rufescens*	0.077	0.084	0.090	0.095	0.096	0.082	0.082	0.081	0.090	0.088	0.080	0.074	0.074	0.087	0.092	0.087	0.088	0.100
12	*hudsonicus*	0.075	0.081	0.079	0.093	0.089	0.079	0.079	0.076	0.091	0.087	0.075	0.072	0.076	0.083	0.087	0.081	0.086	0.092
13	*cinctus*	0.075	0.084	0.080	0.096	0.084	0.073	0.074	0.069	0.088	0.087	0.076	0.067	0.076	0.081	0.081	0.083	0.086	0.086
14	*lugubris*	0.083	0.086	0.089	0.103	0.091	0.082	0.076	0.078	0.091	0.095	0.082	0.078	0.082	0.089	0.089	0.095	0.100	0.098
15	*superciliosus*	0.087	0.087	0.087	0.094	0.097	0.095	0.084	0.088	0.094	0.091	0.078	0.082	0.079	0.084	0.085	0.085	0.097	0.099
16	*davidi*	0.088	0.080	0.091	0.098	0.087	0.081	0.079	0.079	0.084	0.098	0.076	0.077	0.080	0.084	0.090	0.085	0.093	0.098
17	*varius* (Taiwan)	0.094	0.099	0.096	0.108	0.108	0.089	0.090	0.086	0.091	0.097	0.093	0.090	0.090	0.102	0.105	0.103	0.107	0.101
18	*varius* (Korea)	0.086	0.096	0.097	0.095	0.091	0.085	0.084	0.085	0.092	0.087	0.086	0.087	0.087	0.091	0.086	0.091	0.103	0.091
19	*cristatus*		0.059	0.073	0.098	0.092	0.088	0.088	0.085	0.085	0.090	0.078	0.076	0.084	0.087	0.087	0.081	0.089	0.095
20	*dichrous*	0.073		0.081	0.099	0.092	0.079	0.076	0.078	0.086	0.094	0.083	0.078	0.086	0.091	0.092	0.084	0.096	0.096
21	*wollweberi*	0.099	0.113		0.086	0.076	0.090	0.084	0.085	0.082	0.083	0.075	0.075	0.079	0.081	0.087	0.079	0.091	0.095
22	*inornatus*	0.150	0.148	0.122		0.068	0.098	0.097	0.102	0.100	0.100	0.094	0.093	0.095	0.105	0.110	0.102	0.105	0.117
23	*bicolor*	0.139	0.137	0.104	0.086		0.094	0.089	0.093	0.101	0.104	0.092	0.088	0.098	0.098	0.103	0.096	0.096	0.112
24	*ater aemodius*	0.135	0.114	0.139	0.152	0.150		0.033	0.020	0.066	0.082	0.075	0.061	0.070	0.094	0.096	0.080	0.089	0.089
25	*ater ater*	0.133	0.107	0.125	0.149	0.139	0.036		0.027	0.071	0.080	0.068	0.062	0.067	0.090	0.094	0.077	0.087	0.078

TABLE 2. Continued.

		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
26	*melanolophus*	0.129	0.111	0.129	0.160	0.148	0.021	0.028		0.062	0.080	0.069	0.064	0.067	0.090	0.093	0.079	0.090	0.082
27	*rubidiventris*	0.125	0.125	0.120	0.153	0.163	0.081	0.089	0.076		0.065	0.072	0.069	0.068	0.086	0.089	0.080	0.083	0.098
28	*rufonuchalis*	0.148	0.155	0.138	0.176	0.195	0.119	0.115	0.115	0.084		0.082	0.080	0.078	0.086	0.089	0.086	0.094	0.092
29	*elegans* (Luzon)	0.113	0.121	0.107	0.140	0.141	0.098	0.086	0.089	0.092	0.122		0.034	0.032	0.086	0.085	0.074	0.089	0.093
30	*elegans* (Mindanao)	0.110	0.112	0.108	0.142	0.138	0.076	0.079	0.082	0.089	0.121	0.037		0.032	0.082	0.084	0.078	0.081	0.089
31	*amabilis*	0.124	0.126	0.117	0.146	0.159	0.089	0.085	0.087	0.087	0.112	0.034	0.034		0.089	0.089	0.080	0.075	0.092
32	*caeruleus*	0.139	0.145	0.125	0.175	0.165	0.145	0.141	0.142	0.130	0.143	0.129	0.122	0.135		0.025	0.080	0.091	0.100
33	*cyanus*	0.139	0.145	0.135	0.183	0.177	0.149	0.148	0.149	0.137	0.146	0.125	0.122	0.132	0.026		0.079	0.096	0.101
34	*major*	0.127	0.135	0.122	0.180	0.165	0.115	0.112	0.118	0.118	0.145	0.107	0.113	0.119	0.124	0.119		0.052	0.082
35	*monticolus*	0.139	0.157	0.143	0.181	0.164	0.130	0.129	0.137	0.123	0.164	0.134	0.116	0.107	0.143	0.150	0.061		0.094
36	*xanthogenys*	0.158	0.158	0.162	0.227	0.206	0.138	0.118	0.129	0.160	0.153	0.153	0.147	0.150	0.181	0.178	0.125	0.151	
37	*spilonotus*	0.149	0.142	0.130	0.201	0.181	0.139	0.118	0.130	0.144	0.168	0.146	0.141	0.147	0.175	0.173	0.132	0.138	0.061
38	*niger* (AF347967)	0.107	0.141	0.125	0.156	0.154	0.116	0.122	0.116	0.135	0.154	0.116	0.108	0.109	0.126	0.123	0.119	0.128	0.125
39	*niger* (AY308720)	0.102	0.135	0.118	0.151	0.144	0.115	0.119	0.116	0.132	0.151	0.114	0.106	0.107	0.125	0.122	0.112	0.120	0.119
40	*albiventris*	0.129	0.165	0.147	0.163	0.183	0.151	0.147	0.146	0.142	0.155	0.119	0.116	0.123	0.152	0.143	0.136	0.146	0.150
41	*afer*	0.155	0.174	0.138	0.164	0.164	0.147	0.140	0.142	0.155	0.157	0.145	0.136	0.148	0.131	0.140	0.115	0.127	0.141
42	*funereus*	0.164	0.184	0.166	0.178	0.186	0.158	0.144	0.164	0.147	0.167	0.143	0.142	0.125	0.180	0.172	0.157	0.167	0.161
43	*rufiventris*	0.135	0.154	0.151	0.188	0.167	0.123	0.133	0.135	0.136	0.180	0.136	0.120	0.142	0.141	0.156	0.142	0.146	0.162
44	*fasciiventer*	0.153	0.157	0.154	0.214	0.213	0.129	0.127	0.122	0.137	0.162	0.130	0.134	0.126	0.161	0.151	0.146	0.160	0.176
45	*holsti*	0.144	0.154	0.147	0.183	0.168	0.140	0.123	0.135	0.146	0.169	0.119	0.120	0.129	0.157	0.147	0.138	0.139	0.113
46	*Pseudopodoces*	0.162	0.178	0.134	0.186	0.174	0.146	0.151	0.156	0.128	0.155	0.121	0.109	0.130	0.138	0.142	0.123	0.133	0.167
47	*Melanochlora* (India)	0.180	0.184	0.165	0.211	0.201	0.172	0.162	0.168	0.175	0.189	0.143	0.140	0.147	0.159	0.166	0.175	0.186	0.206
48	*Melanochlora* (Vietnam)	0.194	0.193	0.173	0.229	0.211	0.180	0.173	0.179	0.176	0.201	0.153	0.149	0.164	0.164	0.172	0.178	0.194	0.223
49	*Sylviparus*	0.228	0.197	0.212	0.231	0.218	0.222	0.195	0.213	0.200	0.234	0.191	0.175	0.192	0.173	0.180	0.188	0.202	0.243
50	*Remiz*	0.342	0.329	0.328	0.370	0.408	0.318	0.318	0.319	0.309	0.353	0.298	0.295	0.325	0.281	0.271	0.275	0.297	0.396
51	*Auriparus*	0.377	0.395	0.388	0.418	0.465	0.410	0.408	0.399	0.371	0.458	0.346	0.340	0.387	0.357	0.390	0.460	0.453	0.535
52	*Anthoscopus*	0.311	0.331	0.307	0.406	0.393	0.400	0.382	0.375	0.367	0.416	0.328	0.338	0.349	0.293	0.312	0.320	0.336	0.402
53	*Sylvia*	0.401	0.445	0.428	0.456	0.443	0.459	0.432	0.431	0.427	0.475	0.392	0.399	0.390	0.412	0.413	0.460	0.518	0.495

		37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
1	*atricapillus*	0.105	0.085	0.083	0.094	0.096	0.103	0.101	0.108	0.095	0.100	0.096	0.097	0.114	0.130	0.133	0.129	0.144
2	*gambeli gambeli*	0.108	0.092	0.089	0.095	0.095	0.101	0.104	0.105	0.099	0.107	0.105	0.107	0.120	0.140	0.137	0.133	0.141
3	*gambeli baileyi*	0.107	0.090	0.088	0.097	0.099	0.107	0.100	0.108	0.094	0.098	0.106	0.107	0.124	0.129	0.148	0.133	0.144
4	*carolinensis* (east)	0.100	0.082	0.079	0.093	0.082	0.096	0.100	0.104	0.100	0.096	0.103	0.106	0.118	0.141	0.144	0.142	0.151
5	*carolinensis* (west)	0.096	0.083	0.079	0.088	0.076	0.088	0.103	0.097	0.093	0.094	0.112	0.114	0.117	0.135	0.148	0.135	0.150
6	*palustris brevirostris*	0.097	0.077	0.076	0.091	0.088	0.093	0.096	0.099	0.087	0.096	0.105	0.106	0.114	0.132	0.138	0.142	0.141
7	*palustris palustris*	0.095	0.075	0.073	0.090	0.088	0.093	0.094	0.099	0.086	0.094	0.103	0.104	0.115	0.131	0.136	0.142	0.141
8	*montanus songarus*	0.101	0.087	0.086	0.096	0.101	0.100	0.098	0.103	0.091	0.094	0.101	0.102	0.103	0.119	0.139	0.135	0.153
9	*montanus borealis*	0.098	0.084	0.083	0.090	0.091	0.101	0.102	0.096	0.092	0.092	0.096	0.096	0.109	0.128	0.134	0.130	0.143
10	*sclateri*	0.110	0.084	0.084	0.092	0.098	0.104	0.100	0.101	0.098	0.104	0.104	0.107	0.123	0.135	0.142	0.143	0.152

TABLE 2. Continued.

		37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
11	*rufescens*	0.104	0.084	0.082	0.093	0.095	0.097	0.101	0.099	0.091	0.100	0.107	0.109	0.113	0.136	0.142	0.132	0.144
12	*hudsonicus*	0.095	0.081	0.079	0.088	0.089	0.095	0.098	0.097	0.092	0.091	0.098	0.101	0.107	0.129	0.134	0.129	0.145
13	*cinctus*	0.090	0.077	0.075	0.085	0.087	0.092	0.095	0.095	0.081	0.093	0.097	0.099	0.104	0.131	0.136	0.134	0.141
14	*lugubris*	0.099	0.080	0.078	0.095	0.092	0.099	0.090	0.093	0.083	0.093	0.113	0.112	0.110	0.139	0.151	0.138	0.144
15	*superciliosus*	0.097	0.083	0.082	0.098	0.084	0.099	0.096	0.099	0.097	0.095	0.110	0.111	0.112	0.129	0.152	0.137	0.152
16	*davidi*	0.094	0.077	0.076	0.084	0.086	0.094	0.095	0.098	0.087	0.097	0.108	0.109	0.116	0.129	0.140	0.140	0.140
17	*varius* (Taiwan)	0.102	0.095	0.093	0.103	0.096	0.113	0.106	0.101	0.105	0.108	0.120	0.121	0.137	0.142	0.153	0.143	0.142
18	*varius* (Korea)	0.101	0.078	0.076	0.089	0.083	0.092	0.095	0.105	0.102	0.100	0.102	0.108	0.120	0.136	0.158	0.139	0.133
19	*cristatus*	0.092	0.075	0.072	0.088	0.096	0.103	0.090	0.096	0.089	0.098	0.103	0.107	0.116	0.128	0.140	0.122	0.140
20	*dichrous*	0.091	0.090	0.087	0.102	0.102	0.110	0.098	0.098	0.095	0.104	0.104	0.108	0.109	0.123	0.145	0.130	0.150
21	*wollweberi*	0.081	0.082	0.079	0.095	0.088	0.102	0.096	0.095	0.090	0.085	0.099	0.101	0.111	0.125	0.138	0.126	0.147
22	*inornatus*	0.108	0.095	0.093	0.101	0.092	0.105	0.110	0.120	0.102	0.108	0.116	0.121	0.117	0.139	0.152	0.150	0.158
23	*bicolor*	0.102	0.095	0.091	0.110	0.095	0.109	0.101	0.118	0.096	0.101	0.110	0.112	0.114	0.140	0.157	0.141	0.153
24	*ater aemodius*	0.088	0.080	0.079	0.097	0.093	0.101	0.085	0.087	0.088	0.093	0.103	0.105	0.117	0.131	0.145	0.146	0.153
25	*ater ater*	0.079	0.082	0.080	0.096	0.090	0.094	0.090	0.086	0.080	0.094	0.097	0.100	0.106	0.128	0.146	0.139	0.149
26	*melanolophus*	0.083	0.078	0.077	0.094	0.090	0.102	0.089	0.083	0.085	0.095	0.099	0.102	0.112	0.127	0.144	0.137	0.144
27	*rubidiventris*	0.089	0.089	0.087	0.095	0.096	0.096	0.091	0.092	0.091	0.084	0.104	0.103	0.110	0.126	0.141	0.137	0.150
28	*rufonuchalis*	0.090	0.091	0.090	0.094	0.088	0.099	0.104	0.097	0.092	0.088	0.102	0.105	0.116	0.130	0.154	0.144	0.151
29	*elegans* (Luzon)	0.090	0.079	0.077	0.082	0.093	0.096	0.092	0.089	0.078	0.081	0.091	0.094	0.107	0.124	0.134	0.129	0.145
30	*elegans* (Mindanao)	0.085	0.075	0.074	0.082	0.088	0.095	0.084	0.090	0.076	0.076	0.090	0.093	0.101	0.121	0.131	0.132	0.146
31	*amabilis*	0.089	0.076	0.074	0.084	0.095	0.086	0.095	0.087	0.081	0.085	0.094	0.099	0.106	0.129	0.141	0.135	0.146
32	*caeruleus*	0.095	0.081	0.081	0.094	0.084	0.108	0.093	0.098	0.094	0.089	0.096	0.098	0.100	0.119	0.139	0.125	0.140
33	*cyanus*	0.097	0.081	0.080	0.090	0.090	0.105	0.100	0.096	0.092	0.091	0.100	0.103	0.101	0.121	0.145	0.132	0.138
34	*major*	0.083	0.079	0.075	0.087	0.077	0.099	0.093	0.091	0.086	0.081	0.100	0.101	0.100	0.117	0.149	0.123	0.148
35	*monticolus*	0.088	0.085	0.081	0.093	0.085	0.104	0.096	0.100	0.089	0.087	0.107	0.110	0.107	0.124	0.147	0.132	0.159
36	*xanthogenys*	0.051	0.081	0.078	0.092	0.090	0.098	0.099	0.103	0.078	0.096	0.106	0.110	0.117	0.138	0.159	0.140	0.154
37	*spilonotus*		0.090	0.088	0.098	0.092	0.109	0.103	0.106	0.086	0.094	0.115	0.113	0.118	0.135	0.148	0.137	0.144
38	*niger* (AF347967)	0.150		0.001	0.050	0.078	0.083	0.070	0.086	0.082	0.090	0.093	0.096	0.111	0.129	0.143	0.136	0.149
39	*niger* (AY308720)	0.144	0.001		0.050	0.076	0.081	0.068	0.086	0.081	0.086	0.092	0.096	0.107	0.129	0.143	0.133	0.149
40	*albiventris*	0.165	0.057	0.058		0.087	0.094	0.085	0.091	0.088	0.095	0.096	0.101	0.119	0.130	0.150	0.148	0.149
41	*afer*	0.152	0.114	0.109	0.133		0.088	0.093	0.091	0.085	0.091	0.106	0.112	0.109	0.134	0.161	0.142	0.155
42	*funereus*	0.196	0.117	0.112	0.136	0.127		0.098	0.104	0.095	0.098	0.111	0.118	0.123	0.151	0.167	0.153	0.166
43	*rufiventris*	0.176	0.093	0.090	0.117	0.140	0.143		0.095	0.096	0.097	0.096	0.097	0.128	0.143	0.150	0.150	0.151
44	*fasciiventer*	0.184	0.128	0.128	0.135	0.137	0.159	0.142		0.098	0.106	0.097	0.099	0.116	0.128	0.155	0.145	0.147
45	*holsti*	0.127	0.126	0.123	0.136	0.127	0.149	0.153	0.159		0.091	0.102	0.104	0.116	0.135	0.149	0.139	0.154
46	*Pseudopodoces*	0.161	0.139	0.131	0.150	0.141	0.153	0.150	0.179	0.147		0.100	0.099	0.099	0.126	0.162	0.139	0.158
47	*Melanochlora* (India)	0.244	0.152	0.151	0.160	0.187	0.191	0.155	0.159	0.184	0.172		0.009	0.109	0.133	0.153	0.139	0.156
48	*Melanochlora* (Vietnam)	0.240	0.162	0.164	0.173	0.207	0.214	0.162	0.164	0.190	0.171	0.009		0.115	0.133	0.155	0.139	0.155
49	*Sylviparus*	0.247	0.207	0.196	0.236	0.212	0.243	0.247	0.225	0.235	0.172	0.202	0.223		0.136	0.156	0.132	0.167
50	*Remiz*	0.375	0.335	0.337	0.343	0.357	0.443	0.383	0.331	0.359	0.322	0.335	0.329	0.379		0.149	0.119	0.176
51	*Auriparus*	0.445	0.413	0.420	0.442	0.526	0.528	0.402	0.440	0.453	0.547	0.449	0.459	0.418	0.454		0.150	0.172
52	*Anthoscopus*	0.372	0.380	0.365	0.431	0.416	0.443	0.422	0.431	0.372	0.392	0.376	0.368	0.311	0.252	0.456		0.165
53	*Sylvia*	0.485	0.447	0.451	0.432	0.477	0.508	0.452	0.427	0.481	0.488	0.494	0.496	0.512	0.658	0.598	0.603	

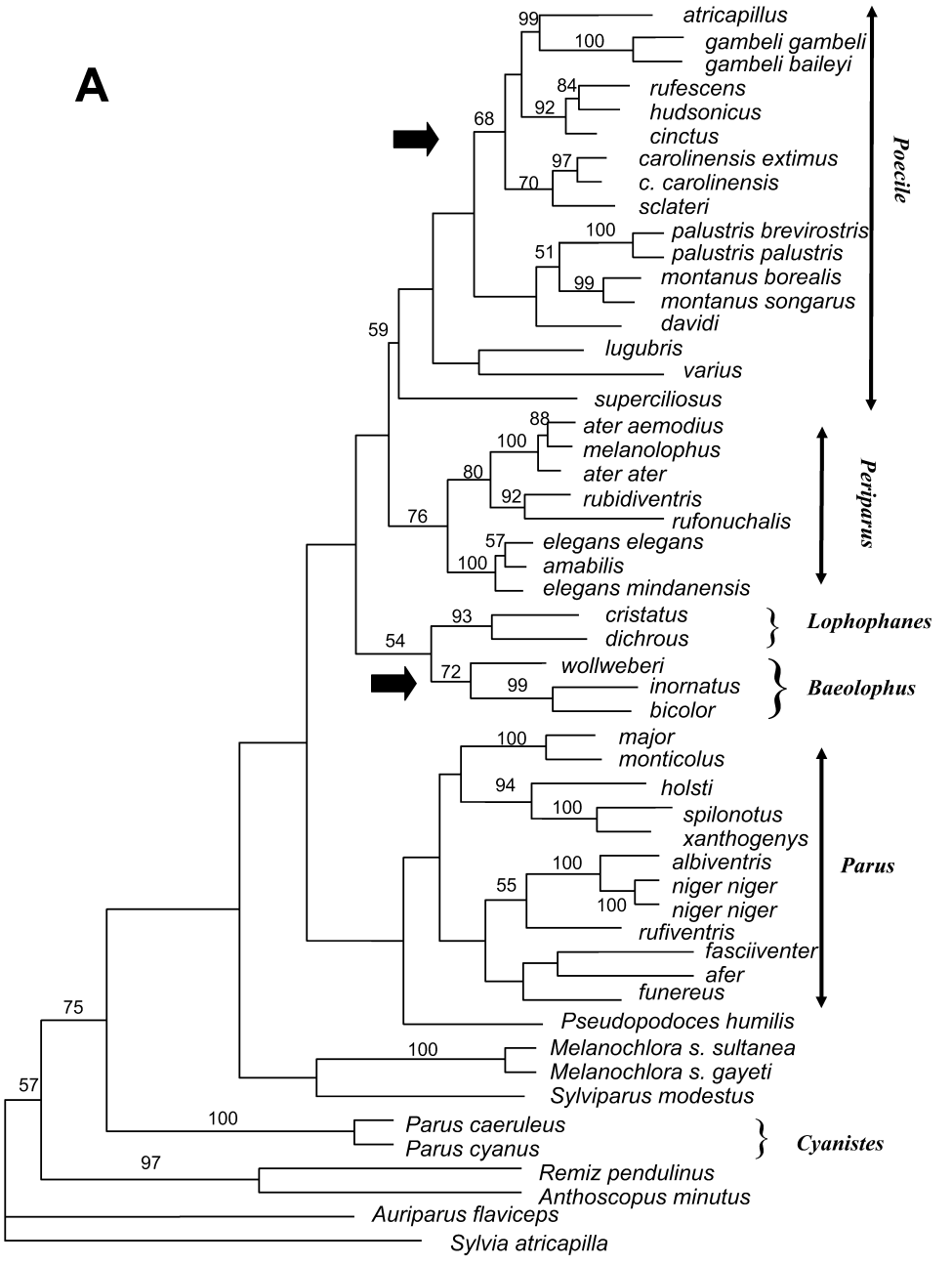

FIG. 1. Relationships among species of Paridae. (A) Single most-parsimonious tree from a heuristic search in PAUP* (Swofford 2002), with TBR branch swapping and random addition-sequence of taxa (10 repetitions). Transversions were weighted 5:1 over transitions at first- and third-codon positions. Numbers on the branches are bootstrap percentages from 1,000 replicates. Hypothesized invasions of North America are indicated by black arrows. Recommended genera are indicated on right side of figure. (Continued on next page.)

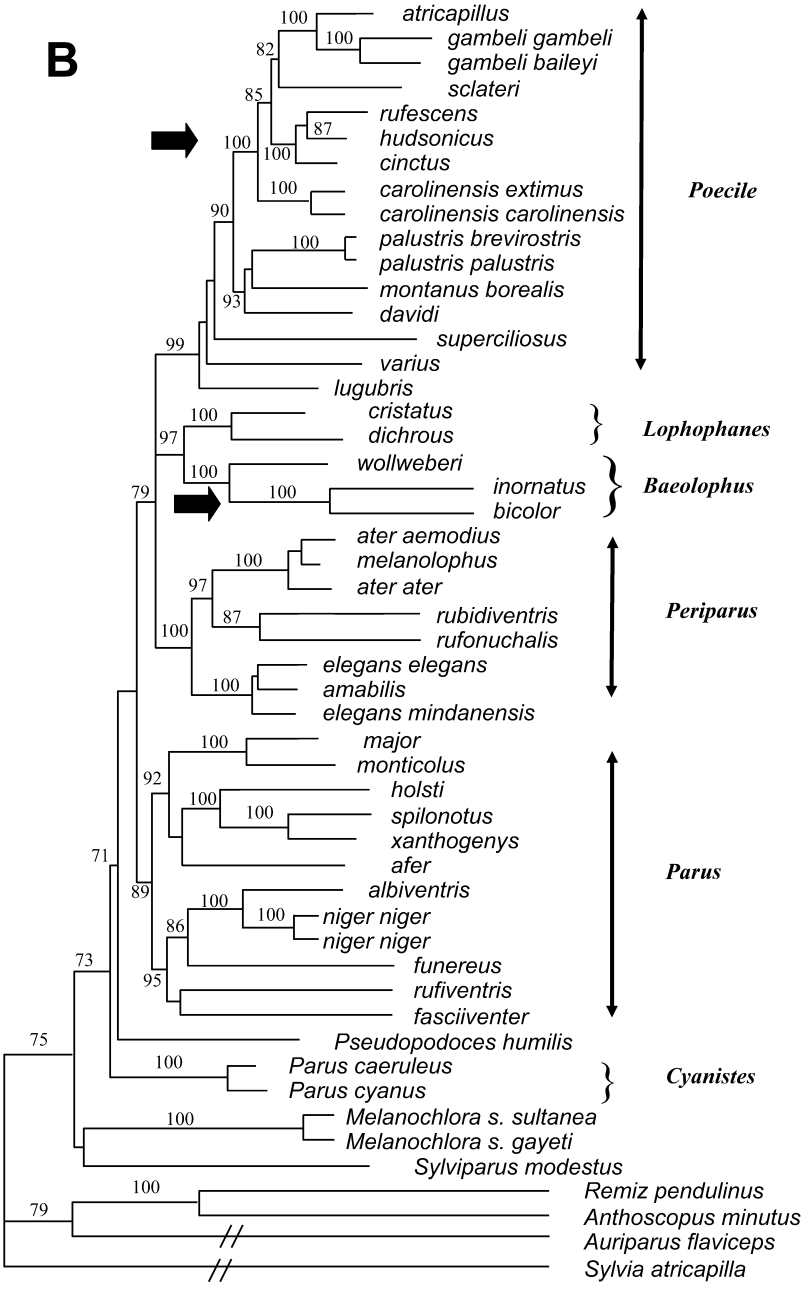

Fig. 1. (*Continued*.) Relationships among species of Paridae. (B) Maximum-likelihood phylogram from a Bayesian analysis using MRBAYES (Huelsenbeck and Ronquist 2001). Search was run with 4 chains for 500,000 generations, with a sampling frequency of 100 generations. Number of cycles before the chain reached stationarity ("burnin" in MRBAYES) was selected as 39,999 (i.e. first 39,999 cycles of the chain were discarded). Numbers on branches are posterior probabilities >70%. Hypothesized invasions of North America are indicated by black arrows. Recommended genera are indicated on right side of figure.

substantial genetic divergence between eastern and western populations of *P. carolinensis* and between Arizona and California populations of *P. gambeli* was reported previously on the basis of mitochondrial RFLP data (Gill et al. 1993, 1999). Finally, a sequence obtained from a feather of *P. varius castaneoventris* from Taiwan differed by 6% from a (toepad) sequence of *P. varius varius* from Korea. We included only the latter in our analyses, but point this out as a result that requires both confirmation and sampling of other island populations of this species.

Phylogeny.—A heuristic MP search yielded a single most-parsimonious tree (Fig. 1), rooted with *Sylvia atricapilla*. That tree is identical to the ML tree in most nodes. Differences are limited to a few weakly supported nodes, including (1) the position of *sclateri* within the clade of chickadees and gray tits (subgenus *Poecile*) and (2) the relationships among the African tits (e.g. *Parus afer, P. funereus, P. rufiventris*). The consensus tree from the Bayesian analysis is identical to the ML tree, except that a few nodes that are resolved in the ML tree are polytomies in the Bayesian consensus tree.

In all optimal trees, the family Paridae—including *Pseudopodoces, Melanochlora,* and *Sylviparus*—is monophyletic (bootstrap support = 75%, Bayesian posterior probability = 75%). *Sylviparus* and *Melanochlora* position as sister species to the rest of the Paridae, the latter including *Pseudopodoces* and species in the genus *Parus*. Within *Parus*, six clades corresponding to named subgenera appear in all optimal trees, with good support in the Bayesian tree and weaker support in the MP tree. Relationships among major clades are the same in all optimal trees, but the nodes defining those relationships have weak support. One exception is the pairing of the European and North American crested tit clades (subgenera *Lophophanes* and *Baeolophus*, respectively), which is well supported in the Bayesian tree. That pairing also was found in trees based on nuclear DNA–DNA hybridization data, though with weak support (Sheldon et al. 1992, Slikas et al. 1996).

Previously published phylogenies based on nuclear DNA–DNA hybridization data showed a basal split in the parid family, with *P. caeruleus* and *P. major* forming a clade that is the sister group of all other parids (Sheldon et al. 1992, Slikas et al. 1996). In the optimal trees based on the cytochrome-*b* sequence data, *P. caeruleus, P. major,* and their close relatives do not form a monophyletic group. *Parus caeruleus* and *P. cyanus* pair together with strong support; that pair is the sister clade to all other parids, excluding *Sylviparus* and *Melanochlora*. *Pseudopodoces* and *Parus major* and its close relatives branch off next. In the MP tree, *Pseudopodoces* is sister to the great tit clade (subgenus Parus); whereas in the consensus tree from the Bayesian analysis, *Pseudopodoces* and the great tit clade (subgenus Parus) branch off sequentially.

Relationships within subgenera.—Chickadees and gray tits (subgenus *Poecile*) are monophyletic in all optimal trees (Bayesian posterior probability [BPP] = 99%, bootstrap [BOOT] = 59%). Three Eurasian and Oriental species—*superciliosus, lugubris,* and *varius*—branch off sequentially as sisters to the remaining chickadees, though the branching order differs between the MP and ML–Bayesian trees. The remaining chickadees form a clade with relatively weak support (BPP = 90%, BOOT < 50%). Within that clade, most relationships are consistent across optimal trees. *Parus palustris, P. montanus*, and *P. davidi* group together with weak support. North American chickadees (including *P. cinctus*) form a clade (BPP = 100%, BOOT = 68%). Within that clade, *P. atricapillus* is sister to *P. gambeli.* The brown-backed chickadees are monophyletic: *P. rufescens* is sister to *P. hudsonicus*, and that pair is sister to *P. cinctus.* Those latter relationships have strong support. *Parus sclateri* pairs differently in the two trees—that is, with *P. atricapillus* and *P. gambeli* in the ML–Bayesian tree and with *P. carolinensis* in the MP tree.

The Eurasian crested tits, *P. dichrous* and *P. cristatus*, pair as sister species (BPP = 100%, BOOT = 93%). In all optimal trees, the North American crested tits form a clade: *P. bicolor* pairs with *P. inornatus* (BPP = 100%, BOOT = 99%), and *P. wollweberi* is their sister (BPP = 100%, BOOT = 72%). The Eurasian and North American crested tit clades are sister groups in all optimal trees (BPP = 97%, BOOT = 54%).

Among the coal tits, *P. ater* is paraphyletic with respect to *P. melanolophus.* In all optimal trees, *P. ater aemodius* (China) pairs with *P. melanolophus,* and *P. ater ater* (Greece) is sister to that pair. According to Martens and Eck (1995), *P. ater* and *P. melanolophus* are a single species, because the two forms hybridize extensively in west-central Nepal. In all optimal trees, the sister

to the *ater–melanolophus* clade is a pair of species from the Himalayas, *P. rufonuchalis* and *P. rubidiventris*. That clade of Eurasian tits (*P. ater, P. melanolophus, P. rufonuchalis, P. rubidiventris*) is sister to a pair of species endemic to the Philippines, *P. elegans* and *P. amabilis*. The optimal trees suggest that the latter two species are paraphyletic, but the node suggesting paraphyly has weak support.

The great tit clade includes both Eurasian and African taxa. *Parus major* and *P. monticolus* pair as sister taxa with strong support. *Parus holsti*, a species endemic to Taiwan, pairs with *P. spilonotus* and *P. xanthogenys* of the mainland Orient, also with strong support. Five African species (*P. albiventris*, *P. niger, P. funereus, P. rufiventris,* and *P. fasciiventer*) form a clade in all optimal trees; in the MP tree, that clade also includes *P. afer,* another African species. In the ML tree, however, *P. afer* pairs weakly with *P. holsti*, *P. spilonotus*, and *P. xanthogenys*. *Parus niger* and *P. albiventris* pair with strong support, but other relationships among the African taxa have weak support. *Parus caeruleus* and *P. cyanus* pair as sisters, with strong support.

Saturation.—When uncorrected cytochrome-*b* sequence divergences were plotted against DNA hybridization distances (Fig. 2), sequences exhibited a fast initial rate of change (~10× the scnDNA rate) up to ~7% divergence, after which the rate slowed as a result of saturation by back mutations. Saturation is partly responsible for the packing of uncorrected distances at about 8–10% (range of genetic distances among subgenera), making it difficult to resolve phylogenetic relationships. The packing effect of saturation can be ameliorated by correcting the cytochrome-*b* distances using the HKY95 model, but the corrections introduce another problem. Instead of falling out in a linear array with respect to relative time, HYK95 distances show considerable scatter, reflecting an increased variance as compared with uncorrected distances. An increase in variance is expected when relatively short sequences are corrected using a parameter-rich method (Swofford et al. 1996). The lack of linearity with time also may suggest the formation of (subgeneric) lineages in a short time.

The plot of transition to transversion ratio (Ti/Tv) against DNA hybridization distance (Fig. 2) indicates large variance in Ti/Tv near the origin, a pattern that apparently results from a combination of small sample size and relatively few transversions between closely related species. At longer distances (2–4% scnDNA divergence), the Ti/Tv settles at about two.

Plots of partitioned cytochrome-*b* sequence distances versus DNA hybridization distances (Fig. 2) show the expected patterns with respect to relative rates and saturation (Irwin et al. 1991, Hackett 1996). Third-position transitions have an initial rate of evolution ~20× that of single-copy nuclear DNA (scnDNA) before rapid saturation. The relatively unsaturated partitions display the following rate patterns vis-à-vis nuclear DNA: first-position transitions have about the same rate as scnDNA; second-position transitions and first- and second-position transversions have slower rates than scnDNA; and third-position transversions change at about twice the scnDNA rate.

When sequences are further partitioned according to protein structural regions (Fig. 3; Griffiths 1997; Sheldon et al. 1999, 2000), third-position transversions are again the least saturated, most rapidly changing subset of the data. First-position transitions and transversions from the transmembrane region also display fairly consistent, though slower, increases over time.

Discussion

Phylogenetic history.—The family Paridae comprises a mixture of regionally endemic species groups and widely distributed species and species groups. Our phylogenetic analyses support the traditional view that the family Paridae has an Old World center of origin (Mayr and Short 1970). Areas of high diversification and endemism are the Himalayas, Africa, and North America. Some of the older lineages, such as the blue tits (subgenus *Cyanistes*) and the great tits (subgenus *Parus*), show no particular regional pattern of diversity and endemism. Both are widespread and ubiquitous in the Old World. Extinction, dispersal, and other agents of time have obscured the trail of their diversification. An invasion of Africa and subsequent radiation there of one lineage of great tits seems apparent, as does vicariant evolution in the Himalayas. Trees based on both cytochrome-*b* and DNA hybridization data strongly indicate that parids invaded and radiated in the New World from the Old World on at least two occasions.

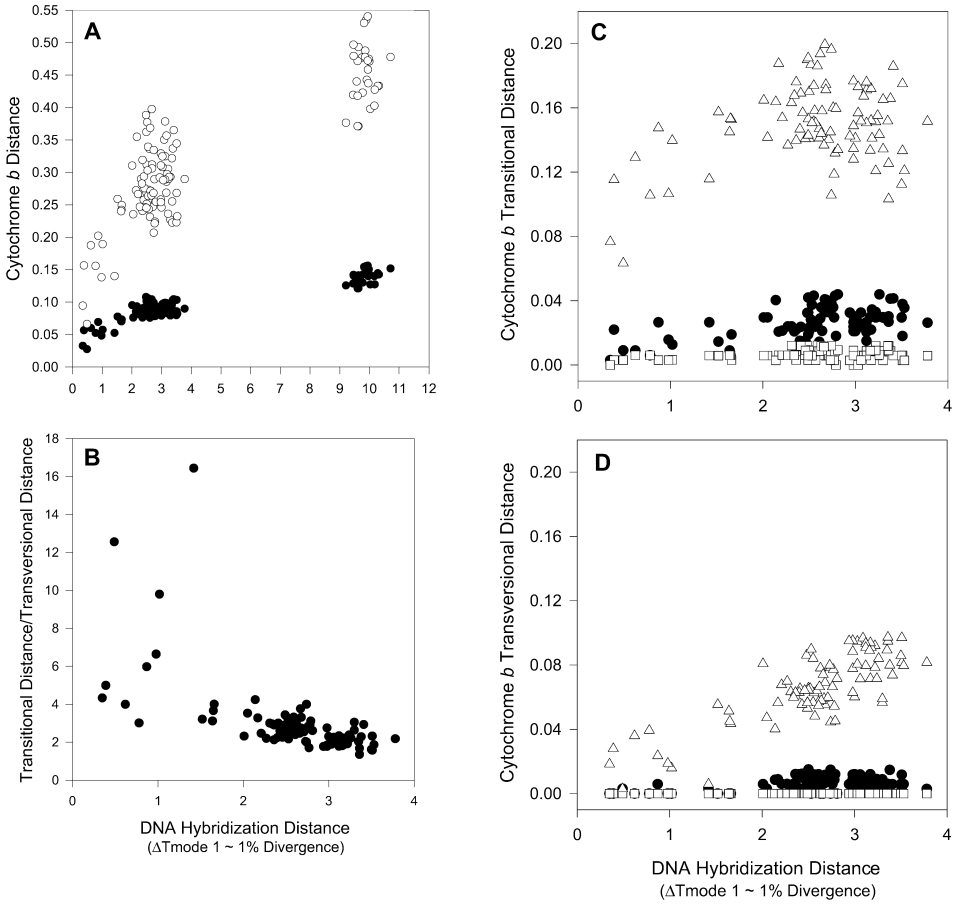

FIG. 2. Cytochrome-*b* sequence data compared with DNA hybridization distance. (A) Uncorrrected cytochrome-*b* sequence distances versus DNA hybridization distances. Closed circles = uncorrected proportional distances; open circles = HKY95 distances (GTR rates: 1.7351446, 11.368306, 1.0266284, 0.32832423, 10.329434; codon site rates: 0.319910, 0.066472, 2.613617). (B) Cytochrome-*b* Ti/Tv values, based on uncorrected Ti and Tv distances, versus DNA hybridization distances. Uncorrected cytochrome-*b* distances, partitioned as (C) transitions and (D) transversions and by codon site positions, versus DNA hybridization distances. Closed circles = first position distances; open squares = second position distances; open triangles = third position distances.

North American crested titmice (subgenus *Baeolophus*) form the sister group of the Old World crested tits (subgenus *Lophophanes*). Linking those two groups morphologically and vocally is the Bridled Titmouse (*P. wollweberi*) (e.g. Thielcke 1968, Hailman 1989). Previously, on the basis of its similarity to Old World species in plumage pattern, *P. wollweberi* was considered a North American representative of the subgenus *Lophophanes*, but phylogenetically it is clearly sister to the New World species of subgenus *Baeolophus*. That conclusion is supported by DNA hybridization as well as cytochrome-*b* evidence. The similarity in plumage pattern between *P. wollweberi* and the Old World Crested Tit (*P. cristatus*) may be the result of symplesiomorphy.

Some parid relationships were not resolved either by the cytochrome-*b* or by DNA hybridization studies—specifically, the positions of the chickadees (subgenus *Poecile)*, coal tits (subgenus *Periparus*), and the crested tits (subgenera *Baeolophus* + *Lophophanes*) in relation to one another. That trichotomy likely

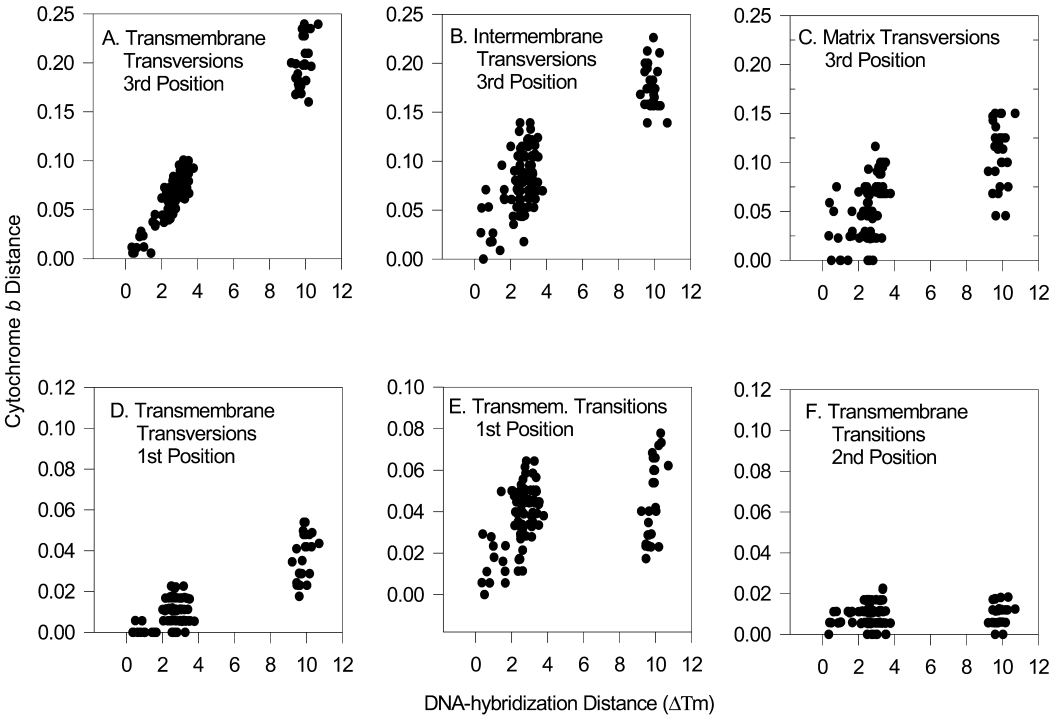

Fig. 3. Distances computed for cytochrome-*b* protein regions versus DNA hybridization distances. Cytochrome-*b* values are uncorrected proportional distances. Only those cytochrome-*b* data partitions that increased with DNA hybridization distance are shown—with the exception of (F)—for comparison with Griffiths (1997).

reflects a rapid timing of the divergence among those groups. Resolution of divergence events is impossible if the time between successive splits is too short for the diverging clades to acquire many synapomorphies (Lanyon 1988). For DNA–DNA hybridization distances, if the length of an internode is less than the average standard error in distance (~0.20; Slikas et al. 1996), then resolution is also impossible.

History of Old World taxa.—Supporting an Old World center of origin are the patterns of taxonomic diversity, endemism, and ecological observations in the family. For example, members of all subgenera but one (subgenus *Baeolophus*) are found in the Old World. The oldest lineages in the family are Old World: *Melanochlora, Sylviparus, Pseudopodoces*, subgenus *Cyanistes*, and subgenus *Parus*. Also supporting the conclusion of an Old World origin are the positions of Old World and New World species of chickadees and gray tits (subgenus *Poecile*). The oldest lineages are three poorly known, widely disjunct Eurasian species, *P. lugubris, P. superciliosus*, and *P. varius*. Previous speculations about the affinity between *P. lugubris* and *P. davidi* (Eck 1988, 1994) are not supported here. Similarly, speculations about the affinity of *P. superciliosus* and *P. gambeli* of North America, based on their white eyebrow lines (Eck 1988, Cramp and Perrins 1993), are not supported.

The number of genetically divergent subgenera in the Old World bears directly on Lack's hypothesis (1969, 1971) that the speciose guilds of Old World tits reflected a more ancient evolutionary history than the smaller guilds in the New World. The trees based on our molecular data support that ecological hypothesis. Old World species have diverged over longer periods of time, evolving morphological and behavioral traits that facilitate sympatry of species from phylogenetically divergent subgenera (McCallum et al. 2001).

History of New World taxa.—The nearly complete DNA samples of New World parid

species provide insights as to the timing of their evolution. Given a rough calibration of 2% divergence per million years (Arbogast and Slowinski 1998, Voelker 1999), we hypothesize that parids colonized the New World on two separate invasions in the late Tertiary. First, a colonization by the ancestor of modern North American crested tits (subgenus *Baeolophus*) occurred ~4 mya, on the basis of the estimate of 8.5% sequence divergence between the subgenera *Baeolophus* and *Lophophanes*. Second and soon thereafter (~3.5 mya), the ancestor of all North American chickadees (subgenus *Poecile*) colonized the continent, based on the average divergences of ~7% (6.81 ± 0.76% [mean ± SD]) between North American species in *Poecile* and the two Eurasian species, *P. montanus* and *P. palustris,* the sister group to North American *Poecile* in our optimal trees.

Soon after their invasion, the North American crested titmice occupied the Madro Tertiary flora of the continent (Cicero 1996). They also underwent at least two successive cycles of speciation. First, the separation of *P. inornatus* and *P. bicolor* from *wollweberi* (and loss of black and white face pattern) occurred. Then, during the mid- to late Pliocene, *P. inornatus* and *P. bicolor* split. Pre-*inornatus* evolved "in conjunction with increased segregation and impoverishment of western sclerophyllous woodlands in the late Pliocene and early Pleistocene" (Cicero 1996). More recently, *bicolor* fragmented into the distinct *P. atricristatus* in Texas–Mexico and *P. bicolor* in the eastern half of North America. That split is hypothesized to have taken place in the late Pleistocene, ~250,000 years ago, by Dixon (1978) and Gill and Slikas (1992). Similarly, *P. ridgwayi*, of the western interior, separated from *P. inornatus* of the west coast, as a result of regional desertification in southeastern California during the late Pleistocene and Holocene transition (Cicero 1996).

Our comparisons of cytochrome-*b* sequences include all currently recognized New World species of chickadees (subgenus *Poecile*). Several of those sort into two well-defined groups: ([*hudsonicus, rufescens*], *cinctus*) and (*atricapillus, gambeli*). But the positions of those groups *vis-à-vis* one another, and relative to *P. carolinensis* and *P. sclateri*, remain unclear. The relationships of *P. sclateri* of the Mexican highlands and southwestern U.S. remain unresolved. On the basis of cytochrome-*b* evidence, *P. sclateri* paired inconsistently with *P. carolinensis* and (*atricapillus, gambeli*). *Parus sclateri* associated weakly with (*hudsonicus, rufescens)* in a previous study of mtDNA restriction sites (Gill et al. 1993).

The present study confirmed the sister relationship between *P. atricapillus* and *P. gambeli* reported earlier by Gill et al. (1993). *Parus atricapillus* speciated from *P. gambeli* ~2.5 mya. *Parus atricapillus* is not most closely related to *P. carolinensis,* even though the two morphologically similar species hybridize in a narrow zone of secondary contact in the eastern United States (Brewer 1963, Gill et al. 1993, E. D. Sattler unpubl. data 1996).

Three species of chickadees of northwestern North America (*P. hudsonicus*, *P. rufescens*, *P. cinctus*) traditionally have been affiliated with each other (Mayr and Short 1970). *Parus hudsonicus* and *P. rufescens* clearly are sister species, despite speculation that *P. cinctus* of the Old World and *P. hudsonicus* of the New World were conspecific (Eck 1980). Those three species formed early in the Quaternary, ~1.5 mya, on the basis of their sequence divergence of 3%. Given the placement of *P. cinctus* well within the clade of North American chickadees in the subgenus *Poecile*, we hypothesize that its immediate ancestor colonized Eurasia from North America. The small modern population of *cinctus* in Alaska likely represents either a remnant of that ancestral population or a subsequent recolonization of North America.

Comparative divergence of parid taxa.—Initially, we noted that the parids exhibit a mosaic pattern of population divergence within species and larger-than-expected divergences between species. Our comparisons of parid cytochome *b* addressed mainly the second issue. To investigate the patterns of species divergence further, we plotted cytochrome-*b* divergence within and between species and within and between genera of parids in Figure 4. The figure may be compared to figure 1 in Johns and Avise (1998) to obtain a relative impression of divergence in parids versus other groups of birds.

Parids are remarkably similar to one another in morphology and behavior and are distinct from other groups of passerines. As such, they appear to be a closely related, recently evolved group, and for that reason all species have been considered congeneric. However, the longest cytochrome-*b* distances between parid species

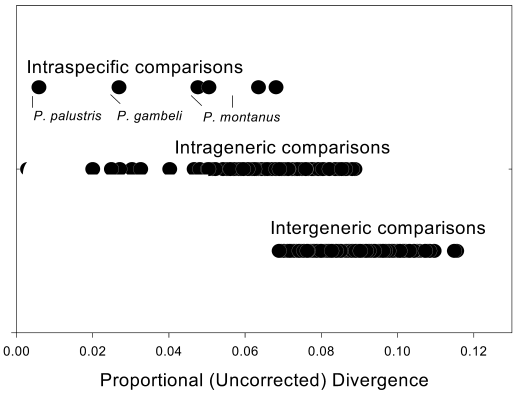

FIG. 4. Relative ranges of divergence for intraspecific, intrageneric, and intergeneric comparisons of cytochrome *b* among parids. "Intrageneric comparisons" are between species in the same parid subgenus and "intergeneric comparisons" are between parid species in different parid subgenera.

reach ~12% in uncorrected divergence, which suggests that parids are an older group than previously realized. Their relative age is evident when parid divergence is compared to intrageneric distances plotted by Johns and Avise (1998). They examined 88 genera, of which 70 (80%) exhibit less pairwise divergence than parids. Even if we consider only those genera with multiple species comparisons, 31 of 48 (65%) have lower between-species divergence than the most distantly related parids. Although such comparisons assume a molecular clock and are biased by the subjectivity of generic allocations, it is clear that the titmice are a relatively old group of songbirds characterized by morphological stasis, with one stunning exception.

The enigmatic Tibetan Ground-Jay (*Ps. humilis*) was long thought to be a corvid, though with doubt (Borecky 1978, Hope 1989). This terrestrial, small thrasher-like species is restricted to a limited region of the high Tibetan plateau. It builds nests in crevices and rodent burrows and in cavities that it excavates in banks (Schäfer 1938). On the basis of separate parsimony analyses of 55 osteological traits and a limited set of DNA sequences (James et al. 2003), *Pseudopodoces*—it turns out—is an unconventional titmouse. Phylogenetic analyses based on nuclear (*c-myc*) and mitochondrial (cytochrome-*b*) gene sequences place *Pseudopodoces* within the Paridae, not the Corvidae. In all optimal trees based on the cytochrome-*b* sequences of this study, as in James et al. (2003), *Pseudopodoces* falls within the family Paridae, sister to the great tits (subgenus *Parus*). The cytochrome-*b* divergence between *Pseudopodoces* and other species of titmice and chickadees is ~9.4% (uncorrected), roughly the level of divergence among parid subgenera. Thus, *Pseudopodoces* is the most morphologically and ecologically divergent of all tit species. Such divergence contrasts with the morphological stasis that is the surface hallmark of the Paridae. Ground Tit would be the appropriate English name for this unusual species.

Adaptive radiation of the Paridae.—The 51 species of titmice and chickadees constitute a well-defined, monophyletic assemblage of sylvioid passerine birds on the basis of both morphological and molecular characters (Moreno 1985, Harrap and Quinn 1995, Sheldon and Gill 1996, James et al. 2003). Their feeding behavior of hammering open seeds with their short bills is associated with diagnostic skull osteology (James et al. 2003).

More than in their morphology, parids exhibit substantial diversification in key features of behavior and in their vocalizations. Two of the oldest clades (subgenera *Parus* and *Cyanistes*) do not cache and recover seeds as the majority of parid species do. That difference in behavior is correlated with differences in brain morphology and social systems (Ekman 1989, Sherry 1992, Slikas et al. 1996). Even more striking is the diversification of vocal repertoires among clades (Thielcke 1968, Hailman 1989).

Parids generally have elaborate vocal repertoires with dozens of elements that include differentiated whistled songs, "gargles," alarm calls, and a combinatorial "*chick-a-dee*" call complex. Hailman (1989, fig. 4) suggested that the primary evolutionary direction of the repertoires was toward increasingly complex vocalizations, from unit vocal patterns, to contextual use of songs, to repertories of equivalent songs, followed by reduced songs in chickadees and gray tits (subgenus *Poecile)*. Our phylogeny of parid taxa suggests different polarities of change among the major features of vocal organization, because at least three taxa with complex repertoires (i.e. *Sylviparus*, *Parus*, and in the genus *Parus*, the subgenera *Cyanistes*) are among the oldest lineages in our trees.

Blue tits of the subgenus *Cyanistes* produce

many of the note types found in other parid species (but not the whistled songs of species of the subgenus *Poecile*; J. Martens unpubl. data), which implies that the entire note repertoire of the Paridae was present early in the diversification of the family. Blue tits also employ combinatorial syntax (Hailman 1989), which we project has been a feature of vocal behavior since the early origin of *Parus sensu lato.* Thus, certain repertoires (i.e. that of *hudsonicus*) are secondarily simplified to isolated notes and unit vocal patterns. We defer the formal analysis of vocal repertoire changes, including additions and deletions of whistled songs, to a separate paper (McCallum et al. unpubl. data). We hypothesize here that cultural restructuring and simplification of complex vocal repertoires may have played a major role in parid speciation. Our phylogenetic framework of parid taxa will help guide future decisions about the polarities of change among the major features of vocal organization.

Taxonomy.—To be descriptively useful, a genus should be monophyletic, reasonably compact, and ecologically, morphologically, or biogeographically distinct. Thus, Sheldon and Winkler (1993) advocated the splitting of the large swallow genus *Hirundo* into monophyletic groups, such as cliff swallows (*Petrochelidon*), house martins (*Delichon*), crag martins (*Ptyonoprogne*), and so forth, on the basis of distinctive nesting habits. Given that logic, it may be desirable to split the genus *Parus*, which currently consists of ~51 species and is one of the largest genera of birds. The AOU (1998) started the process by elevating the North America crested titmice (subgenus *Baeolophus*) and chickadees (subgenus *Poecile*) to genera. As mentioned above, we have attempted a comprehensive review of relations among species based on the traditional primacy of the large genus *Parus*, with explicit and secondary use of historical subgeneric names. On the basis of the results presented here, we now recommend the elevation of five subgenera to genera, and restricted use of *Parus* for members of the clade of great tit species only. Those changes would result in the adoption of six genera of parids in addition to continued recognition of the monotypic *Melanochlora*, *Sylviparus*, and *Pseudopodoces.* Restructuring the traditional genus *Parus* into six clades of generic status should facilitate future analyses of evolution among parids. Each of the six recommendend genera is a monophyletic group that displays distinctive behavioral and morphological characteristics. Those six genera elevated from subgenera are:

(1) *Poecile*. The familiar chickadees of North America and gray tits of Eurasia also include *P. varius* of the Orient. Hellmayr (1903), Thielcke (1968), and Harrap and Quinn (1995) separated *varius* as the monotypic genus–subgenus *Sittiparus*.

(2) *Baeolophus*. We support recognition of this genus, as adopted by the AOU (1998), for the North American crested titmice, including *wollweberi*.

(3) *Lophophanes*. We recommend recognition of this strictly Eurasian genus to include *P. cristatus* and *P. dichrous*, but not *P. wollweberi*.

(4) *Periparus*. In addition to the traditional species of coal tits, two Oriental species are members of this group. *Parus elegans* and *P. amabilis* were previously separated as *Pardaliparus*. Löhrl (1988) also allied *P. venustulus* to *P. amabilis* as the third member of *Pardaliparus*. We recommend including all three species in *Periparus* and would not recognize *Pardaliparus* at this time.

(5) *Parus*. In addition to the core species (*P. major*, *P. monticolus*), the great tits include the Oriental species, *P. xanthogenys* and *P. spilonotus*, as proposed by Hellmayr (1903) and Thielcke (1968); the African tits (*Melaniparus,* Harrap and Quinn 1995); and *P. holsti*, previously assigned to the monotypic *Machlolophus*. We do not have any insight into the relationship of *P. semilarvatus,* which Harrap and Quinn (1995) placed in the *Sittiparus* with *P. holsti*, so we retain it in *Parus* next to *P. holsti.*

(6) *Cyanistes*. The blue tits include the traditional widespread *P. caeruleus* and the north Asian species *P. cyanus* (including *flavipectus*).

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