Phylogenetic positions of Nahan's Francolin *Francolinus nahani* and the Stone Partridge *Ptilopachus petrosus*: enigmatic African gamebirds

Julia Wakeling
Department of Botany and the Percy FitzPatrick Institute
University of Cape Town

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Supervisors: Professor T.M. Crowe and Professor T.A. Hedderson

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Nahan's Francolin
Francolinus nahani

The Stone Partridge *Ptilopachus petrosus*

ABSTRACT

Nahan's Francolin *Francolinus nahani* has both been given its own subgenus and has been associated with and compared to Latham's Forest Francolin (*Peliperdix lathami*). Using sequences of two mitochondrial genes, cytochrome b and NADH dehydrogenase subunit 2 (ND2) as well as a nuclear gene, avian ovomucoid intron G (OVOG), we found that *Francolinus nahani* is sister to the Stone Partridge (*Ptilopachus petrosus*). This relationship is revealed using parsimony analysis, and is supported by high jackknife values. *Peliperdix lathami* is part of the "Quail-Francolins" however its relationship within this clade is unresolved due to insufficient sampling. A clade consisting of *Gallus*, *Bambusicola* and the "Quail-Francolins" is shown, and the use of *Gallus* as an outgroup for pheasant and partridge phylogenetics is questioned.

INTRODUCTION

Nahan's Francolin *Francolinus nahani*, a rare and endangered gamebird found in northeastern Congo, and in west and south central Uganda, was first described by Dubois in 1905 (Urban *et al.*, 1986; Hall, 1963; Johnsguard, 1988, Madge & McGowan, 2002). It was placed by Hall (1963) within the Genus *Francolinus*, which has traditionally been recognized as comprising 36 African and 5 Asian species, prior to molecular studies.

In a reworking of the francolins, Crowe *et al.* (1992) placed Nahan's Francolin in a monotypic subgenus, *Acentrortyx*, within Genus *Pternistis* with 27 other francolins.

F. nahani is found only in dense primary forest up to 1400m, is very shy and usually seen in pairs (Urban *et al.*, 1986). Hall (1963) hypothesized that *F. nahani* could be closely related to Latham's Forest Francolin *Peliperdix lathami*, but also stated that similarities such as small size, general dark colouration and spotting, may be the result of convergence due to habitat. These two species have overlapping distributions, but that of *P. lathami* is much larger and extends further west, with *F. nahani* being rarer and more localized (Urban *et al.*, 1986). The species are also not ecologically segregated, which may suggest that they do not have a close relationship (Hall, 1963).

Hall (1963) proposed that *F. nahani* was part of her "Scaly" group, consisting of *F. ahantensis*, *F. squamatus* and *F. griseostriatus*, forest edge and savanna species. This is because, although much smaller it does share some characteristics with this group. For example, in *F. nahani* and all the species of the "Scaly" group, the sexes are similar, legs are red and there is a small area of bare skin below and behind the eye (although this is not brightly coloured and often overlooked). *F. nahani* is also similar to *F. squamatus* in that they both have an unpatterned head and vermiculated back. *F. nahani* and *F. ahantensis* have white streaking, and *F. nahani* and *F. griseostriatus* both have a crimson base to the bill.

In an analysis of its position using Euclidean distances, Crowe & Crowe (1985) confirmed that *F. nahani* fell near the "Scaly" group and was grouped closest to *F.*

ahantensis. Whilst a number of other studies have used molecular evidence to examine francolin phylogenetics (e.g. Crowe *et al.*, 1992 and Bloomer & Crowe, 1998), the DNA of *F. nahani* has not been used for phylogenetic analysis, and its relation to other galliforms is not confirmed. The "Scaly" group is currently placed in the genus *Pternistis* (Crowe *et al.*, 1992). More recently, unpublished observations on the basis of behaviour and calls have suggested a close link to the Stone Partridge (B. Finch, M. Mills & C. Cohen, pers. comm.).

Aims and Hypotheses

In this study I analyse variation at a number of DNA loci to establish the phylogenetic relationships between *Francolinus nahani* and *Peliperdix lathami*, the "Scaly" group (*Pternistis*) as well as with the Stone Partridge, *Ptilopachus petrosus*.

METHODS

Approach and Materials

Mitochondrial DNA (mtDNA) markers are often used for resolving vertebrate phylogenies because they are haploid and maternally inherited. They evolve rapidly enough to resolve closely related taxa, and yet slowly enough at some sites to resolve deeper relationships (Moore, 1995). However, mitochondrial genes are inherited as a single linkage group, and thus phylogenetic estimates from different mitochondrial genes are not independent (Moore, 1995). Recently, a number of studies have used nuclear genes, which may occur on distinct chromosomes, thus providing independent estimates of phylogeny (Moore, 1995; Johnson & Clayton, 2000; Armstrong *et al.*, 2001).

In this study, two mitochondrial genes (Cytochrome b and NADH dehydrogenase subunit 2 (ND2)) and one nuclear gene (Ovomucoid Intron G (OVOG)), are used to hypothesize the phylogenetic position of *Francolinus nahani* (& PP?). Cytochrome b is a region frequently used for avian phylogenetics, and thus there are many sequences available for comparison. ND2 has been used for a number of galliform studies (e.g. Dimcheff *et al.*, 2002), and was easily amplified. It was thus also used so that a larger character set was available for mitochondrial analysis. OVOG has been shown to be useful for avian

phylogenetics, especially in comparison with mitochondrial genes (Armstrong, *et al.*, 2001). A broad selection of galliforms has also been sequenced for this region (Armstrong, *et al.*, 2001), making it useful for this study.

Species sequenced in this study, and information pertaining to them, are listed in Table 1. Sequences extracted from GenBank are listed in Table 2.

Laboratory Techniques

Total genomic DNA was extracted from blood, heart and liver tissue using the DNeasy animal tissue protocol provided with the DNeasy® tissue kit (Qiagen). Primers used for amplification and sequencing and indicated in Table 3. The initial Cytochrome b primers amplified 1337 base pairs. Due to the length of this region, an internal primer was also used (Table 3). The initial cytochrome b primer pair did not amplify the *Pternistis griseostriatus* and *Pternistis leucoscepus* DNA extractions, so galliform specific primers were used.

Double stranded DNA templates were amplified by polymerase chain reaction (PCR) using 0.75 units of BIOTAQ™ DNA polymerase (Bioline) in 30µl reactions. Reactions also contained 1 x NH_4 buffer, 2.5mM $MgCl_2$, each dNTP at 0.1mM and each primer at 0.3µM. Three µl of un-quantified stock DNA was used as a template. The thermal profile used for all three DNA regions comprised an initial denaturation step at 94°C for two minutes, followed by 30 cycles of 94°C for one minute, 52°C for one minute and 72°C for two minutes, with a final poymerisation step of 72°C for seven minutes. The PCR's were performed by a GeneAmp® PCR System 2700 (Applied Biosystems) Version 2.07.

Amplified products were cleaned from solution or gel using the GFX™ PCR DNA and gel band purification kit (Amersham Biosciences), prior to cycle sequencing with the ABI PRISM® Big Dye™ Terminator V3.1 cycle sequencing Ready Reaction Kit (Applied Biosystems). Amplification primers were also used for sequencing, and the reaction conditions were as per the ABI protocol. The thermal profile used for all three DNA regions comprised 25 cycles of 96°C for 30 seconds, 50°C for 15 seconds and 60°C

for four minutes. Sequencing products were resolved on an ABI PRISM® 3100 Genetic Analyser. Sequences were assembled and checked for incorrect base calling and the presence of stop codons using SeqMan II (LaserGene systems software, DNAstar, inc.). Consensus sequences were aligned by Clustal and adjusted manually using MegAlign (LaserGene systems software, DNAstar, inc.).

Phylogenetic Analyses

Phylogenetic relationships among taxa were analysed with the parsimony optimality criterion (e.g. Swofford *et al.*, 1996), using PAUP4.0b10 (Swofford, 2001) mounted on an Apple Macintosh G4. Parsimony analyses were conducted with only parsimony informative characters included, and no substitution or codon position weighting was applied. 10 000 random stepwise taxon addition replicates were run using a TBR branch-swapping heuristic search, where two trees of length greater than or equal to 10 were kept per replicate. The trees obtained were then used for further TBR branch-swapping search, where the number of trees per replicate was not limited. A strict consensus tree was then computed. Nodal support was assessed by Jackknife (Farris *et al.*, 1996) using 1000 replicates and excluding 33.67% of characters at each replicate. Once again a simple stepwise taxon addition heuristic search using TBR branch-swapping was used, and if it was limited at all, 100 trees were kept per replicate.

RESULTS

Summaries of data and tree statistics are provided in Table 4. For cytochrome b, a total of 1337 base pairs were sequenced for *Francolinus nahani*, *Scleroptila shelleyi*, *Peliperdix sephaena*, *Pternistis afer* and *Peliperdix lathami* and 776 base pairs for *Pternistis griseostriatus* and *Pternistis leucoscepus*. These sequences exceeded those from GenBank, so only 660 base pairs could be used in the analysis of 75 sequences. The strict consensus of all most parsimonious trees was found for each analysis, and jackknife values were added to each topology. Phylogenies of Cytochrome b, ND2, combined mitochondrial analysis, OVOG and nuclear and molecular combined analysis are shown in Figures 1, 2, 3, 5 and 6 respectively. The strict consensus of the two most parsimonious trees was also presented as a phylogram (Figure 4). This shows that *F.*

nahani and *P. petrosus* are distinct from each other, but variation between them is comparable to that of other closely related species (Figure 4).

Close Relationship between *Francolinus nahani* and *Ptilopachus petrosus*

There is very high (Jk = 100) support for the *Francolinus nahani* and *Ptilopachus petrosus* sister relationship (*Ptilo-nahani*) in all phylogenies (Figures 1-6). This has never been published, but has been hypothesized based on behaviour and morphology (Finch et al. pers. comm.).

In the cytochrome b phylogeny, *Ptilo-nahani* is sister to the New World Quails, and this larger clade is sister to all other Phasianine galliforms, but this is unsupported (Figure 1). According to analysis of ND2 and of combined mitochondrial genes, there is very low support that the New World Quails are sister to all other Phasianine galliforms (Figure 2). Within this clade, ND2 analysis shows it is weakly supported that *Ptilo-nahani* and the guineafowl (Family Numididae) are sister to the other Phasianine galliforms (Figure 2). However, when mitochondrial genes are combined, there is support for the sister relationship between *Ptilo-nahani* and all other Phasianine galliforms (Jk = 72) (Figure 3). The OVOG phylogeny shows high support (Jk = 97) for a sister relationship between *Ptilo-nahani* and the New World Quails, and lower support (Jk = 75) for this clade being sister to the rest of the Phasianine galliforms (Figure 5). The combined analysis of all three regions shows a polytomy including *Ptilo-nahani*, *Numida meleagris* and the New World Quails, and there is strong support (Jk = 99) that these are then sister to all other Phasianine galliforms (Figure 6).

Odontophoridae Monophyly

There is very high support in all phylogenies (Jk = 94 to 100) that the New World Quails (Family Odontophoridae) are monophyletic (Figures 1-6), in agreement with previous findings (e.g. Dimcheff *et al.*, 2000; Dimcheff *et al.*, 2002).

Partridge-Francolins and Quail-Francolins

Two distinct clades of francolins emerge in all phylogenies (Figure 1-6), however cytochrome b is the only one with relatively complete taxon sampling. In the other

phylogenies, the clades still emerge, but many taxa are missing. All the taxa (except *Francolinus gularis*) of one clade are included in the "Partridge-Francolins" of Bloomer and Crowe (1998) (amended from Milstein and Wolff, 1987). However, it does not include *Pternistis hartlaubi* unless *Ammoperdix heyi* is also included. This clade is then sister to *Tetraogallus himalayensis*, and if this is also included, the larger clade is sister to *Alectoris*, *Coturnix* and *Margaroperdix*. *Pternistis griseostriatus* and *Pternistis leucoscepus* are confirmed to group with the "Partridge-Francolins", as proposed by Milstein and Wolff (1987) and Crowe *et al.* (1992) (Figures 1-6)

All the taxa in the second clade are "Quail-Francolins" according to Milstein and Wolff (1987), except *Bambusicola thoracica Francolinus gularis*, and *Francolinus francolinus*.

Latham's Forest Francolin

Peliperdix lathami and *Francolinus nahani* have been compared to and associated with each other, because of their similar morphological characteristics and shared habitat (e.g. Johnsguard, 1988). *P. lathami* is part of the "Quail-Francolins" in all analyses, but its position within this clade varies. In the cytochrome b analysis, in which taxon sampling was best, its position is unresolved (Figure 1).

"*Bam-Franc-Gallo*" Clade

There is very low support for *Bambusicola* relationships in all except OVOG analysis, where it is sister to *Gallus* (Jk = 78) (Figures 5 and 6).

Kimball *et al.* (1999) found a *Bambusicola*, *Gallus*, *Francolinus* clade, and used it has evidence that pheasants (subfamily Phasianinae) are not monophyletic. Here, this clade is well supported by ND2 analysis, combined analysis of cyt b and ND2, and OVOG analysis. However, *Francolinus francolinus* is not sampled in the ND2 analysis, but other francolins from the Quail-Francolin clade are, and from comparison with cytochrome b analysis, it appears that *Francolinus francolinus* would be included in this clade. There is a 100% jackknife support for this clade when both mitochondrial and nuclear sequences are analyzed together, with *Bambusicola* and *Gallus* appearing sister to the Quail-Francolins (Figure 6).

Monophyletic Grouse and Retention of "Gallo-Trag" clade

All the Grouse (Family Tetraonidae) that are included here, are monophyletic with a minimum jackknife value of 91(Figures 1-6). The larger clade comprising the grouse (Family Tetraonidae), Kimball *et al.*'s (1999) "Gallopheasants and Allies" clade, Kimball *et al.*'s (1999) "Tragopans and Allies" clade (without *Ithaginis cruentus*, which was not sampled) as well as *Perdix perdix* (partridge) and *Meleagris gallopavo* (turkey), was conserved in all phylogenies. It is however, unsupported in the cytochrome b phylogeny (Figure 1). It is weakly supported in the ND2 phylogeny (Jk = 73) (Figure 2), but strongly supported in the combined mitochondrial phylogeny (Jk = 91) (Figure 3). It is weakly supported in the analysis of OVOG (Jk = 58) (Figure 5), and unsupported. It is however, well supported (Jk = 80) in the combined mitochondrial and nuclear analysis (Figure 6).

Xenoperdix* and *Arborophila

Xenoperdix udzungwensis and *Arborphila* are sister species in the cytochrome b analysis, however there is no jackknife support for this relationship.

DISCUSSION

***Francolinus nahani* and *Ptilopachus petrosus* sister relationship**

All markers show a sister relationship between *F. nahani* and *P. petrosus*, as hypothesized by Finch *et al.* (pers. comm.). Both these species have red bare skin around the eye, and sexes are similar (Johnsguard, 1988; Madge & McGowan, 2002). It is well known that *Ptilopachus* has a long vaulted tail, which is regularly cocked (Johnsguard, 1988; del Hoyo *et al.*, 1994; Madge & McGowan, 2002), but it is less well known, because of its rarity, that *Francolinus nahani* also cocks its tail (C. Cohen, pers. comm.). *Peliperdix sephaena* is the only other francolin known to cock its tail (Madge & McGowan, 2002).

Ptilopachus petrosus occurs on rocky outcrops and scrub-covered plains in arid habitats south of the Sahara, from Gambia to Ethiopia, and south to Cameroon and northern

Kenya (Johnsguard, 1988). Its nearest living relatives have been hypothesized to be Asian spurfowls (*Galloperdix*) and bamboo-partridges (*Bambusicola*) (Johnsguard, 1988; Madge & McGowan, 2002). These three taxa were included in the subfamily Phasianinae (Pheasants and Peafowl) by Ogilvie-Grant (1896) (from Johnsguard, 1988), rather than subfamily Perdicinae (Partridges, Quails and Spurfowl), where Madge and McGowan (2002) placed them. However, Madge and McGowan (2002) agree that they probably should be within the Phasianinae. *Galloperdix* has not been sampled here, but its phylogenetic position would be very interesting, as it has been thought of as a link between subfamilies Phasianinae and Perdicinae (Johnsguard, 1988).

The position of the *Ptilo-nahani* clade is unresolved. Its position in the mitochondrial phylogenies is unsupported. However, its position in the nuclear phylogeny, and in the combined analysis of mitochondrial and nuclear data is fairly well supported, and here it is placed sister to the New World Quails. It is clear that Nahan's Francolin is not a francolin, and could be placed in a subfamily with *Ptilopachus petrosus*. It is fairly different to *P. petrosus*, as seen in the phylogram of combined mitochondrial analysis (Figure 4), and thus would not be placed in the same genus, but these two species are very different to anything else, and provide a link between New World Quails (Family Odontophoridae), and Pheasants and Partridges (Family Phasianidae).

Partridge-Francolins and Quail-Francolins

This important division was first hypothesized by Milstein & Wolff (1987), and reworked by Bloomer & Crowe (1998). The phylogenies here may show relationships that are not comparative to studies where different sampling has been used (e.g. Bloomer & Crowe, 1998), but the two clades are clear, and it is evident that *F. nahani* is not closely related to either of them, but rather, it is sister to both.

Peliperdix lathami

The position of *P. lathami* in the "Quail-Francolins" confirms Milstein and Wolff's (1987), and Crowe *et al.*'s (1992) hypothesis. However its relationship within this clade cannot be concluded due to insufficient sampling.

"Bam-Franc-Gallo" Clade

The relationship between *Bambusicola*, *Gallus* and the "Quail-Francolins" is an interesting one, the extent of which may not be fully recognized. It has been shown a number of times (e.g. Kimball *et al.*, 1997; Kimball *et al.*, 1999; Dimcheff *et al.*, 2000; Armstrong *et al.*, 2001; Dimcheff *et al.*, 2002) that there is a clade comprising *Gallus*, *Bambusicola* and either *Francolinus africanus*, or *Francolinus francolinus*, or two of those three. However, in all these examples, only "Quail-Francolins" were sampled, and it appears that francolin monophyly is assumed. However, it is known that there are two distinct clades of francolin, the "Quail-Francolins" and the "Partridge-Francolins" (Milstein & Wolff, 1987; Bloomer & Crowe, 1998). Here it is shown that *Gallus* and *Bambusicola* group with the "Quail-Francolins", and comfirmed that the "Partridge-Francolins" are separate, grouping with *Alectoris* and *Coturnix* (Bloomer & Crowe, 1998). Bloomer and Crowe (1998) used *Gallus* as an outgroup and did not sample *Bambusicola*. The inclusion of these taxa within a phylogeny may be even more evidence that francolins are not monophyletic.

Monophyletic Grouse and Retention of "Gallo-Trag" clade

That this clade is retained in all phylogenies is evidence that they are at least to some extent, in agreement with the literature. Thus other relationships drawn from the phylogenies produced here have some support.

CONCLUSION

This study shows that *Francolinus nahani* and *Ptilopachus petrosus* are very closely related. However, without complete sampling (particularly the lack of *Galloperdix*), it cannot be concluded that they are sister taxa. The position of this "*Ptilo-nahani*" clade is unresolved, however nuclear data supports a sister relationship between it and the New World Quails. The phylogenies presented agree with the literature in that a "Gallo-Trag" clade, as well as the "Quail-Francolin" and "Partridge-Francolin" clades are retained in all (to the extent to which sampling is complete).

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Table 1: The Collection Localities and GenBank accession numbers for the taxa sequenced in this study

Sample Number	Scientific Name	Common Name	Collection locality
1	*Francolinus nahani*	Nahan's Francolin	Budongo Forest, Uganada
2	*Francolinus nahani*	Nahan's Francolin	Budongo Forest, Uganada
3	*Francolinus nahani*	Nahan's Francolin	Budongo Forest, Uganada
	Pternistis afer	Red Necked Francolin	Quiçama National Park, Angola
	Peliperdix lathami	Latham's Forest Francolin	Dzhanga-Sanga, Central African Republic
	Peliperdix sephaena	Crested Francolin	Tsavo Nation Park
	Pternistis leucoscepus	Yellow Necked Spurfowl	Tsavo Nation Park
1	*Pternistis griseostriatus*	Grey-Striped Francolin	Quiçama National Park, Angola
2	*Pternistis griseostriatus*	Grey-Striped Francolin	Quiçama National Park, Angola
3	*Pternistis griseostriatus*	Grey-Striped Francolin	Quiçama National Park, Angola
	Scleroptila shelleyi	Shelley's Francolin	Nelspruit, Mpumalanga
	Ptilopachus petrosus	Stone Partridge	Ghana (FMNH396395)

Table 2: Accession numbers for taxa downloaded from GenBank. Blanks imply the sequence was not available.

Species Name	Common Name	Cytb	ND2	OvoG
Acryllium vulturinum	Vulturine Guineafowl	AF536742	AF536745	
Alectoris rufa	Red-legged Partridge	Z48775		AF170988
Alectura lathami	Australian Brush-turkey	AF082058	AF394616	
Ammoperdix heyi	Sand Partridge	TMC		
Arborophila javanica	Javan Hill-Partridge	TMC		
Arborophila torqueola	Necklaced Hill-Partridge	TMC		
Bambusicola thoracica	Chinese Bamboo-Partridge	AF028790	AF222538	AF170978
Bonasa umbellus	Ruffed Grouse	AY509677	AF222541	
Callipepla gambelii	Gambel's Quail	AF028763	AF028764	
Catreus wallichii	Cheer Pheasant	AF028792		AF170980
Centrocercus urophasianus	Greater Sage-Grouse	AF230177	AF222542	
Chrysolophus pictus	Golden Pheasant	AF028793		
Colinus virginianus	Northern Bobwhite	AF028775	AF222545	
Coturnix japonica	Japanese Quail	AF119094	AB003195	
Crax rubra	Great Curassow	AY274029	AY141935	
Crossoptilon crossoptilon	White Eared-Pheasant	AJ298921		AF170981
Cyrtonyx montezumae	Montezuma Quail	AF028778	AF028779	AF170976
Dendragapus obscurus	Blue Grouse	AF230178	AF222549	
Peliperdix sephaena	Crested Francolin	U90647		
Falcipennis canadensis	Spruce Grouse	AF230168		AF170986
Francolinus coqui	Coqui Francolin	U90646		
Francolinus francolinus	Black Francolin	AF013762		
Francolinus gularis	Swamp Francolin	U90649		
Francolinus pondicerianus	Grey Francolin	U90648		
Gallus varius	Green Junglefowl	AB044988	AF222551	
Haematortyx sanguiniceps	Crimson-Headed Partridge	TMC		
Ithaginis cruentus	Blood Pheasant	AF068193		
Lagopus lagopus	Willow Ptarmigan	AF230170	AF222552	
Lophophorus impejanus	Himalayan Monal	AF028796		
Lophura swinhoii	Swinhoe's Pheasant	AF534558		
Margaroperdix madagarensis	Madagascar Partridge	U90640		
Megapodius reinwardt	Orange-footed Scrubfowl	AF165465	AF394633	

Meleagris gallopavo	Wild Turkey	AY509693	AF222566	AF170984
Numida meleagris	Helmeted Guineafowl	L08383	AF394613	AF170975
Oreortyx pictus	Mountain Quail	AF252860	AF028782	AF170977
Ortalis vetula	Plain Chachalaca	L08384	AF394614	AF170974
Pavo cristatus	Indian Peafowl	L08379	AF394612	AF170990
Perdicula asiatica	Jungle Bush-Quail	AY390778		
Perdix perdix	Grey Partridge	AF028791	AF222560	AF170982
Phasianus colchicus	Ring-Necked Pheasant	AY368060	AF222561	
Pipile jacutinga	Black-fronted Piping-guan	AF165476		
Polyplectron bicalcuratum	Grey Peacock-Pheasant	AF028799		
Pternistis adspersus	Red-Billed Francolin	U90633		
Pternistis afer	Red-Necked Francolin	U90635		
Pternistis bicalcaratus	Double-Spurred Francolin	U90637		
Pternistis capensis	Cape Francolin	U90632		
Pternistis castaneicollis	Chestnut-Naped Francolin	TMC		
Pternistis erckelii	Erckel's Francolin	U90638		
Pternistis hartlaubi	Hartlaub's Francolin	U90639		
Pternistis hildebrandti	Hildebrandt's Francolin	U90631		
Pternistis natalensis	Natal Francolin	U90630		
Pternistis squamatus	Scaly Francolin	U90636		
Pternistis swainsonii	Swainson's Francolin	U90634		
Pucrasia macrolopha	Koklass Pheasant	AF028800		AF170983
Rollulus rouloul	Crested Partridge	TMC		
Scleroptila africanus	Grey-Winged Francolin	U90629	AF222550	
Scleroptila finschi	Finch's Francolin	U90643		
Scleroptila levaillantii	Red-Winged Francolin	U90642		
Scleroptila levaillantoides	Orange River Francolin	U90644		
Scleroptila shelleyi	Shelley's Francolin	U90645		
Syrmaticus reevesii	Reeves's Pheasant	AY368059		
Tetrao tetrix	Black Grouse	AF230174	AF222564	
Tetraogallus himalayensis	Himalayan Snowcock	AY563126		
Tragopan temminckii	Temminck's Tragopan	AF229838	AF222566	
Tympanuchus phasianellus	Sharp-Tailed Grouse	AF068191	AF222569	AF170985
Xenoperdix udzungwensis	Udzungwa Partridge	TMC		

TMC: refers to unpublished sequences obtained from Professor T.M. Crowe. Where there are gaps, the sequences were not available on GenBank.

Table 3: Primers used for amplification and sequencing

Gene Region	Primer Name	Primer Sequence	Reference
Cytochrome b (initial primer pair)	L14578	5′-CTAGGAATCATCCTAGCCCTAGA-3′	
	H5915	5′-AACGCAGTCATCTCCGGTTTACAAGAC-3′	
(internal)	L15087	5′-TTCCTATACAAAGAAACCTGAAA-3′	
(galliform specific)	ML15131	5′-AACGTACAGTACGGCTGACTCAT-3′	
	MH15907	5′-TGTTCTACTGGTTGGCTTCCAAT-3′	
ND2	L5216	5′-GCCCATACCCCRAAAATG-3′	Sorenson *et al.*, 1999
	H6313	5′-CTCTTATTTAAGGCTTTGAAGGC-3′	
OVOG	Forward	5′-CAAGACATACGGCAACAARTG-3′	Armstrong *et al.*, 2001
	Reverse	5′-GGCTTAAAGTGAGAGTCCCRTT-3′	

Table 4: Details of Phylogeny Analyses

Analysis	Base Pairs	No. Seqs	No. Var. Base Pairs	% Var. Base Pairs	PICs	% PICs	No. MPTs	Length MPTs	CI	RI	RCI
Cyt b	660	75	336	50.9	286	43.3	6	2518	0.22	0.46	0.10
ND2	1018	38	580	57.0	494	48.5	28	2639	0.36	0.53	0.19
Mitochondrial	1678	34	863	51.4	722	43.0	2	3669	0.37	0.50	0.19
OVOG	471	31	183	38.9	113	24.0	40	260	0.80	0.85	0.68
Combined	2149	39	1037	48.3	799	37.2	12	4030	0.39	0.51	0.20

Cyt b: Cytochrome b. Mitochondrial: Combined analysis of Cytochrome b and ND2. No. seqs: Number of sequences used in each analysis (varies from number of taxa analysed as there are replicates for several taxa e.g. *Francolinus nahani*). PICs: parsimony informative characters. No. MPTs: Number of most parsimonious trees obtained from heuristic searches. Length MPTs: The length of the most parsimonious trees. CI: Consistency Index. RI: Retention Index. RCI: Rescaled Consistency Index.

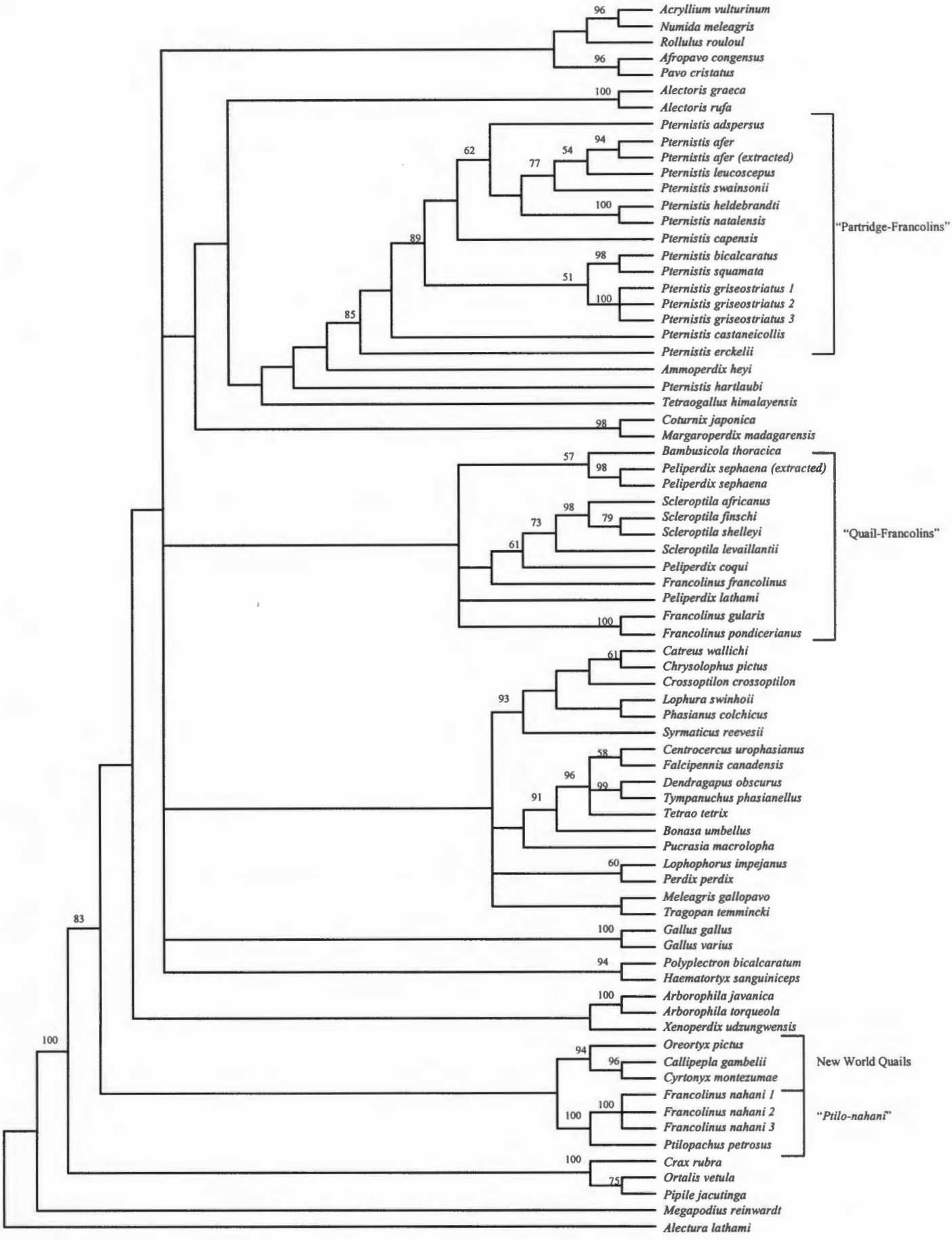

Figure 1: Strict consensus of six most parsimonious trees based on 660 phylogenetically informative cytochrome b characters, showing Jackknife values

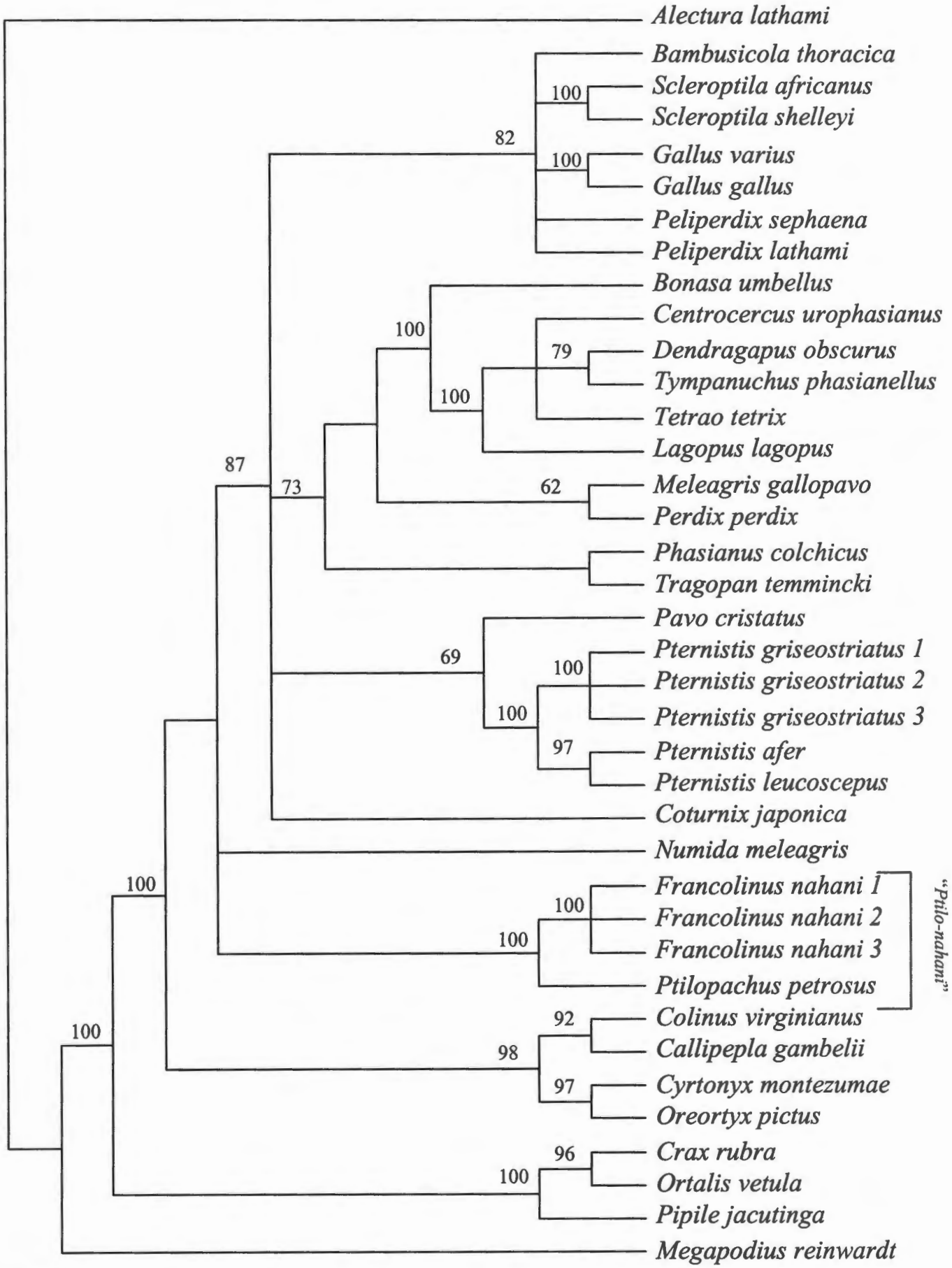

Figure 2: The strict consensus of 28 most parsimonious trees based on analysis of 494 phylogenetically informative ND2 characters, with Jackknife values above branches

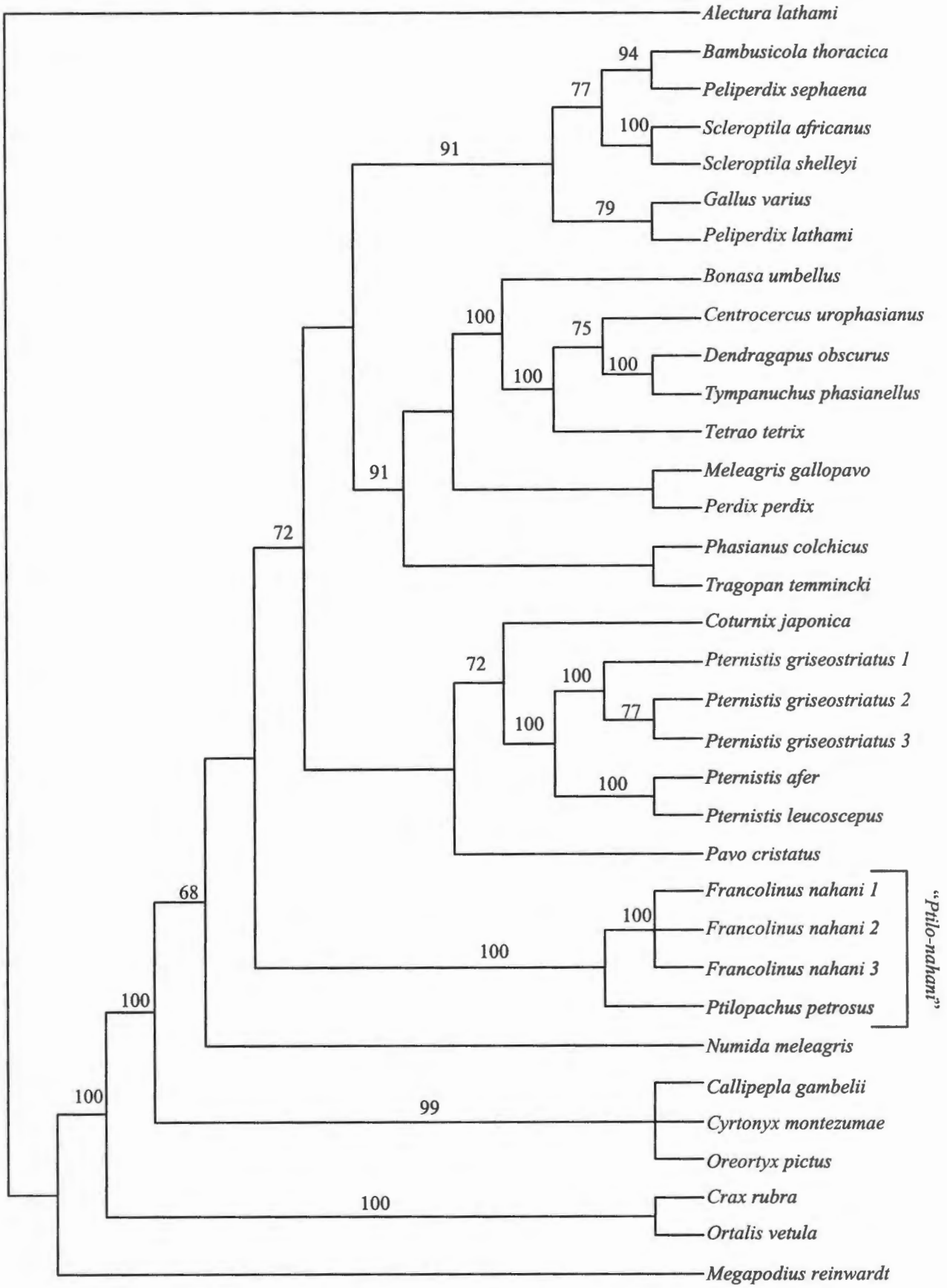

Figure 3: Strict consensus of two most parsimonious trees based on 722 phylogenetically informative cytochrome b and ND2 characters, showing Jackknife values

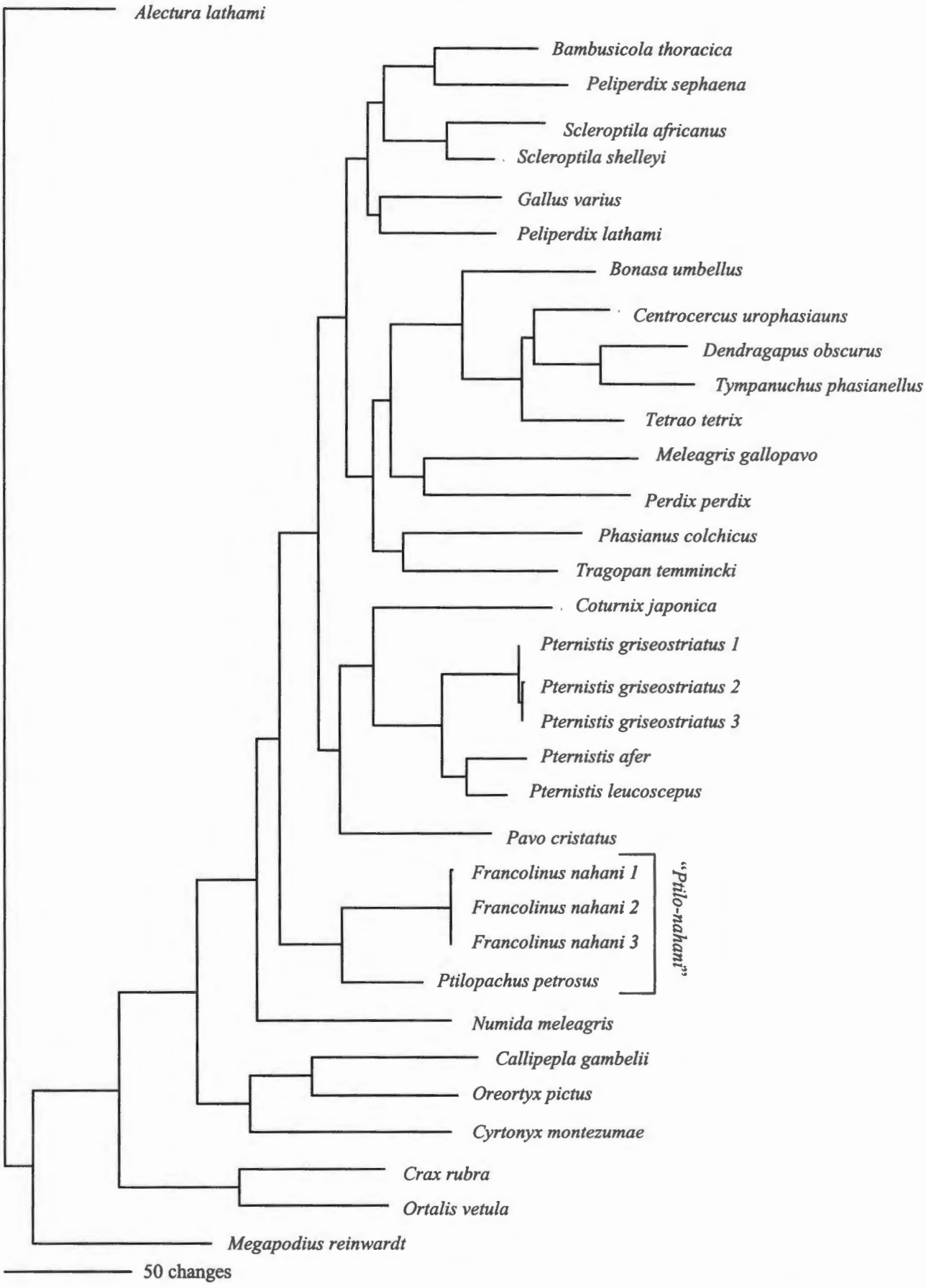

Figure 4: Phylogram of one of the 6 most parsimonious trees of an analysis using 722 parsimony informative cytochrome b and ND2 characters.

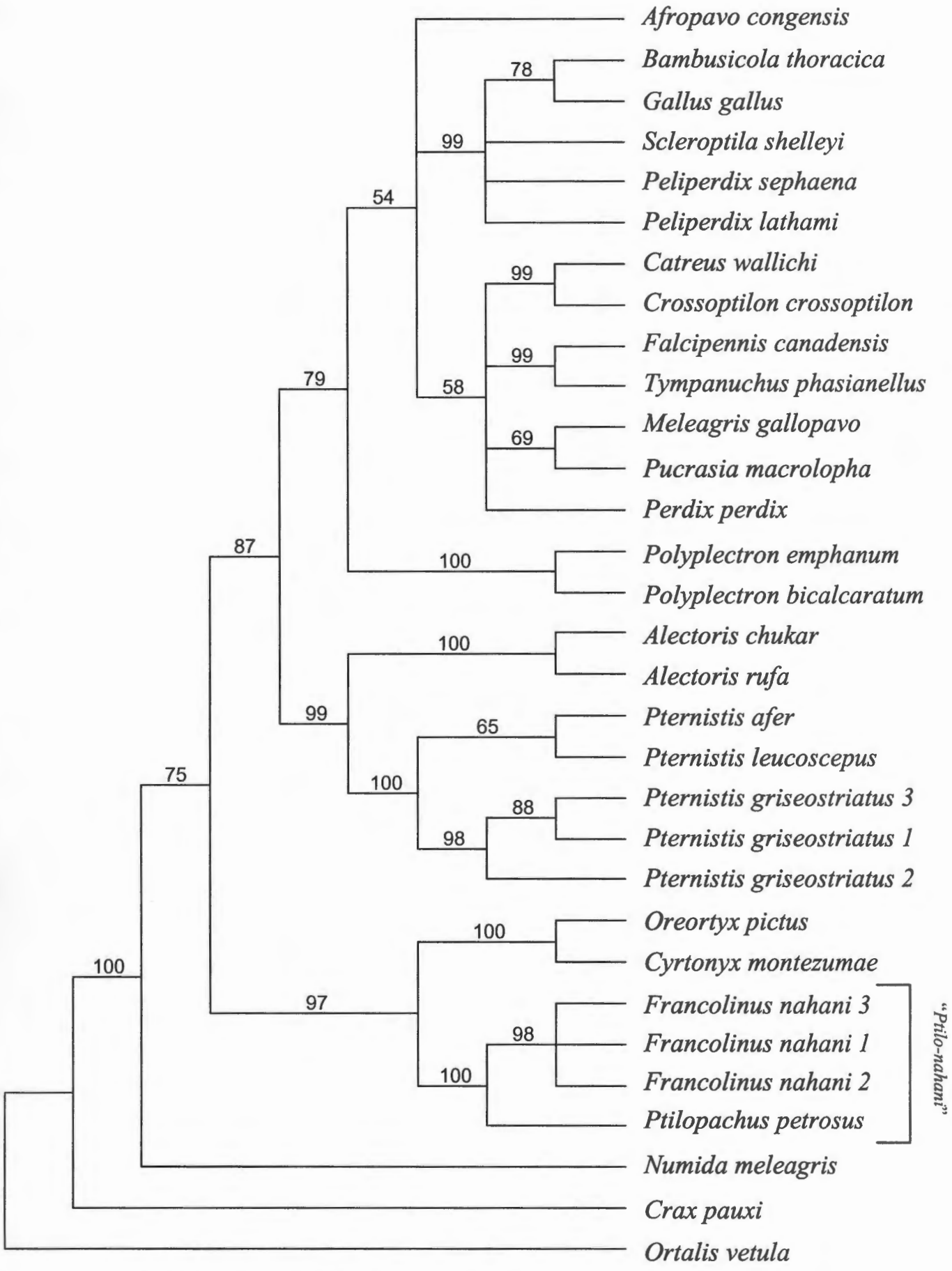

Figure 5: The strict consensus of 40 equally parsimonious trees based on 113 parsimony informative OVOG characters, with Jackknife values added

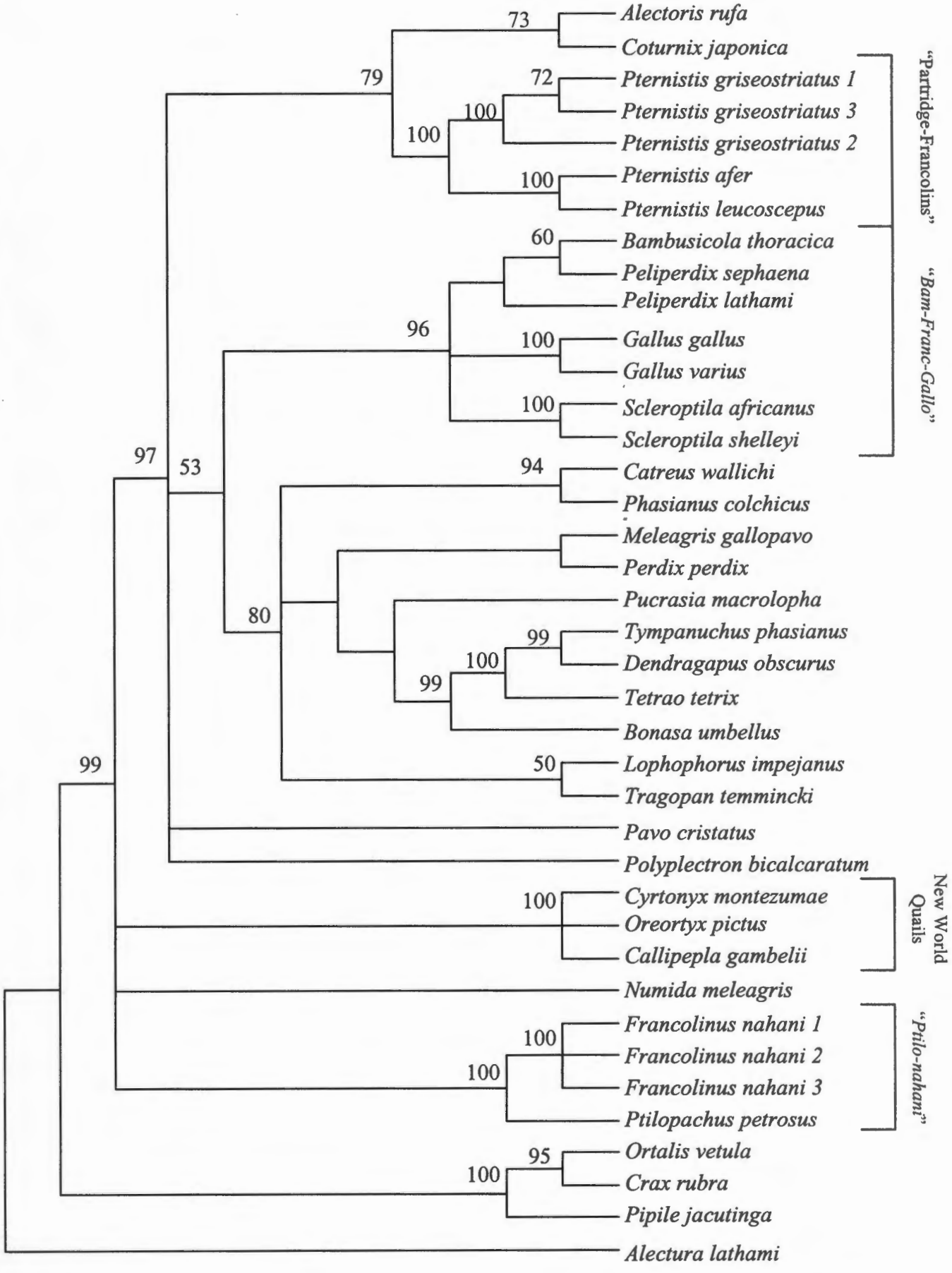

Figure 6: The strict consensus of 12 most parsimonious trees based on 799 parsimony informative characters of cytochrome b, ND2 and OVOG sequences, with Jackknife values above the branches