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DEPARTMENT OF BIOLOGY

EXAMINATION OF THE PHYLOGENETIC POSITION OF THE EXTINCT PASSENGER PIGEON THROUGH THE USE OF NUCLEAR DNA

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Abstract

The phylogenetic position of the extinct passenger pigeon (*Ectopistes migratorius*) remains unclear. Morphological evidence indicates a close relationship with the mourning dove (*Zenaida macroura*) while mitochondrial DNA suggests a common ancestor with *Macropygia*. In this investigation, nuclear DNA of the beta-Fibrinogen gene was analyzed along with previously sequenced mitochondrial data to help resolve this question. DNA was successfully isolated and sequenced from a 140-year-old historic toepad sample using ancient DNA protocols. Phylogenetic and statistical analyses of the new data indicate a result closely corresponding to that suggested by mitochondrial DNA, placing the passenger pigeon as sister to *Patagioenas*. By sequencing DNA from various passenger pigeon samples it may be possible to track rates of population decline that led to the eventual extinction of the species.

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Introduction

Ancient DNA, a History

In 1984 DNA was successfully extracted from the skin of a Quagga, an extinct subspecies of the plains zebra. The recovered mitochondrial sequence was under 250 base pairs (bp) long and confirmed a relationship closer to zebras than horses (Higuchi *et al*., 1984). What was of main interest in this paper was not the taxonomic analysis, but rather the successful recovery of non-modern DNA (140 years old). With this breakthrough, the field of ancient DNA (aDNA) research was born.

Initial aDNA studies were difficult due to the ineffective reaction kinetics that were used in attempting to recover already damaged or minute quantities of DNA (Willerslev and Cooper, 2005). Development of the polymerase chain reaction (PCR) led to an explosion in all forms of genetic research, including aDNA. PCR is a method by which DNA is replicated through the use of an enzymatic reaction (Mullis and Faloona, 1987). Theoretically PCR allows for an infinite replication of DNA molecules from a single template strand. This sensitivity is both a blessing and a curse for aDNA as there are often few undamaged DNA fragments, but the risk of contamination is elevated. For example, a publication claiming to have sequenced hundred-million-year-old bacteria was found to have obtained modern contamination (Vreeland *et al*., 2000). There have been many other unproven claims including Miocene amber (Austin *et al*., 1997) and fossilized dinosaurs (Siddow *et al*., 1991). However, use of stringent protocols, improved reagents, and accepted practices have led to many successful aDNA studies examining new questions in a multitude of areas (Paabo *et al*., 2004).

Ancient DNA has been isolated from items as diverse as plants, eggshells and even paleo-eskimos (Rollo and Amici, 1991; Oskam *et al*., 2010; Rasmussen *et al*., 2010). With this data researchers have been able to rearrange phylogenetic relationships, examine population dynamics, and even verify the validity of religious relics (Nilsson *et al*., 2010). In this thesis the phylogeny of the extinct passenger pigeon is examined with aDNA techniques.

Passenger Pigeon Extinction

When Europeans first arrived in the Americas it has been estimated that the passenger pigeon composed up to 40% of the total avian population (Schorger, 1973). Initial descriptions referred to "infinite" flocks, while later reports described over 2 billion birds flying together (Schorger, 1973). Passenger pigeon numbers began a gradual decline with European settlement and started to fall precipitously in the 19th century culminating in extinction during the early 20th century.

Passenger pigeons depended on large population size as a defense against predators, in a manner similar to buffalo or sea turtle hatchlings (Schorger, 1973). Therefore, while the initial population size was high the minimum threshold for species survival was also elevated. Multiple explanations have been put forward for the extinction of the passenger pigeon, but it is most likely a combination of factors acting in concert that lead to its disappearance. One theory proposed that deforestation eliminated possible nesting sites and destroyed nesting populations as over 100 nests in a tree have been recorded (Allen, 1876). A perception regarding vulnerability to disease has also been propagated, as it was not uncommon for thousands of pigeons to die overnight in captivity (Thompson, 1922). With the introduction of foreign pigeon species and the corresponding

pathogens this theory cannot be completely discounted. However, it seems that these played a minor role compared to that of hunting by man.

Passenger pigeons were so easy to shoot; with up to ten being bought down with a single shot, they were not even considered game birds (Herbert, 1852). A more effective method of hunting was by utilizing massive nets. Hunters using a standard net approximately 30 feet across would capture on average over a thousand pigeons in a single snatch (Schorger, 1973). With the advent of the railroad in the mid-19th century, pigeon harvesting accelerated to feed the growing demand for the bird as a source of food for the lower classes. In 1851 nearly two million birds were taken from a single nesting site in New York (Schorger, 1973). Mating pairs of passenger pigeons only lay one egg per year and as a result with such heavy losses the population could not be maintained. As the population declined hunters became less selective in the birds that they captured and many young individuals barely fit for food were taken. This further exacerbated the decline as the short five-year lifespan of the passenger pigeon meant that these individuals were necessary to maintain the species.

By the 1880s the passenger pigeon had grown scarce in the wild. The flocks that were seen contained only a few hundred individuals. In 1900 it was widely anticipated that the species would go extinct. The last known passenger pigeon was named Martha and died in captivity on September 1, 1914 (Mershon, 1916). Unconfirmed reports of wild passenger pigeons would continue into the 1930s but have been given little stock by researchers (Griscom, 1946).

Proposed Phylogenetic Relationships of the Passenger Pigeon

In 1758 the passenger pigeon was described as *Columba macroura*, a name that at the time it shared with the mourning dove (Linnaeus, 1774). Confusion over its species assignment continued until 1827 when the passenger pigeon was placed in its own distinct genus, *Ectopistes* (Swainson, 1828). Despite several attempts to change the genus the name *Ectopistes migratorius* remains the nomenclature today.

Due to the high level of morphological similarity to the mourning dove *Zenaida macroura,* ornithologists have long assumed a close relationship for the two species. As previously noted these similarities were so strong that the two were confused for a single species by early researchers (Linnaeus, 1774). *Zenaida* and *Ectopistes* have been combined together into a mourning dove group with a suggested closest relationship to the American ground doves (Figure 1; Goodwin, 1967). To understand the lack of clarity in this situation it seems prudent to elaborate on the morphological differences of the two species.

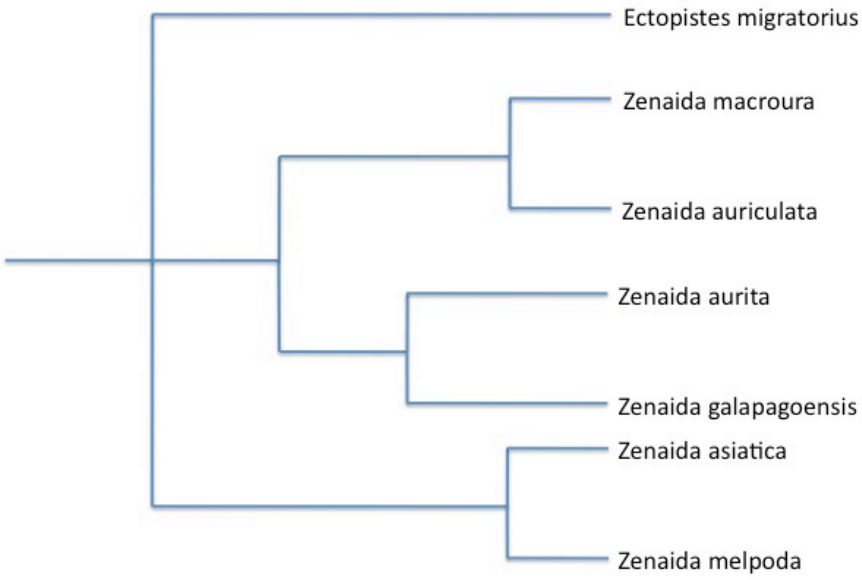

Figure 1. Proposed phylogenetic tree for the mourning dove group. (Figure redrawn from Goodwin, 1967)

Superficially the two species appear very similar. However, several morphological differences between the passenger pigeon and the mourning dove do exist. The most pronounced of these include the presence of only 12 tail feathers in the passenger pigeon compared to 14 in the mourning dove (Schorger, 1973). *Zenaida aurita* and *Z. galapagoensis* both have 12 tail feathers indicating that this morphological difference is not strong evidence for division into two distinct genera (Johnson and Clayton, 2000). The mourning dove is smaller and possesses a facial stripe not present in the passenger pigeon (Baskett *et al*., 1993). Reproductive characteristics were also distinguishing features as the passenger pigeon clutch size was limited to one egg and sex dichromatism (males and females display distinct coloration patterns) was more pronounced (Goodwin, 1967).

Molecular systematic studies have indicated a clade composed of American quail doves and the mourning dove group, which would include *Zenaida* and *Ectopistes* (Johnson and Clayton, 2000). This is in agreement with previous studies (Goodwin, 1967). However, the placement of *Ectopistes* was not addressed using DNA until 2002. Passenger pigeon mitochondrial DNA (mtDNA) was examined as part of a study on the Dodo (Shapiro *et al*., 2002). Analysis of 12S rRNA and cytochrome *b* genes provided support for a relationship of *Ectopistes* with cuckoo-doves of *Macropygia* (Figure 2; Shapiro *et al*., 2002). This is a significant difference with that suggested by morphological data. *Macropygia* are part of the Columbinae subfamily, where *Zenaida* belong in Leptotilinae.

Columbiformes originated in the neotropical (South American) portion of Gondwana during the Cretaceous period (Pereira *et al*., 2007). Lineages began to radiate out of this area sometime between 39 and 55Mya. Divergences between the genera in Leptotilinae occurred at an earlier date then those in Columbinae (Pereira *et al*., 2007). *Macropygia* are indigenous to Oriental and Australasia regions (Goodwin, 1967). Were the passenger pigeon found to be a sister species to *Macropygia* it would indicate that the passenger pigeon took part in a 2nd colonization event of North America independent of that of the other New World pigeons. Understanding the phylogenetic history of *Ectopistes* is an important step towards understanding how a species can expand to an extremely high population size.

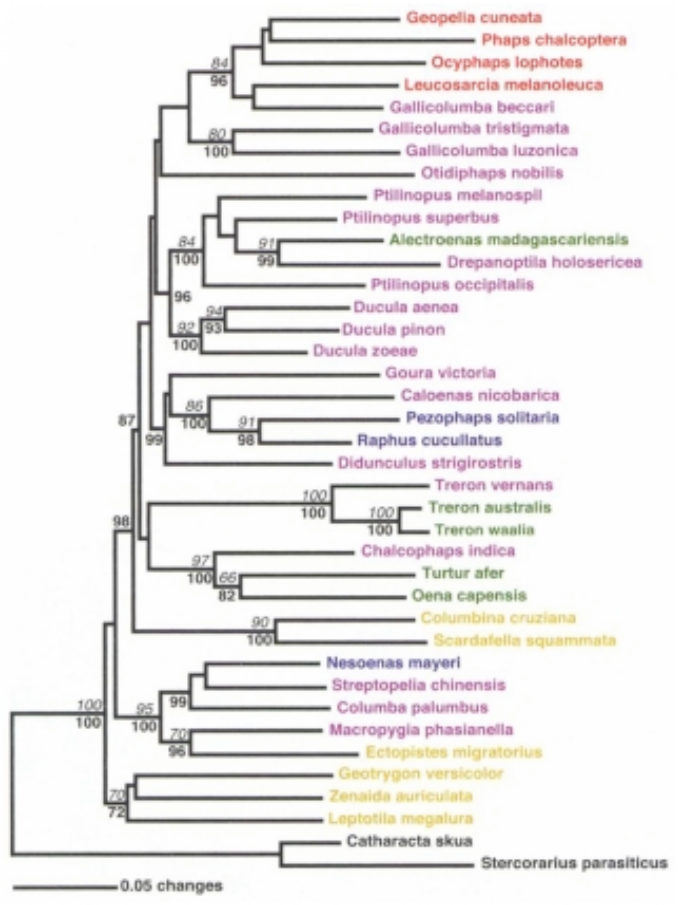

Figure 2. Maximum likelihood tree for Columbidae based on mitochondrial analysis. (Figure from Shapiro *et al.*, 2002)

The discrepancy between morphological and mitochondrial DNA has remained unexplored, thus the phylogenetic position of the passenger pigeon remains unresolved.

Nuclear vs. Mitochondrial DNA

Molecular studies have often relied on both nuclear and mitochondrial DNA when addressing a hypothesis. This is because differences in characteristics between the two types of DNA can result in conflicting information (Hey, 1997). Mitochondrial DNA mutates at a faster rate due to the high number of reactive oxygen species (ROS) in mitochondria that can damage DNA (Brown *et al.*, 1979). An accelerated mutation rate is useful when

examining species suspected of a recent divergence, such as neighboring genera. With hundreds of mitochondria in an individual cell it is much easier to extract this type of DNA as well. This trait has made mtDNA appealing in aDNA studies when high levels of degradation are expected, as the probability of intact copies remains high. Limitations of mtDNA analysis include that due to its mode of inheritance it is only possible to construct a maternal history based on this information.

Nuclear DNA, on the other hand, is bi-parentally inherited, thereby allowing for the more complete construction of an evolutionary picture. With minimal exposure to ROS nuclear DNA mutates at a slower rate (Richter *et al.*, 1988). If there is danger of potential saturation in a molecular study, such as a family wide analysis, nuclear DNA is often more informative. Despite its many advantages over mtDNA the low quantity of nuclear DNA per cell has limited its use in aDNA studies until recently (Huynen *et al*., 2003).

Purpose

This thesis will attempt to resolve the question regarding the phylogenetic position of the passenger pigeon. The advent of aDNA research has made this possible by allowing for the molecular analysis of a species that has been extinct for roughly one hundred years. While the majority of ornithologists have come to a general consensus that if not a member of *Zenaida* then *E. migratorious* is a sister species (Schorger, 1973; Goodwin, 1967), recent mitochondrial analysis has demonstrated a discrepancy with this suggested relationship (Shapiro *et al*., 2002). Through the examination of nuclear DNA, in combination with previous molecular studies, it is hoped a better-supported phylogenetic position for the passenger pigeon can be resolved.

Methods

Samples and DNA Isolation

Toe pad and feather samples of *Ectopistes migratorius* (museum ID: T17065) were received from the World Museum Liverpool. Prior to DNA extraction 25mg of tissue was cut into small pieces utilizing a razorblade. A Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) was used to extract DNA from the samples. Extraction negatives (no sample) were used to ensure no contamination occurred throughout the DNA isolation process. Extractions were performed according to manufacturer's instructions, except proteinase K was used to lyse the tissue over a 24-hour period, during which time the samples were rotated continuously to ensure mixing. Following visual confirmation of thorough lysing, samples were loaded onto DNeasy spin columns where the ensuing steps were undertaken in accordance with manufacturer protocol. Final elution occurred into 200μl of AE buffer.

Locus Selection and Primer Design

In an attempt to perform a comparative analysis including the greatest phylogenetic diversity within Columbidae intron 7 of the nuclear gene beta-fibrinogen (*FIB7*) was selected for analysis, as it has been examined in a wide variety of literature and represented the best possibility for an effective analysis.

Due to the high level of degradation to aDNA PCR primers designed to amplify 1000bp fragments will not work effectively. Therefore it was necessary to design novel primers suitable for aDNA amplification. As such, primers were designed to amplify fragments no longer then 150bp (Table 1). *FIB7* sequences were downloaded from Genbank and aligned utilizing MAFFT v6.0 with a L-INS-i algorithm (Katoh *et al.,* 2009).

Following visual inspection with Se-Al v2.0 the consensus sequence was obtained (appendix A; http://tree.bio.ed.ac.uk/software/seal/). SeqBuilder v8.0.3 was used to design a series of primers with which to isolate a 445 bp region of *FIB7* (DNAstar, Madison, WI, USA).

Table 1. Series of primers designed for extraction of a portion of the *FIB7* gene from aDNA samples.

Primer set	Forward 5' to 3'	Reverse 5' to 3'	Annealing Temperature(C)
F2R3	AGCACTGTTTTCTTGGATCTGAAGT	GTTTATTATGGTTTGAAAATTCCAGT	52
F3R4	TAGATCAACAGAGTACCTAGACCTGC	TTCACCTTCCAAGTGCCCTGTGT	56
F4R5	ACCATAATAAACATTTAAAATCCTCTC	AATTATCAATTGATAAACTAAAATGACA	48
F5R6	AGCAGCTAAGAAAAACAAGTAAAA	GGGAAGACATACATTTCTCATTGTT	48
F6R7	CATAATGATGATTGCAATATCAA	GTGTGCTGTGCCTTTACCTTA	48
F7R8	TTCCTTTATTCATGAATGTGTGA	AAAGTTCTGCCTACTTAGAAGACA	48

DNA Amplification

PCR amplifications were performed in 25μl reactions utilizing 2μg rabbit serum albumin, 6.25nanomol dNTPs, 1μl 25x buffer, .25μl Hi-Fidelity *Taq* (Invitrogen, Carlsbad, CA, USA), H_20, 37.5nanomol $MgSO_4$, 2.5 picomol of each primer, and 1μl template. Amplification reactions were undertaken in an MJ Research PTC-225 thermal cycler. A typical reaction was 94°C for 90 sec, with 50 cycles of denaturing at 94°C for 45 sec, annealing for 45 sec at 48-56°C (Table 1), and extension at 68°C for 90 sec. A final 68°C extension period for 10 min followed the final cycle to ensure the completion of all strands. Due to variable primer stability multiple annealing temperatures were required and can be noted in Table 1. Negative PCRs (including no template) were produced in each series of amplifications.

Gel electrophoresis was run using 5μl of amplified product that was visualized on a 2% agarose gel utilizing ethidium bromide. A BioDoc-It 3UV Transilluminator allowed for visualization of the gel following a run. If bands of an incorrect size or negative contamination were found to have occurred the amplification was repeated.

PCR products underwent a cleanup protocol to remove unincorporated dNTPs and other undesired material. For ease of handling 80μl of H_20 was added to each PCR. The diluted PCR was transferred to a filter plate (Millipore) and pulled through with a vacuum for 10 min or until all the liquid was pulled through. After providing 30 sec to allow for complete drying the vacuum was released, 50μl of H_2O was added, and the vacuuming repeated. To resuspend the DNA 50μl H_20 was added, and the plate shaken for 30min at half speed on a plate shaker (IKA).

Cloning

Cloning for each fragment successfully amplified by PCR was performed with a TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA). Reactions were prepared using 0.25μl salt solution, 0.25μl TOPO vector, 7-10ng of clean PCR product, and 1.5μl of H20. Cloning proceeded following the manufacturer's protocol. Three plates were cultured with 50,20 and18 μl of cell mixture. Flame sterilization of the spreader prevented possible contamination. Plates were incubated overnight at 37°C.

Fifteen white and one blue colony were picked using a pipette tip and placed in 50μl of H_20. PCR reactions with a final volume of 12.5μl were prepared with 1.25μl 10X buffer, 0.125μl Econo-Taq (Lucigen, Middleton, WI, USA), 3.125nanomol dNTPs, 12.5 nanomol $MgCl_2$, 12.5 picomol M13F primer, 12.5picomol M13R primer, 2.25μl H_20, and 5μl colony in H_20. The amplification reaction began at 94°C for 2 min followed by 30 cycles of 94°C for

60 sec, 56°C for 60 sec, and 72°C for 60 sec, with a final 7 min extension period upon completion of the final cycle. Gel electrophoresis served as a check for proper insertion. 1μl of loading dye, 2.5μl of PCR product, and 3μl of H_2O was run and examined as previously noted.

Contamination Prevention

Ancient DNA is highly vulnerable to contamination and several steps were taken to detect and minimize the possibility. All aDNA samples were extracted in a separate building from modern samples. In addition all PCRs from ancient samples were prepared separately as well. In the aDNA facility breathing masks, paper suits, gloves, and shoe covers were worn at all times. Surfaces were sprayed with 3% bleach and then wiped with 70% ethanol to destroy any exogenus DNA. This occurred before and after any work was done in the lab. Negative extraction controls were included and every PCR was prepared with a negative control.

Sequencing PCR, Cleanup and Sequencing

Sequencing reactions utilized 0.25μl BigDye 3.1, 1.6μl M13 (1uM) primer, 1.75μl 5X BigDye buffer, 4.4μl H_20 and 2μl clone PCR product to constitute a 10μl reaction (Applied Biosystems, Foster City, CA, USA). Cycling followed the manufacturer's guidelines and reactions were cleaned using ethanol/EDTA precipitation.

Extraneous material was removed with an ethanol/EDTA precipitation cleanup. 2.5μl of 125mM EDTA and 25μl EtOH was added to each reaction and incubated for 15 min. A Heraeus centrifuge set to 4°C spun the reactions at 2750 rpm for 30min to pellet the sequencing reaction. Reaction plates were then immediately inverted and centrifuged again at 500rpm for 2 min. Plates were removed and 30μl of 70% EtOH added to each well, and

the plates were spun again at 2750rpm for 10min. This was again followed immediately by 2 min at 500rpm with the plate inverted. Evaporation of any remaining EtOH was allowed to occur overnight with the plate set in a dark drawer. Prior to sequencing the sequence reaction was resuspended in 20μl H_20 and one quarter of the reaction was sent to the Penn State Genomics Core Facility (University Park, PA) for sequencing (appendix A) using an ABI Hitachi 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Analysis

Cytochrome *b*, 12S rRNA and *FIB7* sequences for 117 taxa of the Columbidae family were retrieved from GenBank. The name and accession numbers for these individuals are available in appendix B. These individuals were chosen to give the widest possible coverage of the various genera within Columbidae. Multiple species for several genera were selected in order to provide the most thorough dataset possible. *FIB7* was examined individually, and then in combination with the other genes.

Evolutionary models were determined with jModelTest 0.1 (Posada, 2008). A general time reversible (GTR) model with discrete gamma model of heterogeneity (GTR+G) was selected based on AIC for 12S and *FIB7* and GTR+I+G (including a proportion of invariant sites) for cytochrome *b*. Garli0.96 was used to construct maximum likelihood (ML) trees (NESCent, Durham, NC, USA). Bayesian posterior probabilities were estimated using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Chains were run for 10,000,000 iterations with sampling every 1000. The first 10,000 iterations were removed as burn-in after a convergence assessment using the MrBayes tool sump visualized the trace and confirmed PSRF was 1.000 for all parameters. *Aerodramus fuciphagus* and *Phaethon rubricauda* were included as outgroups.

Results

The analysis of 445-bp of *FIB7* resulted in the ML tree depicted below (Figure 3); both bootstrap and Bayesian posterior probabilities are provided. Here, we find strong statistical support that the passenger pigeon falls within the subfamily Columbinae (91% ML bootstrapping support; 100% Bayesian posterior probabilities), and within the group most closely related related to the New World typical pigeons *Patagioenas* (60% ML bootstrapping support; 99% Bayesian posterior probabilities). *Zenaida*, alternatively resides in the Leptotilinae subfamily, which is distinct from Columbinae in which the passenger pigeon falls. Interestingly, this marker finds no support for a close relationship between *Ectopistes* and *Macropygia*, as previous studies have suggested (Shapiro *et al*., 2002).

The ML analysis from the combined sequences of 12S, cytochrome *b* and *FIB7* (Figure 4) supported the results from the independent *FIB7* analysis. *Ectopistes* demonstrates a sister relationship with *Patagioenas* near the root of the Columbinae subfamily. However, the support is not as strong as it was in the analysis solely examining *FIB7*. There remains no evidence supporting a *Zenaida*-*Ectopistes* link.

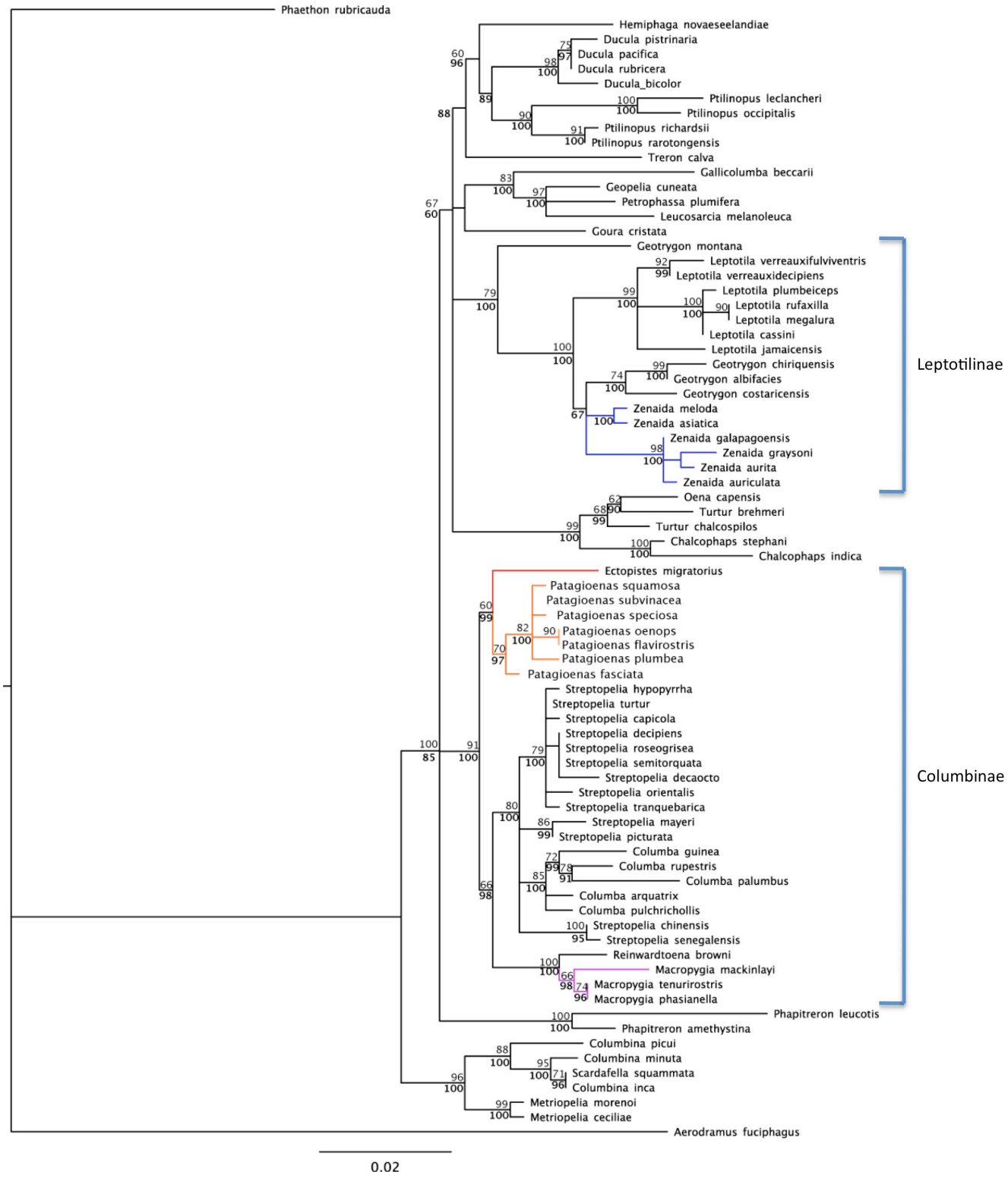

Figure 3: ML tree depicting the phylogenetic position of various species of Columbidae based upon *FIB7.* ML bootstrap values for relationships with ≥ to 60% support and Bayesian posterior support (bold) are given.

Figure 4: ML tree depicting the phylogenetic relationships of 117 species of Columbidae. Cytochrome *b*, 12S, and *FIB7* were used to construct the tree. ML bootstrap values for relationships with ≥ to 60% support and Bayesian posterior support (bold) are given.

Discussion

Relationships of the Passenger Pigeon within Columbidae

To our knowledge this is the largest single study examining the relationships within the family Columbidae. Both figures 3 and 4 are consistent with the idea that the passenger pigeon is most closely related to the genus *Patagioenas*. The large genetic distance between *Patagioenas* and *Ectopistes* maintains the currently accepted view that *Ectopistes* is a unique genus. Interestingly, this phylogenetic result may suggest a neotropical origin for the passenger pigeon with a northern radiation. *Patagienas* remains confined to South America and the lower extremes of North American including the Caribbean and southern Texas.

A *Patagioenas-Ectopistes* link is inconsistent with previous research. There is very strong evidence that the passenger pigeon does not form any type link with *Zenaida* as had been suggested in morphological studies (Goodwin, 1967). Based on this evidence it appears that the passenger pigeon and the mourning dove serve as a remarkable case of convergent evolution.

Previous mitochondrial analysis had suggested that *E. migratorius* formed a clade with *Macropygia* (Shapiro *et al.,* 2002). Figures 3 and 4 indicate this is unlikely. While the passenger pigeon remains in Columbinae it appears to have last shared a relationship with *Macropygia* over 35Mya (Pereira *et al.,* 2007). Since the previous mitochondrial study did not include any specimens from *Patagioenas* it is reasonable to expect the passenger pigeon to be have been linked with the closest relative to *Patagioenas* in that study, *Macropygia* (Shapiro et al., 2002).

Additional Observations

Geotrygon is not a monophyletic group. Every analysis undertaken in this study indicates that *G. montana* and *G. versicolor* appear to have diverged prior to the *Zenaida, Leptotila, Geotrygon* clade. Previous studies have indicated if this were the case these two species last shared a common ancestor with the rest of *Geotrygon* approximately 40 Mya (Pereira *et al*., 2007). This study appears to have been the first to make note of this anomaly. Based on this evidence *G. montana* and *G. versicolor* should remain in Leptotilinae, but be placed in their own unique genus. *Mensis* would be an appropriate name based on the montane habitat preferred by both species.

Further Areas of Research

The population history of the passenger pigeon remains unclear. Estimates of a population size that at one time measured in the billions are not based on a rigorous scientific study, but rather an extrapolation based on testimony (Schorger, 1973). Fortunately, the large number of preserved passenger pigeons in the hands of private collections and museums provides an opportunity to examine the population dynamics of the species over time. By isolating mitochondrial aDNA from a number of these specimens and tracking the time and location of their capture it should be possible to develop a model for how drastically the population size changed over time. Facets of passenger pigeon behavior could additionally be addressed by such a study.

Passenger pigeons were a migratory species with the average nesting site for a flock being nearly 31 miles in area (Schorger, 1973). The passenger pigeon was also known to nest widely in single pairs or groups of several dozen individuals (Grundtvig, 1895). A large

molecular analysis of the species would be able to examine the rate of interbreeding between distinct large colonies, and also that of the small groups.

At a time of heightened concern regarding species extinction due to human influences the passenger pigeon offers a cautionary tale. By further exploring the level of harvesting by mankind over time and the resulting population decline the results could help lead to better ecological management of modern species.

Conclusion

This study analyzed the phylogenetic position of the passenger pigeon by extracting and sequencing a 445bp fragment of DNA from *FIB7*. Previous morphological studies had suggested the passenger pigeon had a close relationship with *Zenaida*, and a molecular study of mitochondrial DNA had indicated it was a sister genus to *Macropygia* (Goodwin, 1967; Shapiro *et al.,* 2002). Successful collection of nuclear DNA was made possible through the use of aDNA protocols. The DNA was examined through a series of statistical analyses. Results indicated that the passenger pigeon was most closely related to the genus *Patagioenas,* within the Columbinae subfamily. A consensus data set including the mitochondrial data from the previous study that was analyzed supported this result as well. Based on this study the accepted phylogenetic position of the *E. migratorius* should be changed to that of a sister genus to *Patagioenas*.

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Appendix A

Consensus sequence of *FIB7* for primer design (5' to 3')

AGCACTGTTTTCTTGGATCTGAAGTTACCCCTGATGAGGGAAAATGAAGTGTTAAGAGTGACAG
ATGAACAGTAAGTAGATCAACAGAGTACCTAGACCTGCCCCAGTACTGGAATTTTCAAACCATA
ATAAACATTTAAAATCCTCTCTGAACTTTCTGTAAAAGATTGACCATCACAAGTGTGTGCTACAC
AGGGCACTTGGAAGGTGAAGCAGCTAAGAAAAACAAGTAAAATTTTAGCTGCATCTCTTTAGGA
ATGTCATTTTAGTTTATCAATTGATAATTACAATTACTTGAAGTTCATAATGATGATTGCAATA
TCAATGCAATTTAAAGATNNNATAAAACAATGAGAAATGTATGTCTTCCCCAATCTAAATGATT
CCTTTATTCATGAATGTGTGATCAAGTAAAGTGATTAAGGTAAAGGCACAGCACACAGTATTAG
AGATAAATTATGTACTTTAGAATTGAGCNNNTGTCTTCTAAGTAGGCAGAACTTTA

Sequence of FIB7 obtained from *E. migratorius* (5' to 3')

TACCCTGATGAGGGAAAATGAAGTGTTAATAGTGACAGATGAACAGTAAGTAGATCAACAGAGT
GCCTAGACCTGCCGCAGTACTGGAATTTTCAAACCRTAATAAACATTTAAAATCCTCTCTGATCT
TTCTGTAAAAGATTGACCATCACAAGTGTGTGCTACACAGGGCACTTGGAAGGTGAAGCAGCTA
AGAAAAACAAGTAAAATTTTAGCTGCATCTCTTTAGGAATCTCATTTTAGTTTATCAATTRATA
ATTACAATTACTTAAAGTTCATAATGATGATTGCAATATCAATGCAATTTAAAGATATAAAACA
GYGAGAAATGTATGTCTTCCCCAATCTAAATGACTCCTATATTCATGAATGTGTGATCAAGTATT
AAAGTAAAGGCACAGCACACAGTTTAGAGATAAATTATGTACTTTAGAATTGAGCTGAT

Appendix B

Genbank accession numbers for species examined in this thesis.

Species	12S	cyt b	FIB7
Aerodramus fuciphagus	AY513106	AY294428	AY513078
Alectroenas madagascariensis	AF483307	AF483344	
Caloenas nicobarica	AF483299	AF483336	
Chalcophaps indica	AF483288	AF483325	AY443694
Chalcophaps stephani	EF373293	AY443673	AY443695
Claravis pretiosa	EF373294	AF182682	AF182649
Columba arquatrix		EU481977	AF353463
Columba guinea		AF279708	AF279718
Columba livia	EF373295	FJ160757	
Columba palumbus	AF483298	AF375960	AF353462
Columba pulchrichollis		AF353413	AF353464
Columba rupestris		AF353410	
Columbina cruziana	AF483281	AF483318	
Columbina inca		AF182683	AF182650
Columbina minuta		AF182685	AF182652
Columbina picui		AF182687	AF182654
Didunculus strigirostris	AF483306	AF483343	
Drepanoptila holosericea	AF483308	AF483345	
Ducula aena	AF483294	AF483331	
Ducula bicolor		AF182705	AF182672
Ducula pacifica		AY443667	AY443689
Ducula pinon	AF483295	AF483332	
Ducula pistrinaria		AY443669	AY443691
Ducula rubricera		AY443668	AY443690
Ducula rufigaster	EF373297	EF373277	
Ducula zoeae	AF483296	AF483333	
Ectopistes migratorius	AF483314	AF483351	
Gallicolumba beccarii	AF483309	AY443675	AY443697
Gallicolumba jobiensis	EF373298	EF373278	
Gallicolumba luzonica	AF483297	AF483334	
Gallicolumba tristigmata	AF483282	AF483319	
Geopelia cuneata	AF483280	AF483317	AF182678
Geopelia striata	EF373299	EF373279	
Geotrygon albifacies		AY443658	AY443680
Geotrygon chiriquensis		AY443659	AY443681
Geotrygon costaricensis		AY443660	AY443682
Geotrygon montana	EF373301	AF182696	AF182663

Geotrygon versicolor	AF483289	AF483326	
Goura cristata	EF373302	AF182709	AF182676
Goura victoria	AF483283	AF483320	
Gymnophaps albertisii		EF373280	
Hemiphaga novaeseelandiae	EF373304	AY443666	AY443688
Henicophaps albifrons	EF373305	EF373281	
Leptotila cassini		AY443661	AY443683
Leptotila jamaicensis		AF279706	AF279716
Leptotila megalura	AF483305	AF483342	AF182664
Leptotila plumbeiceps		AF279707	AF279717
Leptotila rufaxilla	EF373306	AF182698	AF182665
Leptotila verreauxidecipiens		AY443662	AY443684
Leptotila verreauxifulviventris		AF279704	AF279714
Leucosarcia melanoleuca	AF483290	AF483327	AF182679
Lopholaimus antarcticus	EF373308	GQ912619	
Macropygia amboinensis	EF373309	EF373283	
Macropygia mackinlayi		AF353415	AF353466
Macropygia phasianella	AF483302	AF182693	AF182660
Macropygia tenurirostris		AF353416	AF353467
Metriopelia ceciliae		AF182688	AF182655
Metriopelia morenoi	EF373310	AY443677	AY443699
Nesoenas mayeri	AF483285	AF483322	
Ocyphaps lophotes	AF483286	AF483323	
Oena capensis	AF483316	AF483353	AF182674
Otidiphaps nobilis	AF483315	AF483352	
Patagioenas fasciata		AF353414	AF353465
Patagioenas flavirostris		AY443656	AY443678
Patagioenas leucocephala	AY274023	AF182689	
Patagioenas oenops		AF182690	AF182657
Patagioenas plumbea		AF182691	AF182658
Patagioenas speciosa		AF279711	AF279721
Patagioenas squamosa		AY443657	AY443679
Patagioenas subvinacea		AF182692	AF182659
Petrophassa albipennis	EF373314	EF373284	
Petrophassa plumifera		AY443676	AY443698
Pezophaps solitaria	AF483300	AF483337	
Phaethon rubricauda	AY369046	EU167010	EF552776
Phapitreron amethystina		AF182706	AF182673
Phapitreron leucotis		AY443670	AF279722
Phaps chalcoptera	AF483287	AF483324	
Ptilinopus leclancheri		AF182708	AF182675
Ptilinopus melanospil	AF483291	AF483328	

Ptilinopus occipitalis	AF483293	AF483330	AF279723
Ptilinopus pulchellus	EF373317	EF373285	
Ptilinopus rarotongensis		AY443663	AY443685
Ptilinopus richardsii		AY443664	AY443686
Ptilinopus superbus	AF483292	AF483329	
Raphus cucullatus	AF483301	AF483338	
Reinwardtoena browni	EF373318	AF353417	AF353468
Scardafella squammata	AF483310	AF483347	AF182651
Streptopelia bitorquata		AF353406	
Streptopelia capicola	EF373319	AF279709	AF279719
Streptopelia chinensis	AF483304	AF483341	AF182662
Streptopelia decaocto		AF353398	AF353449
Streptopelia decipiens		AF353400	AF353451
Streptopelia hypopyrrha		AF353403	AF353454
Streptopelia mayeri		AF353408	AF353459
Streptopelia orientalis		AF353405	AF353456
Streptopelia picturata		AF353409	AF353460
Streptopelia roseogrisea		AF353399	AF353450
Streptopelia semitorquata		AF353401	AF353452
Streptopelia senegalensis		AF279710	AF279720
Streptopelia tranquebarica		AF353407	AF353458
Streptopelia turtur		AF353404	AF353455
streptopelia vinacea		AF353402	
Treron australis	AF483312	AF483349	
Treron calva	EF373320	AY443674	AY443696
Treron vernans	AF483284	AF483321	
Treron waalia	AF483313	AF483350	
Trugon terrestris	EF373321	EF373286	
Turacoena manadensis	EF373322	EF373287	
Turtur afer	AF483311	AF483348	
Turtur brehmeri		AY151005	AY151006
Turtur chalcospilos	EF373323	AY443671	AY443693
Uropelia campestris	EF373324	EF373288	
Zenaida asiatica			AF258324
Zenaida auriculata	AF483303	AF483340	AF182667
Zenaida aurita		AF182704	AF182671
Zenaida galapagoensis		AF182701	AF182668
Zenaida graysoni		AF182702	AF182669
Zenaida macroura	EF373325	EU834878	
Zenaida meloda		AF182699	AF182666

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Undergraduate Research Assistant — September 2008 – Present
Eberly College of Science-Biology Department — University Park, PA
- Develop and execute experiments to extract and examine aDNA from 150 year old samples
- Report to Principle Investigator Beth Shapiro, PhD

Teaching Assistant for Biology 322 — January 2010 – May 2010
The Pennsylvania State University — University Park, PA
- Organize review sessions and help prepare 150 students for upcoming exams
- Ensure availability to all students 10 hours per week by holding office hours and grading exams in a timely manner
- Report to Professor Paula McSteen, PhD

Primary Care Scholar — May 2009
Penn State Hershey Medical Center — Hershey, PA
- Observed and participated in seminars and shadowing experiences demonstrating the aspects of a career in primary care
- Reported to Dr. Dennis Gingrich

IRTA Summer Fellow — May 2008 – August 2008
National Institutes of Health — Bethesda, MD
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Economics Department Grader — September 2007 – May 2008
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- Proctored tests and graded papers for over 700 students each semester taking Introduction to Micro Economics (ECON 002)
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Volunteer Experience

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- Assist various staff members in their duties
- Train incoming volunteers on their responsibilities
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Habitat for Humanity — August 2009-Present
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- Assist in housing construction and Rent-A-Worker programs
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Honors and Awards

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