THE RELATIONSHIPS OF THE NEW ZEALAND WRENS (ACANTHISITTIDAE) AS INDICATED BY DNA-DNA HYBRIDIZATION

By CHARLES G. SIBLEY, GORDON R. WILLIAMS and JON E. AHLQUIST

ABSTRACT

The relationships of the New Zealand Wrens have been debated for a century but up to 1981 it has not been clear to which suborder of the Passeriformes they should be assigned. Comparisons between the single-copy DNA sequences of *Acanthisitta chloris* and those of other passerine birds indicate that the Acanthisittidae are members of the suboscine suborder Oligomyodi, and that they are sufficiently distant from other suboscine passerines to warrant separation as an Infraorder, Acanthisittides.

INTRODUCTION

The endemic New Zealand family Acanthisittidae contains four species in two genera. The Rifleman *(Acanthisitta chloris)* occurs commonly in many parts of the North and South Islands, and Stewart Island, and on some offshore islands. There are no recent records of the Bush Wren *(Xenicus longipes)*, which once occurred on all three main islands. If not now extinct it occurs only in a few remote forested areas. The Rock Wren *(X. gilviventris)* inhabits rocky terrain in the subalpine and alpine zones of the main South Island mountains, and the Stephens Island Wren *(X. lyalli)*, known only from that small island, has been extinct since 1894.

The Rifleman was described as *"Sitta chloris"* by Sparrman in 1787 and was assigned to various other genera, including *Motacilla, Sylvia,* and *Acanthiza,* until 1842 when Lafresnaye erected the genus *Acanthisitta.*

The distinctive characters of the New Zealand Wrens were discovered by Forbes (1882), who found that the syrinx is located in the bronchi and lacks intrinsic muscles. These conditions are known otherwise only in some of the suboscines. Forbes concluded that the "Xenicidae" must be related to the New World tyrannoid suboscines, to the Old World Pittidae, or to the Philepittidae of Madagascar, although the New Zealand Wrens differ from these groups in several other characters. In spite of important differences, Forbes considered the acanthisittids to be allied to the New World suboscine manakins

(Pipridae) and tyrant flycatchers (Tyrannidae), and to the Old World pittas and philepittas. Forbes (1882) proposed the family name "Xenicidae" for the New Zealand Wrens, but Sundevall (1872) had used " Acanthisittinae," which takes precedence as the basis for the family name.

Furbringer (1888) included *Xenicus* in his "Oligomyodi" with *Pitta* and the Neotropical tyrannoids, and suggested that their wide geographic distribution attests to their extreme age and accounts for their anatomical diversity.

Sclater (1888) reviewed Forbes' study and concluded that " the Xenicidae must be held to be more nearly allied to the Pittidae than to any other Passerine form yet known. But they have only 10 rectrices instead of 12 — the normal Passerine number, and the scutellation of the tarsus is different." Sclater placed the " Xenicidae " between the Pittidae and the broadbills (Eurylaimidae) and Gadow (1893) inserted the " Xenicidae " between the Pittidae and the Tyrannidae.

Pycraft (1905) examined the pterylography, ear region, tarsal scutellation, rhamphotheca, muscles, syrinx and skeleton of *Acanthisitta* and found that some of the " extremely interesting facts " he discovered were in "conflict with the statement made by Forbes." Pycraft differed from Forbes in finding what he thought to be an intrinsic syringeal muscle that " ends, in the form of very degenerate fibrous tissue, on the third bronchial ring." Forbes had reported that, in *Xenicus*, these fibres terminated before reaching the top of the " syringeal box." Ames (1971) showed that the muscle described by Pycraft was the extrinsic M. tracheolateralis and that there are no intrinsic syringeal muscles in either *Acanthisitta* or *Xenicus*.

Pycraft (1905: 608) also described the unusual structure of the ear opening in *Acanthisitta* which is a " narrow horizontal slit " behind the eye that gives access to a pocket-like chamber extending downward to the opening of the auditory meatus.

Pycraft concluded that the skull of *Acanthisitta* " appears to agree most nearly with that of the Synallaxine birds " (p. 615) and its "nearest allies . . . are the Furnariidae [=Neotropical ovenbirds] . . . the same form of the maxillopalatine processes and the schizorhinal nares is present in all the Furnariidae " and in *Acanthisitta* (p. 619). But Pycraft remained in a quandary because the syrinx of *Acanthisitta* " and other small features . . . prevent the introduction of [*Acanthisitta* and *Xenicus*] into the Furnariidae." The haploophone syrinx, " the peculiar aural apertures and the primitive condition of the *deltoides major* muscles, forms a combination . . . to justify the formation of a separate family . . ."

The following year Pycraft (1906) published a study of the osteology of the tracheophone passerines and included the " Xenicidae " in his suborder Tracheophoneae (=Formicariidae, Dendrocolaptidae, Furnariidae, Conopophagidae, Xenicidae). But the " Xenicidae " have

a haploophone [= bronchial] syrinx, and so Pycraft decided that they " would appear to be at the bottom of the tracheophone stem, the members of which split up into holorhinal and schizorhinal types " (p. 157). Thus, in spite of several major discrepancies, Pycraft viewed the New Zealand Wrens as " more or less nearly related " to the Synallaxinae, one of his subfamilies of the Neotropical Furnariidae.

The anatomical studies of Forbes and Pycraft had brought them to substantially different conclusions. They agreed only that the New Zealand Wrens are not oscines and that they deserve recognition as a family. But Forbes thought they were related to the Neotropical tyrannoids and the Old World suboscines, and Pycraft allied them to the New World furnarioids.

Since 1906 the data and opinions of Forbes and Pycraft have been variously interpreted by avian systematists, as the following brief reviews will demonstrate.

Mathews (1927) divided the four species of New Zealand Wrens into three families, Acanthisittidae, Xenicornithidae, and Traversiidae, and placed them between the Pittidae and the Atrichornithidae.

Wetmore (1930), who took " the work of Hans Gadow " as a starting point, placed the " Xenicidae " in the " Superfamily Tyrannides " between the Pittidae and the Philepittidae. The same arrangement was used by Wetmore in his later editions (e.g. 1960).

Stresemann (1934: 845) recognised a Superfamily " Haploophonae " in which he placed the Old World families Acanthisittidae, Philepittidae, and Pittidae, and the New World tyrannids, piprids, cotingids, and phytotomids.

Oliver (1945) observed that the vomer of *Acanthisitta* is distinctive in form and unlike that of *Pitta,* although the " maxillopalatines are entire in both genera."

Mayr & Amadon (1951: 12) viewed the external similarities between the New Zealand Wrens and some of the Neotropical " Tracheophonae," for example, *Conopophaga,* as due to convergence, rather than to close relationship. In their classification they placed the " Xenicidae " in the " Tyrannoidea (Haploophonae) " with the pittas, philepittas, and New World tyrannoid families, thus following Forbes (1882) and Stresemann (1934). Berndt & Meise (1960) also associated the " Xenicidae " with the pittas, philepittas, and the New World tyrannoids.

Sibley (1970) found that the electrophoretic pattern of the egg-white proteins of *Acanthisitta* differed " in many ways from those of the New World non-oscine groups," and suggested that the true relatives might be the oscines. Sibley (1970: 39) noted that " the egg-white patterns are useful in suggesting lack of relationship and in demonstrating the cohesion of a closely related group but they cannot,

alone, provide a firm basis for suggesting an alliance between groups for which there is no other evidence of relationship." Sibley concluded that the evidence, from all sources, indicated " that it is improbable that the Eurylaimidae, Acanthisittidae and Pittidae are closely related to one another."

Ames' (1971) study of the passerine syrinx included comparisons between the syringes of *Acanthisitta* and *Xenicus,* and those of many other groups of passerines. Ames confirmed Forbes' (1882) descriptions and concluded (p. 155) that " The . . . genera *Xenicus* and *Acanthisitta,* were placed by Forbes (1882) in the " Oligomyodae " (the Tyrannoidea of later writers) solely on the basis of the insertion of the syringeal muscles . . . . These two peculiar genera show no clear relationship to any New World tyrannoid group. Without knowledge of other anatomical and behavioral features it is unlikely that the true position of the Acanthisittidae can be determined."

In 1974 Feduccia published the first of a series of papers on the morphology of the avian bony stapes (columella). He examined the stapes in " more than 1000 species " of birds and found that "most of the non-passerine orders," and the entire " oscine passerine assemblage " have a " primitive," reptile-like, stapes " with a flat footplate, and a straight bony shaft." The major groups of suboscines, however, have a stapes " characterized by a large bulbous footplate area perforated usually by one large (often one large and one small) fenestra . . . ." This " derived " condition was found in the Eurylaimidae, the Pittidae, and in all of the New World suboscines.

Feduccia (1975a) found that *Acanthisitta* has a stapes which is " typically oscine, but with a shaft relatively more robust " than those of undoubted oscines. Feduccia concluded that " additional *new* evidence will be needed to draw conclusions of the relationships of the New Zealand wrens, but . . . I suggest that their closest living relatives are to be found among the oscines."

Feduccia (1975b) next assembled his data on the avian stapes in a comprehensive study. With reference to the New Zealand Wrens he wrote (p. 29) that although " possession of the primitive condition of the stapes . . . does not prove . . . oscine affinities, it suggests that they are not close allies of the modern suboscines, or at least would have had to evolve before the derived stapes type." Feduccia (1975b: 33) concluded that " it is improbable that . . . *Acanthisitta* is a suboscine."

In 1977 Feduccia presented a " new model for the evolution of the perching birds," based mainly upon the morphology of the stapes. His new classification split the traditional Passeriformes into two orders. The suboscines became the " Tyranniformes " which, Feduccia suggested, had shared a common ancestor with another new order of non-passerines, the " Alcediniformes" (bee-eaters, kingfishers, motmots, todies, trogons). Feduccia placed the typical oscines in his restricted order Passeriformes

because " the oscines and suboscines could not have shared a common ancestor." With this conclusion, Feduccia put aside his earlier caution and declared the Acanthisittidae to be oscines, hence to be included in his order " Passeriformes." He speculated (p. 27) that the ancestors of the oscines might be " the primitive piciforms " or some group " intermediate between coraciiform and piciform birds . . ."

The " new model " of passerine phylogeny was soon questioned by Feduccia and his colleagues (Henley *et al.* 1978), who found that oscine spermatozoa have an "undulating membrane " and occur in precisely aligned " bundles," whereas the spermatozoa of the woodpeckers (Picidae) lack the undulating membrane, do not occur in precisely oriented bundles, and differ in other respects. Feduccia (1979) then used the scanning electron microscope to examine the spermatozoa of the suboscine Tyrannidae and found them " to have the sperm bundles characteristic of . . . the oscines." The scanning electron microscope also revealed " many differences, especially in the footplate region " between the stapes of suboscines and members of his " alcediniform " assemblage. He reported that the differences " do not argue for homology of the two and, in fact, would seem to indicate a high probability that the two morphologies evolved independently." Feduccia concluded (p. 694) that " it now seems . . . more probable that the order Passeriformes is monophyletic " although the " oscines and suboscines are very distinctive groups . . . separated by a broad and ancient evolutionary gulf."

Although Feduccia himself discovered the flaws in his " new model " of the passerine birds, he left the New Zealand Wrens among the oscines. However, Feduccia cast considerable doubt on the significance of the stapes as an index to relationships and, as noted above, showed (1975a) that the stapes of *Acanthisitta* actually differs from that in *all* other groups of passerine birds.

Wolters (1977), without explanation, gave the New Zealand Wrens their own suborder, Acanthisittae, between the suboscine Tyranni and the oscine suborder Passeres, and Mayr (1979) indicated his uncertainty by placing the Pittidae, Philepittidae, and Acanthisittidae in a " Suborder Incertae Sedis " between the New World Tyranni and the Australian Menurae. Sibley (1974), Feduccia (1975a), and Sibley & Ahlquist (in press, h) have provided evidence that the Australian lyrebirds (Menuridae) are oscines.

The taxonomic history of the Acanthisittidae demonstrates the difficulties encountered in the attempts to determine the phylogeny and relationships of birds using morphological characters. During the period from 1882 to 1975, there was a consensus that the New Zealand Wrens are suboscines, although there was a wide range of opinion within that boundary. Feduccia expanded the arena to include the oscines but the lack of confidence in any of the opinions noted above is epitomised by Mayr's (1979) assignment of the group to the limbo

of " incertae sedis." After a century of study and debate we have returned to the starting point — the Acanthisittidae are passerine birds of unknown affinities.

In this paper we offer new data from comparisons of single-copy DNA sequences, which provide objective measurements of the degrees of difference between the genetic complements of different species. These data indicate that the Acanthisittidae are the only survivors of a lineage that probably branched from the other passerines in the Cretaceous. They are suboscines, but they have no close living relatives.

METHODS

We have used the DNA-DNA hybridization technique to examine the taxonomic relationships between the New Zealand Rifleman and other passerines. The genetic material, deoxyribonucleic acid (DNA), is a double-stranded molecule composed of linear sequences of four " nucleotides," which differ in the chemical structures of their nitrogenous bases, namely, adenine (A), thymine (T), guanine (G), and cytosine (C). In double-stranded DNA the bases occur as complementary pairs: an A in one strand pairs only with a T in the other strand, a G pairs only with a C. Genetic information is encoded in the *sequences* of the bases. The two strands of native DNA molecules will separate if heated in solution to c.100 °C, which dissociates ("melts") the hydrogen bonds between base pairs. Upon cooling, the double-stranded molecules reform because the complementary bases on the two strands reassociate. If the temperature is maintained at or near 60 °C, base pairing occurs only between *long* homologous sequences of nucleotides. This is because only long sequences of complementary bases have sufficient bonding strength to maintain stable duplexes at that temperature, and only homologous sequences have the necessary degree of complementarity. Thus, under appropriate conditions of temperature and salt concentration, the dissociated single strands of conspecific DNA will reassociate only with their homologous partners and the matching of complementary base pairs will be essentially perfect.

Similarly, if the single-stranded DNA molecules of two different species are combined under conditions favouring reassociation, " hybrid " double-stranded molecules form between homologous sequences. However, these hybrid molecules will contain mismatched base pairs because of the differences in their nucleotide sequences that have evolved since the two species diverged from their most recent common ancestor. The mismatched bases reduce the bonding strength holding the two strands together and cause them to dissociate at a temperature lower than that required to melt conspecific double-stranded DNA molecules. The reassociation of homologous sequences, and the decreased thermal stability of partly mismatched hybrid sequences, form the basis of the DNA-DNA hybridization technique.

The extent to which the bases in the homologous nucleotide sequences of any two single strands of DNA form complementary A-T and G-C pairs can be determined by measuring (1) the percentage of hybridization and (2) the thermal stability of the reassociated double-stranded molecules. Following is a synopsis of the technique, which is described in more detail by Sibley & Ahlquist (1981) and based on procedures described by Kohne (1970) and Britten *et al.* (1974).

DNAs of the species in Table 1 were obtained from the nuclei of avian erythrocytes, purified according to the procedures of Marmur (1961) and Shields & Straus (1975), and " sheared " into fragments with an average length of c.500 nucleotides by sonication.

The single-stranded DNA of the New Zealand Rifleman, which was to be " labelled " with radioiodine, was allowed to reassociate to a Cot of 1000 at 50 °C in 0.48M sodium phosphate buffer. (Cot = the concentration of DNA in moles/litre times the duration of incubation in seconds — Kohne 1970: 334.) This period of reassociation permitted most of the repeated sequences to form double-stranded molecules while the slowly reassociating single-copy sequences remained single stranded. The latter were recovered by chromatography on a hydroxyapatite column. This process produced a single-copy DNA preparation consisting of one copy per genome of each original single-copy sequence and *at least* one copy per genome of each different repeated sequence. Such a single-copy preparation contains at least 98%, and probably 100%, of the " sequence complexity " of the genome, i.e., the total length of *different* DNA sequences (Britten 1971). Kohne (1970: 334-347) has discussed the reasons for using only single-copy DNA in studies designed to determine " the extent of nucleotide change since the divergence of two species."

The single-copy DNA sequences of the New Zealand Rifleman were labelled with radioactive iodine (^{125}I) according to the procedures of Commorford (1971) and Prensky (1976). DNA-DNA hybrids were formed from a mixture composed of one part (=250 nanograms) radioiodine-labelled single-copy DNA and 1000 parts (=250 micrograms) sheared, whole DNA. The hybrid combinations were heated to 100 °C for 10 min to dissociate the double-stranded molecules into single strands, then incubated for 120 hours (=Cot 16 000) at 60 °C to permit the single strands to form double-stranded hybrid molecules.

The DNA-DNA hybrids were bound to hydroxyapatite columns immersed in a temperature-controlled water bath at 55 °C and the temperature was then raised in 2.5 °C increments from 55 °C to 95 °C. At each of the 17 temperatures the single-stranded DNA produced by the melting of double-stranded molecules was eluted in 20 ml of 0.12M sodium phosphate buffer.

The radioactivity in each eluted sample was counted in a Packard Model 5220 Auto-Gamma Scintillation Spectrometer, optimised for ^{125}I.

A computer program determined the best fit of the experimental data to one of four functions: (1) the Normal, (2) the dual-Normal, (3) the " skewed " Normal, or (4) a modified form of the Fermi-Dirac equation.

To obtain a comparison between the homologous DNA hybrid *(Acanthisitta chloris* x *A. chloris)* and each of the heterologous hybrids (e.g. *A. chloris* x *Pipra coronata,* etc.) we have used the $T_{50}H$ statistic of Bonner *et al.* (1981), which, in a normalised cumulative frequency distribution function, is the temperature in degrees Celsius at which 50% of the single-copy DNA sequences are in the hybrid form. The $T_{50}H$ (or $T_{50}R$) statistic was first suggested by Kohne (1970: 349). In the calculation of $T_{50}H$ it is assumed that all of the single-copy sequences in the genomes of each of the two species being compared have homologs in the other species, that all single-copy sequences potentially can hybridise with their homologs, and that all degrees of divergence can be detected. For DNA-DNA hybrids with normalised percentages of hybridization greater than 50%, $T_{50}H$ may be determined by a graphic extrapolation of the most linear portion of the cumulative (sigmoid) thermal dissociation curve to find the temperature corresponding to its intercept with the 50% hybridization level. All of the DNA hybrids in this study had percentages of hybridization above 50%. The delta $T_{50}H$ is the difference in degrees Celsius between the $T_{50}H$ of the homologous hybrid and the $T_{50}H$ of a heterologous hybrid. For additional discussion of $T_{50}H$, and other aspects of data analysis, see Sibley & Ahlquist (1981).

RESULTS AND DISCUSSION

Tables 1 and 2 and Figures 1 and 2 contain the data from the DNA-DNA hybrids between the radioiodine-labelled single-copy DNA sequences of *Acanthisitta chloris* and the DNAs of 57 other species. These include four species of New World suboscine tyrannoids, five species of New World suboscine furnarioids, two species of broadbills, three species of pittas, 41 species of oscines, and two species of non-passerines. Because only the DNA of *Acanthisitta* was labelled with radioactive iodine, the delta $T_{50}H$ values are distances between it and the other taxa, but not among the other taxa. Two species that have the same delta $T_{50}H$ values are phylogenetically equidistant from the labelled species, but may be any distance from one another which is equal to, or less than, their common distance from the labelled species.

From our comparisons of the DNAs of other groups, we have found that delta $T_{50}H$ values from c. 7-9 are usual between subfamilies, from 9-12 between families, 13-15 between superfamilies, 16-19 between suborders, and 20-25 between orders. These values are preliminary and subject to an error of c. $\pm$ 1.0 (Sibley & Ahlquist, 1980, 1981; in press, a-h).

Table 2 contains the averages, standard errors, and standard deviations for the $T_{50}H$ values of the DNA-DNA hybrids between

TABLE 1 — DNA-DNA hybridization values for comparisons between the New Zealand Rifleman and other species of birds. The abbreviations under the heading Group Index are: A=Acanthisitta, T=Tyrannoidea, F=Furnarioidea, E=Eurylaimidae, P=Pittidae, O=Oscine (Passeres), NP=Non-passerine.

COMMON NAME	SCIENTIFIC NAME	$\Delta T_{50}H$	GROUP INDEX
New Zealand Rifleman	*Acanthisitta chloris*	0.0	A
Ornate Umbrellabird	*Cephalopterus ornatus*	16.4	T
Blue-crowned Manakin	*Pipra coronata*	16.6	T
Chestnut-crowned Leaf-gleaner	*Automolus rufipileatus*	17.1	F
Black and Red Broadbill	*Cymbirhynchus macrorhynchos*	17.2	E
Barred Antshrike	*Thamnophilus doliatus*	17.2	F
Green Broadbill	*Calyptomena viridis*	17.4	E
Straight-billed Woodhewer	*Xiphorhynchus picus*	17.4	F
Yellow-bellied Elaenia	*Elaenia flavogaster*	17.7	T
Willow Flycatcher	*Empidonax traillii*	17.7	T
Dusky-throated Antshrike	*Thamnomanes ardesiacus*	17.8	F
Striped Leaf-gleaner	*Philydor subulatus*	17.9	F
Noisy Pitta	*Pitta versicolor*	18.2	P
Banded Pitta	*Pitta guajana*	18.3	P
Green Broadbill	*Calyptomena viridis*	18.4	E
Noisy Pitta	*Pitta versicolor*	18.5	P
Blue-headed Pitta	*Pitta baudi*	18.6	P
White-winged Chough	*Corcorax melanorhamphos*	18.6	O
Striated Pardalote	*Pardalotus striatus*	18.6	O
Chirruping Wedgebill	*Psophodes cristatus*	18.6	O
American Crow	*Corvus brachyrhynchos*	18.8	O
Australian Magpielark	*Grallina cyanoleuca*	18.8	O
Frilled Monarch	*Arses telescophthalmus*	19.1	O
Dusky Woodswallow	*Artamus cyanopterus*	19.1	O
Pale-billed Scrubwren	*Sericornis spilodera*	19.1	O
Lesser Bird-of-Paradise	*Paradisaea minor*	19.2	O
King of Saxony Bird-of-Paradise	*Pteridophora alberti*	19.2	O
Village Weaver	*Ploceus cucullatus*	19.2	O
Black-faced Woodswallow	*Artamus cinereus*	19.3	O
Brown Treecreeper	*Climacteris picumnus*	19.3	O
Fan-tailed Berrypecker	*Melanocharis versteri*	19.3	O
Yellow-rumped Thornbill	*Acanthiza chrysorrhoa*	19.4	O
Mistletoebird	*Dicaeum hirundinaceum*	19.4	O
Splendid Wren	*Malurus splendens*	19.4	O
White-browed Scrubwren	*Sericornis frontalis*	19.4	O

COMMON NAME	SCIENTIFIC NAME	$\Delta T_{50}H$	GROUP INDEX
Western Thornbill	*Acanthiza inornata*	19.5	O
Varied Sittella	*Daphoenositta chrysoptera*	19.5	O
Chiffchaff	*Phylloscopus collybita*	19.5	O
Red-eyed Vireo	*Vireo olivaceus*	19.5	O
Eastern Yellow-robin	*Eopsaltria australis*	19.6	O
White-throated Warbler	*Gerygone olivacea*	19.7	O
House Sparrow	*Passer domesticus*	19.7	O
Lesser Whitethroat	*Sylvia curruca*	19.9	O
Willie Wagtail	*Rhipidura leucophrys*	20.0	O
Yap White-eye	*Rukia oleaginea*	20.0	O
Common Starling	*Sturnus vulgaris*	20.2	O
Silvereye	*Zosterops lateralis*	20.3	O
River Flycatcher	*Monachella muelleriana*	20.4	O
Common Bulbul	*Pycnonotus barbatus*	20.4	O
Clapper Lark	*Mirafra apiata*	20.5	O
Red-winged Blackbird	*Agelaius phoeniceus*	20.7	O
Oriental White-eye	*Zosterops palpebrosa*	20.8	O
Fairy-bluebird	*Irena puella*	20.9	O
Scarlet Robin	*Petroica multicolor*	20.9	O
Rufous Bristlebird	*Dasyornis broadbenti*	21.0	O
Skylark	*Alauda arvensis*	21.2	O
Little Grassbird	*Megalurus gramineus*	21.3	O
Song Sparrow	*Zonotrichia melodia*	21.7	O
Sacred Kingfisher	*Halcyon sancta*	22.5	NP
Downy Woodpecker	*Picoides pubescens*	25.4	NP

Acanthisitta chloris and the groups represented in Table 1. It is clear that *Acanthisitta* is a member of the suboscine suborder Oligomyodi, which also includes the New World suboscines, the broadbills, the pittas, and, presumably, the philepittids. *Acanthisitta* is distant enough from the other oligomyodian groups (Tyrannides) to be separated from them as an infraorder, Acanthisittides.

It is obvious that the delta $T_{50}H$ values for the members of the groups in Tables 1 and 2 have narrow ranges. The nine values for the DNA hybrids between *Acanthisitta* and the New World suboscines range from 16.4 - 17.9, a difference of 1.5, and the 41 oscine values range from 18.6 - 21.7, a difference of 3.1. The New World suboscine values represent nine genera, at least three families, and two superfamilies, and the oscines represent 18 of the 54 living groups recognised as families by Wetmore (1960). The three broadbill values differ by 1.2, the four pitta values by 0.4. This clustering indicates that the members of each group are genetically equidistant from *Acanthisitta*. Each such cluster is a " relative rate test " (Sarich &

Wilson 1967) in which an external reference species (i.e. *A. chloris*) is used to compare the rates of evolutionary change in members of at least two taxa that diverged first from the external reference species and later from one another. The clustering of the species within each group indicates that the *average* rate of evolutionary change (=nucleotide substitution) has been the same in the lineages within each cluster since the time when the most recent common ancestor of the members of that lineage branched from the lineage that led to *Acanthisitta.*

These data also suggest that the *same average rate* of DNA evolution occurs in *all* lineages. This is indicated by the data for the nine New World suboscines and for those of the 41 oscines. The two major lineages of New World suboscines, the Tyrannoidea and the Furniarioidea, probably branched from one another at least 65 million years ago (mya) but, relative to *Acanthisitta,* their average $T_{50}H$ values differ by only 0.4 °C. Similarly, the 18 " families " of oscines represented in Table 1 probably diverged from one another at various times between c. 60 and 20 mya but, relative to *Acanthisitta,* the range of their $T_{50}H$ values is only 3.1 °C for the 41 species. Most, possibly all, of this variation may be due to experimental error. The same clustered patterns have been found in the DNA hybridization data for the ratites (Sibley & Ahlquist 1981), the Hawaiian honeycreepers (Sibley & Ahlquist, in press, a), and the Australian fairy-wrens (Sibley & Ahlquist, in press, h).

TABLE 2 — Group averages, standard errors (S.E.), and standard deviations (S.D.) for the delta $T_{50}H$ DNA-DNA hybridization values of the comparisons between the New Zealand Rifleman and the members of the other groups in Table 1.

DNA-DNA HYBRIDS	Delta $T_{50}H$	S.E.	S.D.
1. Acanthisitta chloris x A. chloris			
Four homologous hybrids.....	0.0	---	---
2. A. chloris x nine New World suboscines			
Four tyrannoids + five furnarioids.....	17.3	±0.17	±0.5
3. A. chloris x four tyrannoids.....	17.1	±0.35	±0.7
4. A. chloris x five furnarioids.....	17.5	±0.18	±0.4
5. A. chloris x three broadbills.....	17.7	±0.35	±0.6
6. A. chloris x four pittas.....	18.4	±0.10	±0.2
7. A. chloris x 41 species of oscines.....	19.8	±0.13	±0.8
8. A. chloris x two non-passerines (Halcyon and Picoides).....	24.0	±1.42	±2.0

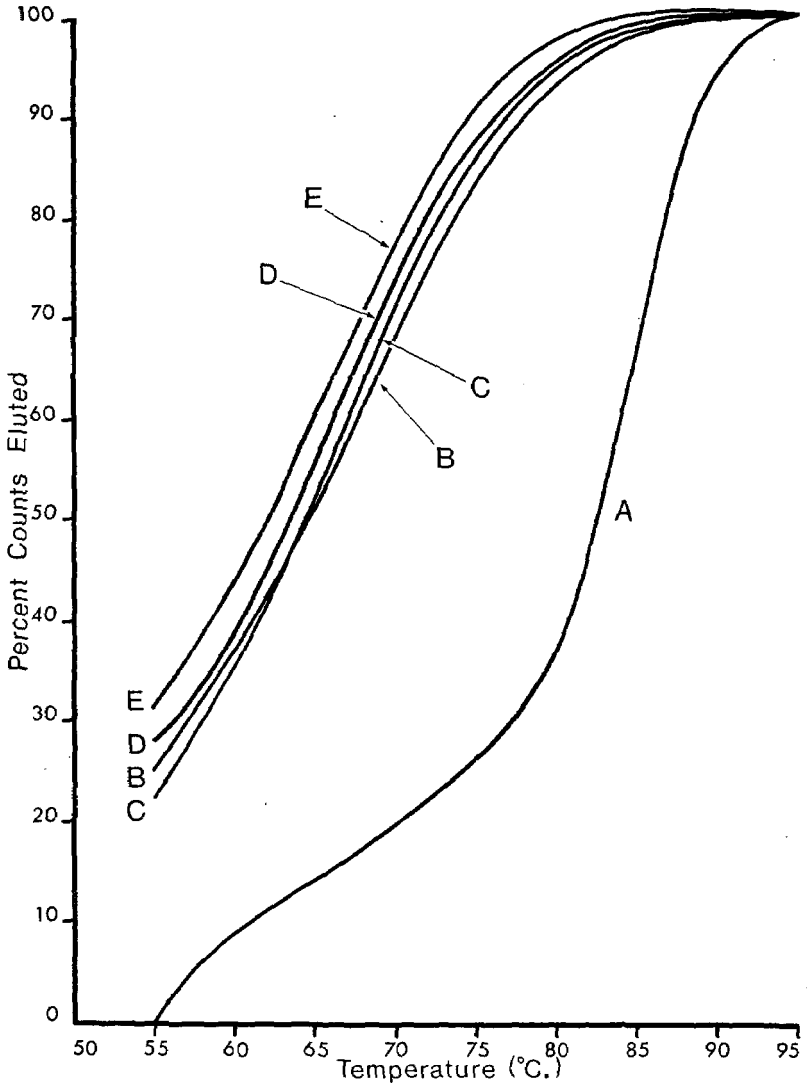

FIGURE 1 — Cumulative thermal dissociation curves of DNA-DNA hybrids in which the New Zealand Rifleman was the radioiodine-labelled species. Curve A is the average of four homologous hybrids; B= average of nine New World suboscines; C= average of three broadbills; D= average of four pittas; E= average of 41 oscines.

That DNA evolves at the same average rate in all lineages, may seem to disagree with our perception of the variable rates of evolution in morphological characters. However, since 1962 when Zuckerkandl & Pauling suggested that the amino acid sequences of proteins evolve at "constant" rates of change, the proposal that a "clocklike" rate of evolution occurs in both proteins and DNA has gained support from many studies. Fitch (1976), Wilson *et al.* (1977), and Doolittle (1979) have provided reviews.

The evidence indicates that each protein (hence each structural gene) evolves at its own rate and that the rates for different proteins differ up to c. 600-fold between the slowest histone and the most rapidly evolving immunoglobulin (Wilson *et al.* 1977: 610). An average protein molecule composed of 400 amino acids is coded for by a DNA sequence (a "gene") of 1200 nucleotides. But the avian haploid genome contains c. 1.7 x 10^9 nucleotides, of which c. 60-70% are in the single-copy fraction as prepared for radioactive labelling. The DNA hybridization values are averages across this large number and so they reflect the net amount of nucleotide-sequence divergence due to the *average* rate of change in approximately one thousand million (10^9) nucleotides over long periods of time. Thus the uniform average rate of DNA evolution in different lineages is the statistical result of the averaging of large numbers of variables operating under the same, relatively narrow, constraints. Each nucleotide, and each gene, in each individual organism is evolving at its own rate, but when these many different rates are *averaged* over such a large number of events, and over time, the uniform *average* rate is the inevitable result. This problem has also been discussed by Sibley & Ahlquist (1981, in press, a).

Because the DNA hybridization values index the average rate of DNA evolution they are proportional to the relative times of divergence between the taxa being compared. Therefore, if the DNA delta values can be calibrated against an external dating source they will provide a measure of absolute time. In our study of the ratite birds (Sibley & Ahlquist 1981) we assumed that the divergence between the lineages that produced the living African ostriches and South American rheas began when the Gondwanaland rift between West Africa and Brazil became an impassable barrier. The minimum dating for this event was estimated as c. 80 mya and, since the ostrich-rhea delta $T_{50}H$ = 15.7, 1.0 delta $T_{50}H$ = c. 5 million years.

A similar calculation for the time of divergence between *Acanthisitta* and the other suboscines may be made by assuming that the ancestor of the New Zealand Wrens was a passenger on the drifting block that became New Zealand. The geological evidence indicates that the Tasman Sea opened up in the late Cretaceous, c. 80 mya (Fleming 1975: 16). The average delta $T_{50}H$ between *Acanthisitta* and the other suboscines = 17.7, thus 1.0 delta $T_{50}H$ = c. 4.5 million years. Considering the uncertainties in the assumptions these two values

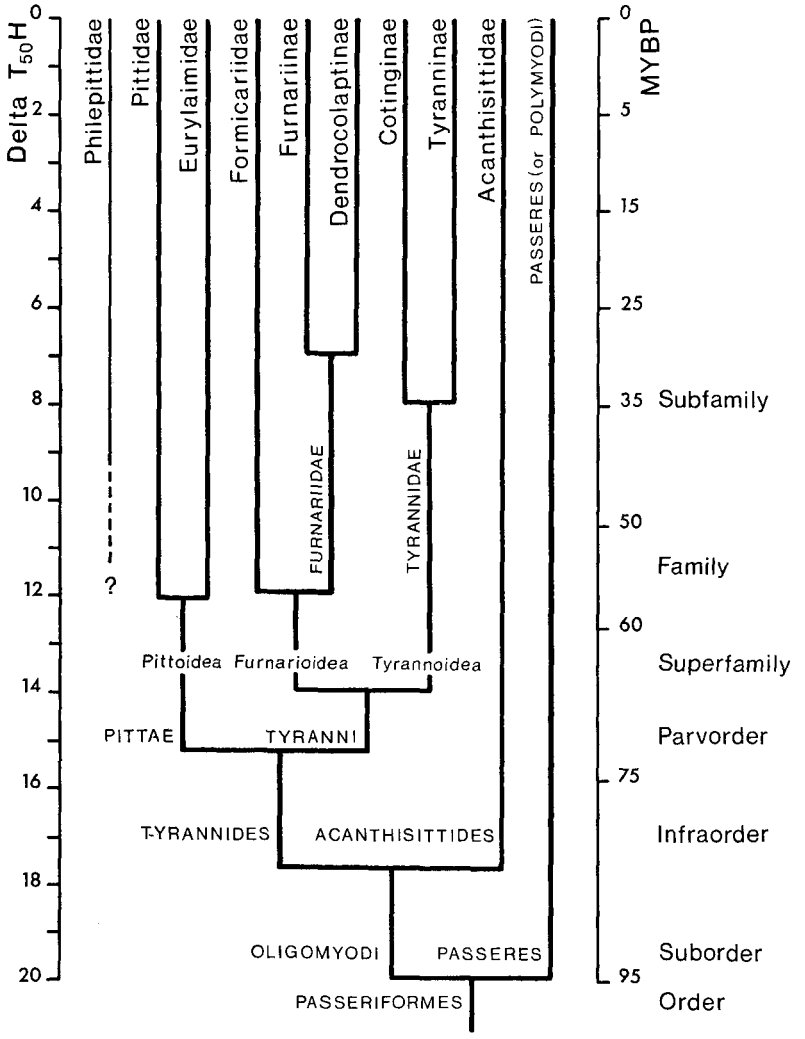

FIGURE 2 — Diagram based, in part, on the data in Tables 1 and 2 and Figure 1. The branching sequence within the Infraorder Tyrannides is based on an unpublished study in which the radioiodine-labelled DNAs of a tyrannid (**Elaenia**), a furnariine (**Automolus**), a dendrocolaptine (**Xiphorhynchus**), a pitta (**Pitta**), and a broadbill (**Calyptomena**) were used. The time scale is in millions of years before the present (MYBP).

are in excellent agreement, but both could be wrong and more and better geological and/or fossil dates of divergence are needed to obtain an accurate calibration of the delta $T_{50}H$ values.

If, for the moment, we accept an average value of 4.75 my = delta 1.0, we obtain a date of c. 70 mya for the separation of the pitta-broadbill lineage (Pittae) from the New World suboscines (Tyranni), c. 84 mya for the divergence between the Acanthisittides and the Tyrannides, c. 94 mya for the dichotomy between the Passeres and the Oligomyodi, and c. 115 mya for the divergence of the Passeriformes from their common ancestry with some non-passerine group. Figure 2 is a diagram of the branching pattern of these groups.

The standard errors (S.E.) of the average delta $T_{50}H$ values (see Table 2) may also be translated into time by using the calibration of 1.0 delta $T_{50}H$ = 4.75 million years. For example, for the divergence between the ancestral lineages of *Acanthisitta* and the 41 oscines, the average delta $T_{50}H$ = 19.8 ±0.13 S.E., which is equivalent to 94 million ±585 000 years. The S.E. is thus less than 1% of the time since the divergence of the two lineages. If the average rates of divergence among the 41 species of Passeres were actually different it is unlikely that, after 20 to 60 million years of independent evolution, all 41 would have arrived at essentially identical delta values. The c. 1% error indicated by the S.E. of 0.13 is probably due to experimental error, not to differences in the average rate of nucleotide substitution. The most reasonable explanation for these data, and for the many similar examples we have observed, is that DNA evolves at the same average rate in all avian lineages.

The New Zealand Wrens may be the oldest living group of endemic New Zealand birds because there is DNA hybridization evidence that the ancestor of the New Zealand ratites did not diverge from the Australo-Papuan ratites until the Eocene, c. 40-50 mya (Sibley & Ahlquist 1981). However, more and better geological and/or fossil divergence dates are needed to obtain an accurate calibration of the DNA hybridization values.

The phylogeny of the major subgroups of the Passeriformes will not be complete until DNA-DNA comparisons have been made among all of the pertinent groups, including the Philepittidae, the DNA of which is not yet available. However, it is apparent that the New Zealand Wrens have no close living relatives and we propose that they be placed in their own Infraorder, Acanthisittides, and be listed among the suboscines, as in the following synoptic classification of some of the major subgroups of the Passeriformes.

Order Passeriformes
  Suborder Oligomyodi
    Infraorder Acanthisittides
      Family Acanthisittidae, New Zealand Wrens
    Infraorder Tyrannides
      Parvorder Pittae
        Superfamily Pittoidea
          Family Pittidae, Pittas
          Family Eurylaimidae, Broadbills

        (Family Incertae Sedis Philepittidae, Asities)

      Parvorder Tyranni
        Superfamily Furnarioidea
          Family Formicariidae, Antbirds
          Family Furnariidae
            Subfamily Furnariinae, Ovenbirds
            Subfamily Dendrocolaptinae, Woodhewers

        Superfamily Tyrannoidea
          Family Tyrannidae
            Subfamily Tyranninae, Tyrant Flycatchers
            Subfamily Cotinginae, Cotingas, Manakins

  Suborder Passeres (or Polymyodi).

The categories Infraorder and Parvorder follow McKenna (1975) and are used to identify the major branching points in the phylogeny depicted in Figure 2.

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CHARLES G. SIBLEY, JON E. AHLQUIST, *Peabody Museum of Natural History and Department of Biology, Yale University, New Haven, Conn. 06511 U.S.A.;* GORDON R. WILLIAMS, *Department of Entomology, Lincoln College, Canterbury, New Zealand.*

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SHORT NOTE

YOUNG BLACK-BROWED MOLLYMAWK INLAND

On 26 May 1981, I was notified of a live albatross that had landed on a farm property at Makahu in Taranaki, approximately 30 km east of Stratford. The landowner had found the bird in a weakened state in a damp gully in pastureland. On collection we found it to be a Black-browed Mollymawk *(Diomedea melanophrys)*. The feather tips, particularly those of the tail and some hind-neck contour feathers, which still retained vestiges of down, indicated that the bird had only recently fledged and flown. C. J. R. Robertson (pers. comm.) inferred that this was probably an early-departing chick of *D. m. impavida* from Campbell Island.

It was penned, supplied with a trough of seawater, and force fed with fresh fish (trevally) pieces, until after 2 days it showed signs of restlessness and greater resistance to being fed. It was released on to the paritutu headland at New Plymouth, from where it flew to sea.

D. P. GARRICK, *Wildlife Service, P.O. Box 96, New Plymouth*