10-2014

The development and function of the nasopharynx and its role in the evolution of primate respiratory abilities

Anthony Santino Pagano
Graduate Center, City University of New York

How does access to this work benefit you? Let us know!
Follow this and additional works at: https://academicworks.cuny.edu/gc_etds
Part of the Biological and Physical Anthropology Commons

Recommended Citation
Pagano, Anthony Santino, "The development and function of the nasopharynx and its role in the evolution of primate respiratory abilities" (2014). CUNY Academic Works.
https://academicworks.cuny.edu/gc_etds/328

This Dissertation is brought to you by CUNY Academic Works. It has been accepted for inclusion in All Dissertations, Theses, and Capstone Projects by an authorized administrator of CUNY Academic Works. For more information, please contact deposit@gc.cuny.edu.
The Development and Function of the Nasopharynx and its Role in the Evolution of Primate Respiratory Abilities

by
Anthony S. Pagano

A dissertation submitted to the Graduate Faculty in Anthropology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2014
Anthony S. Pagano

This manuscript has been read and accepted for the Graduate Faculty in Anthropology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Jeffrey T. Laitman

___________________________  __________________________
Date  Chair of Examining Committee

Gerald Creed

___________________________  __________________________
Date  Executive Officer

Eric Delson
Thomas Plummer
Ekaterina Pechenkina
Samuel Marquez
Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK
Abstract

Development and Functional Morphology of the Nasopharynx and its Role in the Evolution of Primate Respiratory Abilities

By

Anthony S. Pagano

Advisor: Jeffrey T. Laitman, Ph.D.

The nasopharynx is a centrally located region of the upper respiratory tract (URT) integral to several physiological functions. However, few have focused on this area within the context of human evolution. This study investigated osseous morphology, soft tissue histology, development, and evolutionary change of the nasopharynx. Multimodal analyses were performed:

Analysis 1: This study tested hypotheses on the morphological relationships of the osseous nasopharyngeal boundaries with the splanchnocranium and basicranium among dry crania representing humans and non-human primates using 3D geometric morphometrics (3D-GM). Results showed that humans, the most orthognathic group, exhibited the widest nasopharynges. Over human development, the nasopharynx grows vertically taller and anteroposteriorly shorter while the path of the cartilaginous Eustachian tube (CET) grows longer and more vertically oriented. Timing of these growth changes coincide with changes in frequency of otitis media.

Analysis 2: The nasopharynx was hypothesized to warm and humidify air only via its bony, non-contractile surfaces. Air conditioning capacity was assessed by presence of submucosal blood vessels, mucous cells, and serous cells on histological slides of
nasopharyngeal surfaces. Results indicated that all of these microstructures were present on all nasopharyngeal surfaces, rather than being restricted to non-contractile bony surfaces.

Analysis 3: Ambient climate was hypothesized to influence URT growth. Two groups of adult male *Macaca mulatta* raised in Oregon (cold climate) and California (warm climate) outdoor colonies were used. CT imaging and 3D-GM were performed. Results revealed no shape differences but the Oregon individuals exhibited larger airway size and smaller body mass than the California individuals. Thus being raised in cold climates appears related to development of larger URT proportions relative to body size.

Analysis 4: Nasopharyngeal morphology of fossil hominins, including Neanderthals and mid-Pleistocene *Homo* (MPH) from Europe and Africa, were reconstructed using 3D coordinate data and analyzed via 3D-GM. Neanderthals exhibited greater CET length than MPH and modern humans while the horizontal CET orientation of Neanderthals resembled the human infant condition. Results strongly suggest that Neanderthals possessed CET morphology and physiology distinct from modern humans, likely impacting susceptibility to middle ear disease and supporting species-level distinction.
Acknowledgements

I thank the members of my committee, Jeffrey T. Laitman, Eric Delson, Thomas Plummer, Ekaterina (Kate) Pechenkina, and Samuel Marquez. Their suggestions and insights have greatly improved the quality of this dissertation.

In particular, I thank Dr. Laitman, to whom I owe a great debt of gratitude. It is difficult to overstate his pivotal importance in my graduate career. Dr. Laitman was willing to take me on as his student and saw potential in me as a budding anatomist. Over the course of our working together, he became more than an advisor. Dr. Laitman was and remains a true mentor and friend to me and to all of the students he has supervised over his career. Indeed, he helped sculpt me from an inexperienced graduate student to a maturing scientist and educator and I continue to learn from the “macha” of the anatomy world. I look forward to many more years of collaboration and friendship with him as I take my rightful place within the “mishpocha.”

I also owe a great debt of gratitude to Samuel Marquez who has been a steadfast mentor, collaborator, and friend over the years. Sam has been there with me at every step of my graduate career and, like Dr. Laitman, is extremely hands-on in his teaching of both myself and the veritable army of medical students for whom anatomy became so much more than just a core requirement. As collaborators we have worked together closely and have often pulled “all-nighters” in the pursuit of excellence and submission deadlines. I have benefited from his deep knowledge, acute insights, and true commradery.
I would like to thank Eric Delson for sharing his expertise and enriching my graduate career from start to finish. There are few as knowledgeable and as insightful in the field of Biological Anthropology and I am truly grateful for his meticulous attention to this dissertation.

Thomas Plummer and Kate Pechenkina also deserve my sincerest thanks. Upon first arriving to Queens College as an undergraduate student, I already knew that I would pursue a Ph.D. in Biological Anthropology. Dr. Plummer was the first to offer me mentorship in this field and to expose me to professional-level research. His lectures on East Africa and the Oldowan toolmakers were truly inspirational. Dr. Plummer offered me a window into this discipline via participation in his work at the American Museum of Natural History and answered a seemingly never-ending stream of questions on fossil hominin morphology. He also advised me in the completion of my Senior Honors Thesis (on the paleopathology of Neanderthals) and largely prepared me for graduate school. When I had first met Dr. Pechenkina, I was already working on my Senior Honors Thesis and she had lended her insight as a paleopathologist. She gave me a new appreciation for the role of disease processes in human evolution and I had quickly found myself spending much of my time following her around campus to ask questions on skeletal biology and disease. Not only did she answer all of those questions, but she became an unwavering ally and friend. These two wonderful scientists and educators fostered my passion for biological anthropology during my formative years and it is my great honor to have had them participate in my dissertation committee.

I am greatly indebted to Timothy Smith for providing me open access to his laboratory, specimens, and expertise. It was a privilege to work alongside a scientist engaged in truly
Anthony S. Pagano

pioneering work. After making several trips out to his facility in Slippery Rock University, I have gained a new appreciation for the importance of histology and the difficulty involved in executing histological research on large upper respiratory tract structures. Without his invaluable help, a whole chapter of this dissertation would not have been possible. Also providing immense help on the histology portion of this dissertation was Ki Mak of the Icahn School of Medicine at Mount Sinai. He answered a multitude of questions and selflessly engaged in the tedious task of teaching me cellular morphology. I sincerely thank Dr. Mak for his patient guidance.

A special thank you is warranted by Joy Reidenberg and Richard Stern of the Icahn School of Medicine at Mount Sinai, both of whom reviewed portions of this dissertation and contributed to improving the quality of this work.

I am grateful to Dr. Charles Bluestone of Pittsburgh University Medical Center. Dr. Bluestone is a giant in the field of pediatric otolaryngology and literally wrote the book on the Eustachian tube and its disorders, fittingly titled “The Eustachian Tube.” His pioneering work on otitis media and the evolutionary roots of its etiology have raised important questions that this dissertation attempts to answer. I greatly thank him for the chance to co-author a manuscript and for his peerless expertise.

I would also like to thank Isamu Sando (formerly of the University of Pittsburgh Medical Center) for sending me a signed copy of his “Clinical Atlas of the Temporal Bone and Eustachian Tube.” Dr. Sando has engaged in some of the best and most detailed research on normal and
pathological Eustachian tube morphology. His groundbreaking and meticulous work has also been inspirational to me.

Access to fossils was granted by Robert Kruszynski at the Natural History Museum of Great Britain, Reinhard Ziegler of the Staatliches Museum für Naturkunde of Germany, George Koufos of the Aristotle University of the Thessaloniki in Greece, and Luca Bondioli of the Museo Nazionale Preistorico Etnografico Luigi Pigorini in Rome, Italy. Studying these priceless specimens was a life changing experience and I offer these curators my sincerest thanks for their kindness and hospitality.

I thank the collection managers, curators, and curatorial assistants who allowed me access to extant human and non-human primate collections. These include Gisselle Garcia-Pack and Eileen Westwig of the American Museum of Natural History in New York, Darin Lunde of the National Museum of Natural History in Washington D.C., Lyman Jellema of the Cleveland Museum of Natural History, Carrie Balaban and Gloria Limetti of the University of Pittsburgh Medical Center (for access to the Sando Collection), and Dorothy Dechant at the University of the Pacific School of Dentistry (for access to the Spencer R. Atkinson Collection).

I would like to thank Viktor Deak for collaborating with me on the production of artwork used in this dissertation. After several years of patient insistence, my advisor had finally convinced me to seek out the services of a professional artist. After acquiescing reluctantly, I was surprised to have gained a new perspective on the structures which I had been working to reconstruct. Sitting in his studio and attempting to fit a cartilaginous Eustachian tube onto a digital Neanderthal
Anthony S. Pagano

cranium, I quickly became aware of some fundamental anatomical differences between us and our closest extinct relatives.

Derek Yuan of the Stony Brook University Medical Center has also been a great help to this work. Early on, he and I collaborated in programming the SAS commands behind some of the morphometric techniques used here. As I assisted Dr. Laitman in mentoring him through the Master of Biological Sciences Program at the (then) Mount Sinai School of Medicine, I quickly found a capable researcher and true friend. Also, thank you for teaching me so much about Chinese culture and the delights of Szechuan cuisine! 谢谢

Along the course of my frantic dash to complete this dissertation, several people have helped in their own unique ways. I would like to acknowledge Madeline Olmeda, Torrence Wilson, Tarin Rivera, Steven Yuen, Eddie Lugo, and Herman Suppoyo who have supported The Center for Anatomy and Functional Morphology of the Icahn School of Medicine of Mount Sinai through their tireless work and have mobilized its resources for me at times when I most desperately needed them.

On a personal note, I would like to thank my family. Their love and support have been unconditional and they have shared with me every trial by fire that life has given. My mother, Mercedes, was born in Havana, Cuba. After her father (Manuel Lopez) was executed by the Castro regime for participating in the Bay of Pigs military operation, she endured many long years living under a totalitarian communist regime. After years of interrogation and daily harassment by state police, she and her family were airlifted to the United States. Arriving with
little formal education and speaking no English, she eventually built a life with my father, Richard. By her example, I learned the true meaning of hard work and perserverance. Her story made me feel lucky to have been born where I was and gave me a sense of obligation to ensure that her struggle was not endured in vain. Most of all, I dedicate this dissertation to her.

My father taught me a different lesson. His favorite adage is: “When a door closes, kick it down. When you don’t like the rules, make your own.” From this, he advocates thinking outside of convention to solve problems and never accepting passively what life offers. This way of thinking was a product of his upbringing in a closely knit New York Italian family. It is how he has lived his life, teaching my brother and me by example. My father has been a huge influence into the present time and continues to embody the true meaning of commitment to family.

My father has also influenced me through his curiosity about the natural world. In fact, Paleoanthropology has been one of his great interests. He had enrolled in the ACE (Adult Continuing Education) Program at Queens College and took Introduction to Human Evolution, taught by Sarah Stinson. I was a Junior High School student when he had brought home his class materials and shared them with me. This was when I first became interested in Biological Anthropology and the experience prompted me to take the same class several years later. I had sat in the first row and, before Dr. Stinson began her fist lecture, I told her that I intended to enroll in NYCEP.
My brother, John (J.J.) has also offered constant encouragement and friendship. For the last several years of my graduate career, I lived in his apartment as his roommate. He has been my closest lifelong friend and has always offered support when needed. Thank you, little brother.

Lastly, I would like to thank Marissa Blando for her love, devotion, and support through the writing process. She constantly encouraged me and reminded me that there is life after graduate school. Marissa also “fueled” my work with excellent home cooking and free occupational therapy when I needed it (especially after my trip to London). I look forward to many more happy years with her by my side.

To all the above named, thank you for your support and for the important roles you all played in shaping both my personal life and career.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Scope of study</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Background</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Overview of Chapters</td>
<td>3</td>
</tr>
<tr>
<td>1.3.1 Chapter 2</td>
<td>3</td>
</tr>
<tr>
<td>1.3.2 Chapter 3</td>
<td>3</td>
</tr>
<tr>
<td>1.3.3 Chapter 4</td>
<td>4</td>
</tr>
<tr>
<td>1.3.4 Chapter 5</td>
<td>5</td>
</tr>
<tr>
<td>1.3.5 Chapter 6</td>
<td>6</td>
</tr>
<tr>
<td>2. Background on the nasopharynx</td>
<td>7</td>
</tr>
<tr>
<td>2.1 What is the nasopharynx?</td>
<td>7</td>
</tr>
<tr>
<td>2.1.1 Is it nose? Is it pharynx? Is it both?</td>
<td>7</td>
</tr>
<tr>
<td>2.1.2 Functional and histological differences among nasopharyngeal components</td>
<td>11</td>
</tr>
<tr>
<td>2.1.3 Relation to head segmentation: A composite evolutionary history</td>
<td>11</td>
</tr>
<tr>
<td>2.1.4 Developmental origins of the nasopharyngeal boundaries</td>
<td>14</td>
</tr>
<tr>
<td>2.2. Comparative Anatomy: A survey of air-breathing vertebrates</td>
<td>17</td>
</tr>
<tr>
<td>2.2.1 Choanae and the origins of the tetrapods: Assessing the lungfish as a model for primitive choanal morphology</td>
<td>17</td>
</tr>
<tr>
<td>2.2.2 Nasopharyngeal anatomy of amphibians</td>
<td>23</td>
</tr>
<tr>
<td>2.2.2.1 Evolutionary origins of the Eustachian tube</td>
<td>25</td>
</tr>
<tr>
<td>2.2.3 Nasopharyngeal anatomy of reptiles</td>
<td>26</td>
</tr>
<tr>
<td>2.2.4 The evolution of the pterygoid complex and the rise of mammals</td>
<td>27</td>
</tr>
<tr>
<td>2.2.5 Reconstructing the evolution of the mammalian nasopharynx and its boundaries</td>
<td>31</td>
</tr>
<tr>
<td>2.2.6. The nasopharyngeal morphology of primates: Macrosmats versus microsmats</td>
<td>35</td>
</tr>
<tr>
<td>2.2.7. The nasopharyngeal anatomy of humans</td>
<td>39</td>
</tr>
<tr>
<td>2.2.7.1 Diversity among human populations</td>
<td>43</td>
</tr>
<tr>
<td>2.2.7.2 Normal human development</td>
<td>49</td>
</tr>
<tr>
<td>2.2.7.2.1 Midline nasopharyngeal structures</td>
<td>49</td>
</tr>
<tr>
<td>2.2.7.2.2 The cartilaginous Eustachian tube</td>
<td>55</td>
</tr>
<tr>
<td>2.3 Early study of the nasopharyngeal boundaries</td>
<td>57</td>
</tr>
<tr>
<td>2.4 Clinical relevance for studying the morphology of the nasopharyngeal boundaries</td>
<td>62</td>
</tr>
<tr>
<td>2.4.1 Eustachian tube dimensions and otitis media</td>
<td>63</td>
</tr>
<tr>
<td>2.4.2 Cleft Palate</td>
<td>64</td>
</tr>
<tr>
<td>2.4.3 Nasopharyngeal carcinoma</td>
<td>66</td>
</tr>
<tr>
<td>2.4.4 Sleep apnea</td>
<td>67</td>
</tr>
<tr>
<td>2.5 Conclusions</td>
<td>69</td>
</tr>
<tr>
<td>3. Geometric morphometric analysis of the osseous nasopharyngeal boundaries: Testing for functional morphologic relationships with adjacent cranial structures</td>
<td>70</td>
</tr>
</tbody>
</table>
3.1 Aim of the chapter
3.2 Background on previous morphometric studies of the nasopharyngeal boundaries in relation to adjacent structures.
  3.2.1 Studies of basicranial flexion
  3.2.2 Facial orientation and kyphosis
  3.2.3 Vertical Facial growth and choanal dimensions
3.3 Hypotheses
3.4 Materials and Methods
  3.4.1 Materials
  3.4.2 Sexual dimorphism
  3.4.3 Methods
3.5 Results
  3.5.1 Principal Components Analysis
  3.5.2 Developmental patterns
  3.5.3 Intergeneric variation among adults
  3.5.4 Intergeneric variation among Stage 2 crania
  3.5.5 Univariate measures distinguishing human adults and newborns from non-human hominoids
  3.5.6 Measuring variability with coefficient of variation
  3.5.7 Correlations
  3.5.8 Growth in estimated Eustachian tube dimensions
  3.5.9 Description of variation among humans
    3.5.9.1 Nasopharyngeal morphology of individuals with cleft palate, microencephaly and hydrocephalus
    3.5.9.2 Population differences in nasopharyngeal dimensions
3.6 Discussion
  3.6.1 Assessment of Hypothesis #1
  3.6.2 Assessment of Hypothesis #2
  3.6.3 Hypothesis #3
  3.6.4 Variation among humans
3.7 Conclusion
4. Histology of the primate nasopharyngeal boundaries: Testing for a relationship between air conditioning capacity and surface contractility
  4.1 Aim of the chapter
  4.2 Background
    4.2.1 The respective roles of mucus-producing cells, serous cells and blood vessels in conditioning air.
    4.2.2 Previous studies of nasopharyngeal histology
    4.2.3 Contractility and the distribution of nasopharyngeal epithelia
  4.3 Hypothesis
  4.4 Materials and Methods
    4.4.1 Materials
    4.4.2 Methods
  4.5 Results
    4.5.1 Homo sapiens
4.5.2 *Hylobates* sp  
4.5.3 *Macaca fascicularis*  
4.5.4 *Ateles* sp.  
4.5.5 *Tarsius bancanus*  
4.5.6 *Otolemur* sp.

4.6 Discussion  
4.6.1 The nasopharyngeal septum  
4.6.2 Epithelial distribution and submucosal glands  
4.6.3 Relevance to reconstruction of the Neanderthal nasopharynx

4.7 Conclusions

5. Testing for the epigenetic influence of local climate on development of nasopharyngeal hard and soft tissue dimensions: A CT and geometric morphometrics study of two *Macaca mulatta* colonies

5.1 Aim of chapter

5.2 Background  
5.2.1 Previous studies of the nasal complex and its relationship with climate  
5.2.2 Animal models  
5.2.3 Hypothesis: Climatic stresses, namely temperature and humidity, influence the growth of the nasopharyngeal boundaries via an epigenetic process.

5.3 Materials and Methods  
5.3.1 Materials  
5.3.2 Methods  
5.3.2.1 Radiology  
5.3.2.2 Geometric Morphometrics and Procrustes Scaling

5.4 Results  
5.4.1 Generalized Procrustes Analysis  
5.4.2 Univariate Measures

5.5 Discussion

5.6 Conclusions

6. Geometric morphometric analysis of the osseous nasopharyngeal boundaries of modern humans, Neanderthals, and mid-Pleistocene Homo

6.1 Aim of chapter

6.2 Background  
6.2.1 Previous studies of osseous nasopharyngeal boundary anatomy in Plio-Pleistocene hominins  
6.2.1.1 Choanal morphology of fossil hominins  
6.2.1.2 The nasopharyngeal roof and midline basicranial axis  
6.2.2 Previous studies on upper respiratory tract morphology among Neanderthals and mid-Pleistocene hominins  
6.2.2.1 Cold adaptation in the Neanderthal upper respiratory tract  
6.2.2.2 Reconstructions of vocal tract dimensions and articulatory
abilities among Neanderthals and mid-Pleistocene hominins 292

6.3 Hypotheses 296
6.4 Materials and Methods 298
6.4.1 Materials 298

6.4.1.1 Kabwe 1 299
6.4.1.1.1 State of preservation and estimation of landmark locations 299
6.4.1.1.2 Subjective assessment of morphology 302

6.4.1.2 Petralona 1 303
6.4.1.2.1 State of preservation and estimation of landmark locations 304
6.4.1.2.2 Subjective assessment of morphology 307

6.4.1.3 Steinheim 1 308
6.4.1.3.1 State of preservation and estimation of landmark locations 308
6.4.1.3.2 Subjective assessment of morphology 312

6.4.1.4 Atapuerca 5 313
6.4.1.4.1 State of preservation and estimation of landmark locations 313
6.4.1.4.2 Subjective Assessment 316

6.4.1.5 Gibraltar Forbes' Quarry 1 317
6.4.1.5.1 State of preservation and estimation of landmark locations 317
6.4.1.5.2 Subjective assessment of morphology 320

6.4.1.6 Monte Circeo 1 321
6.4.1.6.1 State of preservation and estimation of landmark locations 322
6.4.1.6.2 Subjective assessment of morphology 326

6.4.2 Methods 331
6.4.2.1 Measurement technique and landmarks 331
6.4.2.2 Mirror reflection of missing landmarks 332
6.4.2.3 Generalized Procrustes Analysis 334
6.4.2.4 Univariate measures 334
6.4.2.5 Identifying differences between fossil crania and modern humans 335

6.5 Results 336
6.5.1 Generalized Procrustes Analysis and multivariate shape diversity 336
6.5.2 Hypothesis #1: Neanderthals possessed taller and narrower nasopharynges as an adaptation to respiration in cold environments 343
6.5.3 Hypothesis #2: Neanderthals possessed longer, more horizontally oriented cartilaginous Eustachian tubes than human adults 352

6.6 Discussion 358
6.6.1 Discussion of Principal Components Analysis (PCA) and multivariate shape diversity 358
6.6.2 Discussion of Procrustes-corrected (scale-free) univariate measures 359
6.7 Conclusions 368

7 Summary and Conclusions 370
  7.1 Osteology 370
  7.2. Histology 372
  7.3 Epigenetic influence of climate on naspharyngeal morphology 373
  7.4 Analysis of nasopharyngeal morphology among fossil hominins 374
  7.5 Nasopharyngeal morphology within the context of primate evolution 376

References 378
List of Figures

2.1 A mid-sagittal section through a human head illustrating key structures within the nasopharynx and adjacent areas. 10
2.2 A coelacanth fish (*Latimeria chalumnae*), which exhibits shared derived aspects of limb morphology with basal tetrapods. 18
2.3 The coelacanth does not possess choanae, instead primitively bearing its anterior and posterior nares on the external rostrum. 19
2.4 The coelacanth exhibits no choanal opening in the oral cavity. 20
2.5 Australian lungfish (*Neoceratodus forsteri*) exposing its oral cavity. 20
2.6 a) Frontal view of a bullfrog (*Rana catesbeiana*) with the right anterior naris indicated by a black arrow. b) Basal view of the same specimen with the right choana indicated by a black arrow. 24
2.7 a) Frontal view of a sea turtle (*Lepidochelys* sp.) with the enlarged choanal opening visible through the anterior naris (arrow on left choanal communication). b) A basal view of the same specimen illustrating the position of the choanae opening into the oral cavity (black arrow indicating the position of the left choana). 27
2.8 Basal views of a red kangaroo (*Macropus rufus*; a) and whitetail deer (*Odocoileus virginianus*; b). Note the location of the choanae is posterior and superior to the hard palate, even among distantly related mammals. This creates a separation of oral and nasal cavities not present among reptiles. 34
2.9 Microsmatic primates exhibit choanae that are relatively tall and narrow, leading directly into the posterior nasal cavity (as in *Cercocebus torquatus*; Top image). Macrosmatic primates instead exhibit choanae that are vertically short and lead into a nasoharyngeal duct that is superiorly bounded by a transverse lamina (as in *Daubentonia madagascariensis*; Bottom image). 37
2.10 Most mammals such as Macaca (left) exhibit an epiglottis (E) that interlocks with the soft palate (S), lying within the nasopharynx (delimited by the blue rectangle). Adult Homo sapiens exhibits a low laryngeal position so that the epiglottis is far inferior to the soft palate and nasopharynx. 40

3.1a Basal landmarks used in the study of hominoids and papionins. 94
3.11b Facial landmarks used in the study of hominoids and papionins. 95
3.2 A midsagittal section through a *Macaca mulatta* cranium. 103
3.3 The position of the Eustachian point used in this study is demonstrated. 108
3.4 A mid-sagittally sectioned human cranium with a wireframe model superimposed. 116
3.5 Principal components plot of multivariate shape variation within the sample along PC1 and PC2. 117
3.6 Principal components plot of multivariate shape variation within the sample using a reduced set of landmarks plotted along PC1 and PC2. 119
3.7 Principal components plot of multivariate shape variation within the sample using a reduced set of landmarks plotted along PC1 and PC2. 121
3.8 Wireframe models representing growth from infancy (Stage 1) to adulthood (Stage 5). A mid-sagittal view is represented.

3.9 Wireframes representing average Stage 1-5 humans all superimposed at the point hormion.

3.10 Box-whisker plots of significant \( P=0.005 \) as per the Bonferroni formula growth changes among *Homo*.

3.11 Box-whisker plots of significant \( P=0.005 \) as per the Bonferroni formula growth changes among *Homo*.

3.12 Box-whisker plots of significant \( P=0.005 \) as per the Bonferroni formula growth changes among *Homo*.

3.13 Box-whisker plots of significant \( P=0.00833 \) as per the Bonferroni formula growth changes among *Pan*.

3.14 Box-whisker plots of significant \( P=0.00833 \) as per the Bonferroni formula \( \{ P=0.00833 \text{ for (a)-(b)}; \ P=0.005 \text{ for (e)} \} \) growth changes among *Gorilla*.

3.15 Box-whisker plots of significant \( P=0.00833 \) as per the Bonferroni formula growth changes among *Pongo*.

3.16 Box-whisker plots of significant \( P=0.00833 \) as per the Bonferroni formula growth changes among *Hylobates* from pairwise t-tests.

3.17 Box-whisker plots of significant \( P=0.00833 \) for \( (b)-(d); \ P=0.005 \text{ for (a), (e), and (f)} \} \) growth changes among *Macaca*.

3.18 Box-whisker plots of significant \( P=0.00833 \) as per the Bonferroni formula growth changes among *Cercocebus*.

3.19 Box-whisker plots of significant \( P=0.005 \) for \( (a), (c), \text{ and (e)}; \ P=0.00833 \text{ for (b), (d), and (f)} \} \) growth changes among *Papio*. For each plot, the X axes represent growth stages.

3.20 Box-whisker plots of measures that significantly \( P<0.00125 \) distinguish all humans (a)-(c), human newborns (d) or human adults (e)-(f) from non-human hominoids.

3.21 Box-whisker plots of measures that are significantly \( P<0.00125 \) different between newborn and adult humans but do not distinguish human adults from non-human hominoids.

3.22 Box-whisker plots of significant \( P=0.005 \) as per the Bonferroni formula growth changes in estimated dimensions and angulation of the cartilaginous Eustachian tube of humans. For each plot, the X axes represent growth stages.

3.23 Note that the individual with cleft palate appears to have relatively wider choanae and laterally rotated medial pterygoid plates (likely related to the preponderance of cases in which the muscular sling of the tensor veli palatini muscle fails to contact the hamulus).

3.24 Specimens with hydrocephalus did not exhibit any significant differences \( P>0.05 \) from normal crania in the relative dimensions of the choanae or overall nasopharynx.

3.25 Individuals with microencephaly exhibited no observable differences in choanal or upper respiratory tract proportions from normal humans.

3.26 Even edentulous specimens with complete alveolar resorption exhibit no observable difference from normal humans in relative choanal or upper respiratory tract dimensions. This suggests that the functional demands of
respiration maintain the proportions of the nasal cavity and nasopharynx throughout postnatal life.

3.27 Box-whisker plots of significant ($P=0.00076$ as per the Bonferroni formula) differences among human populations.

4.1 Illustration of a midsagittally section human cranium with nasopharyngeal soft tissues.
4.2 A slice through the nasopharyngeal floor (dorsal aspect of the soft palate). (Magnification 10x; Slice #1540; *Homo sapiens*)
4.3 A section through the nasopharyngeal roof. (Magnification 10x; Slice #1540; *Homo sapiens*)
4.4 Pseudostratified columnar epithelium lining the nasopharyngeal roof (Top), floor (Bottom), and lateral wall with torus tubarius (right). (Magnification 4x; Slice #1477; *Homo sapiens*)
4.5 Mitotic cells lining the surface of the lateral pharyngeal recess. (Magnification 40x; Slice #769; *Homo sapiens*)
4.6 A section through the nasopharyngeal floor. (Magnification 40x; Slice #1040; *Homo sapiens*)
4.7 A section through the posterior nasopharyngeal wall. (Magnification 10x; Slice #500; *Homo sapiens*)
4.8 A section through the lateral nasopharyngeal wall, anterior to the torus tubarius. (Magnification 40x; Slice #304; *Hylobates* sp.)
4.9 A section through the anterior portion of the torus tubarius. (Magnification 60x; Slice #1455; *Hylobates* sp.)
4.10 A section through the posterior edge of the torus tubarius. (Magnification 10x; Slice #1785; *Hylobates* sp.)
4.11 Section through the posterior nasopharyngeal wall. (Magnification 40x; Slice #2166; *Hylobates* sp.)
4.12 Section through the nasopharyngeal septum. (Magnification 4x; Slice #325; *Hylobates* sp.)
4.13 A section through the base of the nasopharyngeal septum where it meets the soft palate (bottom of the image). (Magnification 4x; Slice #335; *Hylobates* sp.)
4.14 A section through the nasopharyngeal floor. (Magnification 40x; Slice #1511; *Macaca fascicularis*)
4.15 A section through the lateral wall overlying the torus tubarius. (Magnification 20x; Slice #1852; *Macaca fascicularis*)
4.16 A slice through the torus tubarius in the lateral pharyngeal wall at the level of the Eustachian tube orifice. (Magnification 4x; Slice #1852; *Macaca fascicularis*)
4.17 The above image is a section through the superior portion of the post-tubal portion of the lateral nasopharyngeal wall. (Magnification 40x; Slice #1144; *Macaca fascicularis*)
4.18 Section through inferior portion of the post-tubal nasopharyngeal wall. (Magnification 10x; Slice #1144; *Macaca fascicularis*)
4.19 The above is a section through the epithelial surface of the nasopharyngeal
A section through the nasopharyngeal septum, which appears to be comprised primarily of connective tissue and is covered in respiratory epithelium. (Magnification 4x; Slice #1543; *Macaca fascicularis*)

Sagittal view of a *Macaca fascicularis* specimen demonstrating the position of the nasopharyngeal septum.

The above is a section through the posterior nasopharyngeal wall. (Magnification x40; Slice #1034; *Macaca fascicularis*)

A magnified view of submucosal serous cells under the posterior nasopharyngeal wall. These cells allow for the humidification of airflow. (Magnification 40x; Slice #895; *Macaca fascicularis*).

A section through the nasopharyngeal floor at the anteroposterior level of the medial pterygoid plates. (Magnification 60x; Slice #225; *Ateles sp.*)

A slice through posterior nasopharyngeal wall, preserving only an irregular, circular patch of epithelial surface area. (Magnified 4x; Slice #1345; *Ateles sp.*)

Section through the posterior nasopharyngeal wall of *Ateles*. (Magnification 40x; Slice #1345; *Ateles sp.*)

Section through the serous gland in Figure 4.25. (Magnification 40x; Slice #1345; *Ateles sp.*)

Note the constricted recess inferolaterally (left) and the close proximity of the tympanic bulla to the superolateral boundary of the nasopharynx (Magnification 4x, Slice# 1995; *Tarsius bancanus*).

Basal view of a *Tarsius* sp. dry cranium.

The above slice demonstrates the distal portion of the cartilaginous Eustachian tube and its pharyngeal orifice, which are oriented at a nearly vertical position. (Magnification x40; Slice #1685; *Tarsius bancanus*)

The anterior portion of the nasopharynx is visible with lateral wall against the medial pterygoid plate. (Magnification 4x; Slice #1675; *Tarsius bancanus*).

At the anterior portion of the nasopharyngeal floor, ciliated respiratory epithelium is present with thick goblet cells providing mucins to protect this surface from dessication. (Magnification 20x; Slice #1675; *Tarsius bancanus*)

Anterior portion of the post-tubal nasopharyngeal wall lined with respiratory epithelium with goblet cells. (Magnification 40x Slice #1825; *Tarsius bancanus*)

Slice located in the posterior half of the post-tubal nasopharyngeal wall. (Top Magnification 4x, Bottom Magnification 40x; Slice #1965; *Tarsius bancanus*)

The above is a section through the torus tubarius. (Magnification 40x; Slice #1295; *Otolemur sp.*)

The above is a section through the nasopharynx at the craniocaudal level of the Eustachian tube pharyngeal orifice. (Magnification 10x; Slice #1433; *Otolemur sp.*)

A section through the posterior pharyngeal wall. (Magnification 40x; Slice #1665; *Otolemur sp*)

Section through the distal-most (caudal) nasopharynx. (Magnification x10; Slice #1655; *Otolemur sp.*)

Adults were identified via an erupted third molar. The location of the parasagittal plane is demonstrated in (a). From this view, the unerupted third molar is visible.
maxillary molar is visible (b). The below CT is from a subadult specimen of the Oregon sample. Note that the slice is through the right side of the head.

5.2 A sagittal slice through the head of an adult Macaca mulatta from the California National Primate Research Center. The red line represents the respective level and the plane of images in Figure 5.3.

5.3 Two separate axial slices through the nasopharyngeal air sinuses of Macaca raised in an outdoor colony in California.

5.4 Images from a CT scan and an MRI of an adult male Macaca mulatta at the Mount Sinai School of Medicine.

5.5 The wireframe model represents a midsagittal view of the nasal cavity (green), left choana (red), cartilaginous Eustachian tube (blue), and harnion-basion chord (black).

5.6 Principal components plot of shape variation among the cold- and warm-reared Macaca colonies without correction for the measurement artifact.

5.7 Principal components plot of shape variation among the cold- and warm-reared Macaca colonies. Only principal components 2 and 3 are used here, which are appear unaffected by the distortion.

5.8 Principal components ploth of shape variation among the cold- and warm-reared Macaca colonies used here. Principal component 2 is plotted against centroid size.

6.1 Frontal view of the Kabwe 1 cranium from Zambia, Central Africa.

6.2 Basal view of the Kabwe 1 specimen from Zambia, Central Africa. It is in excellent condition, save for the anterior rim of the foramen magnum.

6.3 Frontal view of the Petralona 1 specimen from Greece.

6.4 Basal view of the Petralona 1 cranium.

6.5 A frontal view of the Steinheim 1 cranium from Germany.

6.6 A basal view of the Steinheim 1 specimen from Germany.

6.7 Frontal view of the Atapuerca 5 cast.

6.8 Basal view of the Atapuerca 5 cast.

6.9 A frontal view of the Forbes' Quarry 1 specimen from Gibraltar.

6.10 A basal view of the Forbes' Quarry 1 specimen.

6.11 Frontal view of the Monte Circeo 1 cranium from Italy.

6.12 Basal view of the Monte Circeo 1 cranium.

6.13 Frontal view of the Saccopastore 1 cast.

6.14 Basal view of the Saccopastore 1 cast.

6.15 Superimposition of wireframes atop the anatomical structures which they represent in Figures 6.16-6.18.

6.16 Principal Components plot of total fossil morphology plotted among a modern human developmental series, which includes several, geographically diverse populations.

6.17 Wireframe model used to illustrate palatal (in green), choanal (in red), and cartilaginous Eustachian tube (in blue) morphology.

6.18 Wireframes representing a modern human developmental series (top row) and the fossil crania.

6.19 Box-whisker plots of choanal width (a) and height (b) among Stage 5 modern humans and the fossil crania.
6.20 Box-whisker plots of choanal shape (a) and orientation (b) and maximum nasopharyngeal depth (staphyion-sphenobasion distance; c) among Stage 5 modern humans and the fossil crania.

6.21 Box-whisker plot of relative bilateral width between the left and right osseous Eustachian orifices (an estimate of nasopharyngeal/basicranial width; a) and width between the petrous apices (b) in Stage 5 modern humans and the fossil crania.

6.22 Box-whisker plots of CET floor length (a) and angulation (b) among the fossil crania and Stage 1 and Stage 5 modern human crania.

6.23 Box-whisker plot of CET length (between the Eustachian point and the midpoint on the medial pterygoid plate edge) among Stage 5 modern human crania representing several populations and the fossil crania.

6.24 Three-dimensional reconstructions of the cartilaginous Eustachian tube tracts of a human newborn, adult, and a Neanderthal (Monte Circeo 1).

6.25 The cartilaginous Eustachian tubes of a modern human infant (top), adult (middle), and an adult Neanderthal (Monte Circeo 1, bottom) are shown abutting the medial pterygoid plate.
List of Tables

3.1 Sample sizes arranged by genus and developmental stage. 85
3.2 Sample sizes for Stage 5 human adults arranged by population and sex. 88
3.3 Analysis of differences between sexes for univariate measures. 90
3.4 Landmarks used in the geometric morphometrics analyses. 91
3.5 Reduced set of landmarks used in PCA of upper respiratory tract landmark coordinate data. 115
3.6 Coefficients of variation for univariate measures used in this study in the total hominoid sample. 154
3.7 Multiple correlations performed on the total sample of hominoids and papionins in testing Hypothesis 1. 155
3.8 Multiple correlations performed on the hominoid sample in testing Hypothesis 1. 156
3.9 Multiple correlations performed in testing Hypothesis 2. 158
3.10 Correlations performed on the hominoid sample in testing Hypothesis 2. 159
3.11 Description of measures used in Table 3.12. 165
3.12 Z-scores of pathological individuals plotted against the normal human range. 168
3.13 Coefficients of variation for facial and nasopharyngeal measures among human adults. 175
3.14 A series of Student's t-tests comparing the relative nasopharyngeal dimensions of human groups pooled into "cold" (Northern Europe, Northern China, Alaskan, Aleutian) and "tropical" (East Africa, West Africa, South East Asia) climates. 176
3.15 Human sexual dimorphism in relative choanal width and height. 177

5.1 Three-dimensional landmarks used in the Generalized Procrustes Analysis. 269
5.2 Descriptive statistics of body mass, centroid size (derived from three-dimensional coordinate data), and the ratio of nasopharyngeal surface area (square root) to volume (cube root) measured in millimeters. 277

6.1 Fossil specimens examined. 331
6.2 Landmarks used in the analysis of fossil morphology. 332
6.3 Z-scores of fossils’ values for univariate measures expressed in standard deviations of distance from the pooled Stage 5 modern human mean. 351
6.4 Z-scores of fossils’ values for univariate measures expressed in standard deviations of distance from the pooled Stage 5 modern human mean. 357
1. Introduction

1.1 Scope of study

This dissertation is focused on the development, evolution, and morphological relationships of the nasopharyngeal boundaries among humans and non-human primates. The basic biology of these structures was studied via a multimodal approach. The shape of the osseous nasopharyngeal boundaries and its respective relationships with facial and basicranial morphology were analyzed via three-dimensional geometric morphometrics of extant humans, non-human hominoids, and papionins. Normal growth of the cartilaginous Eustachian tube tract was also examined as this structure is implicated in otitis media (middle ear disease), which is among the most common pediatric clinical conditions worldwide. Histological analysis was performed on a human and several non-human primate specimens to investigate the relationships of anatomically definable structures of the nasopharyngeal boundaries and their epithelial/submucosal coverings. CT imaging was also used to test for the influence of climate on the nasopharyngeal soft tissue dimensions. Finally, a group of fossils representing Neanderthals and mid-Pleistocene *Homo* was contrasted against contemporary humans in testing for nasopharyngeal shape differences including those in the tract of the cartilaginous Eustachian tube. This study is the first to focus on cartilaginous Eustachian tube morphology among the Neanderthals and mid-Pleistocene *Homo*. Any shape differences may bear implications for several physiological functions and, potentially, the extinction of the Neanderthals.
1.2 Background

The nasopharynx is a centrally located space whose boundaries are crucial to functions of respiration, middle ear aeration, deglutition, and speech. Developmental and evolutionary changes in the osseous components of its boundaries may thus impact a wide array of functional systems of the head. It is well known that some nasopharyngeal boundary structures undergo substantial growth change among humans, yet morphometric study of these changes has been infrequent. Still fewer have focused on the nasopharyngeal boundaries of fossil hominins, who exhibit a wide range of cranial morphology. These may have manifested in nasopharyngeal soft tissue dimensions and function that differed from the condition of any living hominoid.

Cartilaginous Eustachian tube (CET) morphology is of special interest to this study as *Homo sapiens* is alone among living primates in its extremely high rates of otitis media (Bluestone, 2005 a,b). This is likely a consequence of the CET dimensions of human infants, who appear similar to adult Neanderthal and some mid-Pleistocene *Homo* crania in their “non-flexed” presentation. As a result, human infants possess a more horizontally oriented cartilaginous Eustachian tube that may admit greater numbers of pathogens from the nasal cavity. Indeed, its horizontal orientation is one of several features implicated in the development of middle ear infection or otitis media (Bluestone, 2005a,b). Susceptibility to otitis media could have thus impacted the reproductive fitness of Neanderthals as they competed for resources with anatomically modern humans.
1.3 Overview of Chapters

1.3.1 Chapter 2

Chapter 2 provided a historical, anatomical, evolutionary, developmental, functional, and clinical framework from which to view the nasopharynx and its boundaries. The space remains among the most difficult to define in the human body. It spans among several cranial regions and has composite evolutionary origins. One must account for these when assessing developmental and evolutionary change in the nasopharyngeal boundaries.

1.3.2 Chapter 3

Chapter 3 used three-dimensional geometric morphometrics and Procrustes-corrected univariate measures to test hypotheses related to the morphological relationships of the osseous nasopharyngeal boundaries to the facial skeleton and external basicranium. Humans, non-human hominoids, and papionins were used to model different anatomic configurations ranging from extreme prognathism and external basicranial retroflexion to extreme orthognathy and external basicranial flexion. Each genus was represented by a growth series, allowing for assessment of inter-generic differences in growth trajectory as well as the quantification of growth change in cartilaginous Eustachian tube (CET) length and angulation among developing humans. The timing of growth changes in the human CET were assessed in relation to times of peak incidence of otitis media. Comparison of population differences among adult human crania and shape
analysis of crania with birth defects (i.e., cleft palate, hydrocephalus, microencephaly) were also performed.

Morphological relationships were found among the osseous nasopharyngeal boundaries, facial skeleton, and basicranium. Humans of all ages exhibited broader nasopharynges than more prognathic non-human primates. Additionally, the human nasopharynx grows vertically taller and anteroposteriorly shorter in association with well known changes in external basicranial flexion during development. The most pronounced changes occur within the first year of life. These were accompanied by growth changes in the orientation and length of the cartilaginous Eustachian tube pathway, which was more vertically oriented and relatively longer among adults.

1.3.3 Chapter 4

Histological analysis was performed on a human, *Hylobates, Macaca, Ateles, Tarsius,* and *Otolemur* specimen. It was tested whether distribution of respiratory epithelium, goblet cells, mucous glands, and submucosal blood vessels were limited to contractile surfaces. These surfaces were divided relative to several gross anatomical structures: medial pterygoid plate, torus tubarius, pharyngeal constrictor muscle, soft palate, and nasopharyngeal roof (overlying the external basisphenoid bone). If histological parameters were estimable from osseous landmarks, they could be reconstructed on fossil crania. Further, histological patterns exhibited consistently among these specimens despite substantial morphological differences are likely applicable to fossil humans.

Results indicated that, contrary to previous studies, all of the nasopharyngeal surfaces were supplied by blood vessels, serous cells, and mucous cells despite surface contractility.
Despite variation among taxa in the expression of goblet cells or intraproprial mucous cells on some contractile surfaces (e.g., posterior or post-tubal portion of lateral wall), all of the surfaces appeared capable of contributing to postnasal air conditioning. These findings suggest that any differences in relative nasopharyngeal surface area among groups of primates may bear implications for air conditioning capacity.

1.3.4 Chapter 5

It was tested whether nasopharyngeal boundary morphology is influenced epigenetically by ambient temperature over development. Postmortem specimens representing two populations of *Macaca mulatta* living in a warm (California) and cold (Oregon) outdoor colony were examined via CT for such epigenetic differences. A geometric morphometrics analysis was performed using coordinate data collected from CT scans. Nasopharyngeal surface area and volume were also collected from CT slices. The presence of noticeable epigenetic influence over nasopharyngeal shape would require further investigation, especially within the context of inferring climatic adaptations from fossil crania.

Few shape differences were found among the nasopharynges and overall upper respiratory tracts of the two outdoor colonies. However, there was a pronounced difference in centroid size, indicating that the Oregon individuals had relatively larger upper respiratory tracts. Yet, this group also exhibited smaller body mass, suggesting that they had upper respiratory tracts that were larger relative to overall body size. The results indicate that development of upper respiratory tract size may be extremely responsive to local climate.
The osseous nasopharyngeal boundary morphology of fossil crania representing Neanderthals (Forbes’ Quarry 1, Monte Circeo 1, Saccopastore 1) and “mid-Pleistocene Homo” (Kabwe 1, Petralona 1, Steinheim 1, Atapuerca 5) were contrasted against modern human crania representing contemporary populations. Three-dimensional geometric morphometrics analysis and Procrustes-corrected univariate measures were used to test whether these specimens were within the modern human range or exceeded it. Two hypotheses were tested: 1) Neanderthals exhibited taller, narrower nasopharynges than modern humans as a climatic adaptation to increasing nasopharyngeal surface area-to-volume ratio; 2) Neanderthals exhibited longer, more horizontally oriented cartilaginous Eustachian tube tracts than adult humans.

It was found that Neanderthals do indeed exhibit tall, narrow nasopharynges but that Kabwe 1, a mid-Pleistocene hominin representing a tropical African population, also exhibited the same morphology. With respect to choanal dimensions, its choanae were relatively taller and narrower than any Neanderthal specimen. Hypothesis 1 was thus not supported by the data. However, Neanderthals were distinguishable by their possession of long cartilaginous Eustachian tube pathways, which plotted at the upper range of human variation and exceeded all mid-Pleistocene hominins. This was accompanied by vertical orientation of the choanae and cartilaginous Eustachian tube tract within range of human infants. Such morphology may have impacted susceptibility to middle ear disease and, ultimately, ecological marginalization by incoming modern humans. The unique suite of features exhibited by Neanderthals suggests species-level differences from modern humans.
2. Background on the nasopharynx

2.1 What is the nasopharynx?

2.1.1 Is it nose? Is it pharynx? Is it both?

The nasopharynx is a complicated region to define. Its boundaries incorporate elements of the nasal cavity, basicranium, and pharynx. Such an eclectic composition has led to debate over whether the nasopharynx is truly a component of a tripartite pharynx, which also includes an oro- and laryngopharynx. Etymologically, the pharynx (φάρυγξ in its original Greek) is a term for the upper alimentary tract. This definition of the pharynx has long persisted among classical anatomists working with non-human animal models that possess a clear separation of respiratory and digestive tracts (i.e. the two-tube aerodigestive tract). This definition has also been used in descriptions of human anatomy until the late 19th and early 20th centuries (e.g., Owen, 1866; Flower, 1872, 1891; Beddard, 1902; Kingsley, 1926) who considered the soft palate the roof of the "true pharynx" (Cave, 1967a [278]). However, some authors working with human cadavers (e.g., Winslow, 1732; Sappey, 1879) adopted the concept of a tripartite pharynx to describe the one-tube aerodigestive tract (which creates an oropharynx in humans, unlike most other mammals). However, the first explicit definition of the nasopharynx is likely the "Schlundkopf" (roughly translated as "pharynx-head" or pharyngocephalic junction) of Luschka (1868), in his seminal work "Der Schlundkopf des Menschen." The nasopharynx later gained
official recognition in the Basle Nomina Anatomica (1895) as the *pars nasalis pharyngis* (or nasal part of the pharynx).

The concept of a nasal portion of the pharynx is a more recent development, beginning in the 18\textsuperscript{th} century (Cave, 1967a). Classical anatomists such as Galen and Vesalius appear to have conceived of the pharynx as a purely alimentary tract as there is no explicit or indirect mention of a nasal portion of the pharynx within "*De Usu Partium*" or the "*Fabrica Corporis Humani*" (see section 2.4). The first explicit descriptions of tri-partite pharyngeal anatomy in the 18\textsuperscript{th} century coincide temporally with the emergence of detailed descriptions of human upper respiratory tract anatomy. Indeed, the nasopharynx, oropharynx, and laryngopharynx are most clearly visible in the human condition where a descended larynx creates these three grossly identifiable spaces.

Among the first to dispute directly the validity of the tripartite pharynx was Wood Jones (1940). He argued against this concept on the basis of function, citing that the nasopharynx is a respiratory pathway and not a digestive one. Lambert (1960) and Cave (1960, 1967a) later proposed similar arguments that were based on the functional separation of the nasopharynx from the digestive tract. They introduced the terms *postnasal space* and *epipharynx*, respectively.

More recent studies have subdivided the nasopharynx into two structural units using multiple lines of evidence. For example, Leela et al. (1974) define an anterior portion of the lateral nasopharyngeal wall anterior to the Eustachian tube orifice that overlies the medial pterygoid plate (i.e. lacks contractility), is covered in highly vascularized lymphoid tissue and ciliated, pseudostratified, columnar epithelium, and is innervated by the maxillary branch of the trigeminal nerve. In contrast, the post-tubal portion of the lateral wall contains stratified
squamous epithelium overlying the superior pharyngeal constrictor muscle (giving it greater contractility). The former is called the nasal portion while the latter is referred to as the true pharyngeal portion. Tobias (1991a) combines the argument of Leela et al. (1974) with embryological evidence to outline the composite nature of the nasopharynx. He is the most recent author to suggest that the term *pars nasalis pharyngis* of the internationally recognized Nomina Anatomica be modified to account for the structural and developmental complexity of the nasopharynx and its boundaries.

Despite the controversy that has long surrounded definition of the nasopharynx, this dissertation will utilize the term to describe the space bounded superiorly by epithelium overlying the basisphenoid bone, anteriorly by the choanae, posteriorly by epithelium overlying the superior pharyngeal constrictor muscle and its raphe, inferiorly by epithelium overlying the soft palate, and laterally by epithelium overlying the medial pterygoid plate, pharyngotympanic (Eustachian) tube cartilage, and anterior portions of the superior pharyngeal constrictor muscles. The hypotheses tested in this study are mostly focused on developmental and evolutionary change within the osseous and soft tissue components of the nasopharyngeal boundaries. Where necessary, distinction is made between the nasopharynx as a space and its boundaries, which ultimately determine the dimensions and physiology of the nasopharyngeal space.
Figure 2.1 A mid-sagittal section through a human head illustrating key structures within the nasopharynx and adjacent areas. Note: MP= Mucosa overlying the medial pterygoid plate; TT= Torus Tubarius; EO= Pharyngotympanic (Eustachian) tube pharyngeal orifice; PR= Pharyngeal recess (of Rosenmuller); SP= Soft palate; HP= Hard palate; IC= Inferior concha. Image provided courtesy of Jeffrey T. Laitman.
2.1.2 Functional and histological differences among nasopharyngeal components

The nasopharyngeal boundaries may be organized into several functional units. The anterior wall appears to be an extension of the posterior nasal cavity (indeed there is an indistinct separation in some animals; Negus, 1961). It overlies the medial pterygoid plates (a non-contractile surface) and exhibits the same pseudo-stratified ciliated epithelium with mucin-producing goblet cells distributed throughout much of the nasal cavity. The posterior wall and post-tubal portion of the lateral wall resemble the oropharynx in histology and gross structure as it overlies the superior pharyngeal constrictor muscle, the cranial-most component of the alimentary tract. These two portions are punctuated by the pharyngeal orifice of the cartilaginous pharyngotympanic (Eustachian) tube. A zone of intermediate epithelia has been described (von Ebner, 1902; Bryant, 1916; but see Leela and Kanagasuntheram, 1973) around the torus tubarius between zones of respiratory and stratified squamous epithelia. This zone contains agglomerations of both respiratory and squamous stratified epithelia with the predominant cell shape being cuboidal. However, the Eustachian tube lumen is lined with respiratory epithelium with goblet cells.

2.1.3 Relation to head segmentation: A composite evolutionary history

The anterior and posterior segments of the nasopharyngeal walls arise from separate developmental precursors with different evolutionary histories. A review of head segmentation is thus warranted to place these structures within their proper contexts.
The concept of head segmentation was first described in the unpublished letters of Goethe, but was later elaborated in several formally published works (Oken, 1807; Geoffroy St. Hillaire, 1818; Goethe, 1824). These early authors held that the entirety of the axial skeleton and its soft tissues, including the head, grow from iterative segments. The skull was believed to have formed from modified vertebra and, as described by Owen (1848), was derived from as many as four separate cranial vertebrae. Huxley (1858) later challenged this paradigm, citing that only the anterior two thirds of the skull grow from the notochord (which is the main embryologic progenitor of the vertebral column) and that several basicranial cartilages remain unsegmented and continuous throughout vertebrate growth (reviewed by Northcutt, 2008).

Later studies of head segmentation no longer centered on cranial vertebrae, but rather on series of somites and pharyngeal arches. Goodrich (1918) argued that the three anterior-most somites contribute to the pre-otic skull (mostly the facial skeleton) while the posterior four are successively associated with developing branchial arches and cranial nerves. This work is important in contributing to the modern concept of skull segmentation over gastrulation and distinguishing between the pre-otic and peri-otic divisions. These roughly correspond to the division observable in the nasopharyngeal wall between the anterior and posterior portions which are distinct in anatomy, histology, and development (see above).

Pre- and post-otic portions of the vertebrate skull may have separate evolutionary origins. As described by Gans and Northcutt (1983), the pre-otic segment was derived from a series of sensory adaptations for active predation, developing exclusively from neural crest cells while ectodermal placodes contribute to the development of the sensory organs and some nerves. The vertebrate skull was thus an ectodermal addition to the basic protochordate body plan (with the notochord progressing only as anterior as the basicranial phenestra). The distinct origins of the
elements composing the anterior and posterior nasopharyngeal walls may thus be as old as the appearance of the first vertebrates.

The developmental evidence cited by Gans and Northcutt (1983) were corroborated by Couly et al. (1993) who mapped the fates of neural crest, somitic, and mesodermal cells in the cranial development of the chicken. Tissue grafts were taken from quail embryos and implanted into chicken embryos between E8 and E12 (the 8th and 12th days of embryological growth, respectively). It was determined that the splanchnocranium, mandible, frontal bone, and parietal bones were all derived from neural crest cells. The sphenoid was divided into an anterior neural crest-derived half and a posterior mesoderm-derived half. The otic capsule was shown to contain elements from all three sources. These results favor the “new head” hypothesis of Gans and Northcutt (1983) by confirming the neural crest origin of the pre-chordal skeleton and by describing the separate developmental trajectories of areas corresponding to the anterior and posterior nasopharyngeal walls.

Further evolutionary depth is given to the separation of the nasopharyngeal wall components in a synthesis by Baker and Bronner-Fraser (1997). They argue that the homologues of vertebrate neural crest cells and ectodermal placodes may be present in non-vertebrate chordates such as the cephalochordates. These possible homologues are ectodermally derived and tend to migrate over development. It is also argued that homologues for the neural crest and placodes may be found in the neural cords of enterpneust worms, which are considered good models for the condition of the last common ancestor of all chordates.

More recent work focuses on the role of homeobox genes such as CCD1 (Soma et al., 2006) and transcription regulators in the growth of pharyngeal structures. McCauley and Bronner-Fraser (2006) explore the function of the SoxE transcription regulators in lampreys.
When SoxE1 was knocked down (i.e. rendered non-functional), the expression of SoxE2 was also compromised. This led to the malformation of the branchial arches posterior to the mandibular arch as neural crest-derived chondrocytes were lost. In the normal individual, these genes are expressed in the neural fold and in later migrating neural crest cells. It was further shown that the neural crest cells that contribute to ectomesenchymal cells are derived from a location medial to the mesoderm as in gnathostomes. SoxE3 was expressed later in the mesenchyme of all branchial arches. Thus the jawless fish and gnathostomes share the homology of partitioned SoxE function and a common directionality of neural crest migration. The evolution of the pharynx and mandible are argued to have occurred with the duplication of SoxE genes.

2.1.4 Developmental origins of the nasopharyngeal boundaries

To discuss the central importance of the nasopharynx, one must understand its composite embryology. As most of our understanding of this region comes from studies on humans, the following discussion will be based on human embryology.

The nasopharynx is related to the developing nasal cavity, palate, branchial arches and pouches, gut tube and oral cavity, and the developing chondrocranium. At the beginning of the fourth week of human embryological development, the facial primordia have appeared from migrating neural crest cells (from dorsal ectoderm). There is a frontonasal prominence, a pair of maxillary prominences, and a pair of mandibular prominences. The maxillary and mandibular prominences are derived from the first pair of branchial arches. Between the progenitors of the maxillae and mandible, the oropharyngeal membrane is present to separate the stomadeum (i.e. stomodeum).
early oral cavity) from the foregut of the primordial gut tube. This membrane ruptures after the fourth week while the nasal placodes, progenitors of the nasal cavity, first appear as ovular thickenings of surface ectoderm on the inferolateral portions of the frontonasal prominence (Moore and Persaud, 2003).

Medial and lateral nasal prominences develop around each nasal placode, which grows into a nasal pit and gradually gives way to a primordial nasal sac. These are separated from the oral cavity by the oronasal membrane. Early in the sixth week, the primary palate is formed by the merging of the medial nasal prominences to the maxillary prominences while the oronasal membrane ruptures to form the early choanae (creating a communication between the nasal and oral cavities). The secondary palate (which is the progenitor of the hard and soft palate) begins its formation early in the sixth week from mesenchymal projections called the lateral palatine processes. They extend between the inferoposterior borders of the two maxillary prominences. The lateral palatine processes fuse during the eighth week, starting anteriorly and ending at the posterior limit of the newly unified palate. Simultaneously, fusion of the nasal septum to the secondary palate (via migration of neural crest cells from the frontonasal prominence) pushes the choanae posteriorly to a position between the nasal cavity and pharynx. It is with the fusion of the secondary palate that the soft palate develops to separate the newly formed nasopharynx from the oral cavity and oropharynx inferiorly (Moore and Persaud, 2003).

The soft palate is of composite origin as the tensor veli palatini develops from the first branchial arch and the levator veli palatini is derived from the third (along with the superior pharyngeal constrictor muscles, which act as the posterior wall of the nasopharynx). The tensor veli palatini is the first of the uvular muscles to become a distinguishable unit from the mesenchymal mass of the secondary palate. It is followed in order by the levator veli palatini
muscle and the musculus uvulae. After roughly 16 to 17 weeks of gestation, all of the uvular muscular components have been developed (Cohen et al, 1993). During the development of the nasal cavity and soft palate, the sphenoid bone (which is the nasopharyngeal roof) develops from hypophyseal cartilages, forming around the cranial end of the notochord and the developing pituitary gland. It is between the sixth and twelfth weeks that the cartilaginous precursor of the sphenoid bone takes shape (Moore and Persaud, 2003; Heneger and Oas, 1980).

The walls of the nasopharynx are of composite origin, comprising both nasal and pharyngeal components. Anteriorly, the medial pterygoid plates develop from two separate centers of ossification, one dorsally located and the other more ventral. The former is formed from neural crest cells migrating from the maxillary and nasal prominences. The ventral portion, however, is partially formed from endochondral ossification. It was first shown by Fawcett (1905) to ossify well before any other portion of sphenoid bone around the ninth or tenth week. He was the first to describe the appearance of the medial pterygoid hamulus around the third month and that this structure is derived from a cartilaginous precursor.

The cartilaginous Eustachian tube and its orifice are derived from the first pharyngeal pouch (Moore and Persaud, 2003). The pharyngeal recess (i.e., the fossa of Rosenmüller) marks the separation of the first and second pharyngeal pouches. Over the course of its embryological development, the second pharyngeal pouch and adjacent third branchial arch elements are gradually pushed out of this region by the development of the tubotympanic recess into the Eustachian tube (Kanagasuntheram, 1967). This process also creates the tympanic membrane from the only persisting branchial membrane, which closes off the first branchial pouch during much of gastrulation.
2.2. Comparative Anatomy: A survey of air-breathing vertebrates

2.2.1 Choanae and the origins of the tetrapods: Assessing the lungfish as a model for primitive choanal morphology

The following section includes two postmortem specimens examined at the Division of Ichthyology of the American Museum of Natural History. One of the specimens is a coelacanth (*Latimeria chalumnae*; catalog #36941) and the other is an Australian lungfish (*Neoceratodus forsteri*; catalog #55451). Dry crania were also examined at the laboratory of the Center for Anatomy and Functional Morphology, Icahn School of Medicine at Mount Sinai. These included: 1) a bullfrog (*Rana catesbeiana*); 2) a sea turtle (*Lepidochelys* sp.); 3) a red kangaroo (*Macropus rufus*); 4) and a whitetail deer (*Odocoileus virginianus*).

Fossil evidence suggests that the presence of choanae may have been among the earliest occurring synapomorphies characterizing the tetrapods (Janvier, 2004). Panchen and Smithson (1987) gave the first formal anatomical definition of ancestral tetrapodamorph choanae as being constrained laterally by the premaxilla and/or maxilla and medially by the vomer. Such morphology is identifiable among the Osteolepiformes, an early group of fossil lobe-finned fish likely related to stem tetrapods. Possession of choanae distinguished them from other groups of lobe-finned fishes, which possess a pair of anterior and posterior nostrils on the external snout such as *Latimeria*, arguably the most primitive extant lobe-finned fish (Figures 2.1, 2.2, 2.3). Despite sharing synapomorphic choanal morphology with terrestrial tetrapods, Osteolepiformes predated the earliest amphibians by approximately 30 million years (Janvier, 2004).
Figure 2.2 Above is a coelacanth fish (*Latimeria chalumnae*), which exhibits shared derived aspects of limb morphology with basal tetrapods. This individual is a pup with the yolk sac still attached. Note that coelacanths are oviparous, birthing live offspring. Photograph of specimen catalog # 36941, Group 7) from the Division of Ichthyology at the American Museum of Natural History, Collection of Fishes.
**Figure 2.3** The above coelacanth does not possess choanae, instead primitively bearing its anterior and posterior nares on the external rostrum. Photograph of specimen catalog # 36941, Group 7) from the Division of Ichthyology at the American Museum of Natural History, Collection of Fishes.
Figure 2.4 The above coelacanth exhibits no choanal opening in the oral cavity. Photograph of specimen (catalog # 36941, Group 7) from the Division of Ichthyology at the American Museum of Natural History, Collection of Fishes.

Von Bischoff (1840) first described the presence of choanae in the lungfishes and grouped them with amphibians. They were considered excellent models for the respiratory morphology of early tetrapods as they appeared intermediate in morphology between the amphibians and fishes. Some lungfishes exhibit choanal morphology similar to that seen in the primitive tetrapod condition, as spaces that communicate between the nasal sac and oral cavity (Figure 2.5). However, a nasopharynx *sensu stricto* may not be found in lungfish or ancestral tetrapods including lobe-finned fishes as no distinct airway is present. The communicative channel between the anterior and posterior nares remains, as in most fishes, an olfactory pathway lined with specialized epithelia (see the description by Derivot, 1984). These are used specifically for olfaction in aquatic environments and are closed off during air swallowing by
specialized valves (Derivot, 1984). As can be inferred from modern lungfish, air breathing animals that lack a means of nasal respiration may engage in an activity known as air swallowing (see description and review by Smith, 1931) in which air is passed to the lungs through the mouth. Given the antiquity of the choanae and their function in lungfish, it appears that these apertures may not have evolved as respiratory pathways.

The phylogenetic polarity of the lungfish choanae has long been debated (Zhu and Ahlberg, 2004). The Choanata was erected by Save-Soderbergh (1933) as a taxonomic group including all tetrapods, lungfishes, and lobe-finned fishes that possessed choanae or choana-like apertures which communicate with the palate. Similarly, Romer (1937) proposed the inclusion of all choanate fishes into the taxon Choanichthyes. Rosen et al. (1981) were some of the most recent authors to suggest that lungfish choanae are homologous to those of tetrapods. Yet, despite the presence of gross similarities, evidence from both the fossil record and cladistic analysis suggest that the ancestors of the modern lungfish may have homoplastically evolved choanae. Chang (1995) first described *Diabolepis*, an extinct lungfish that exhibits the primitive piscine morphology of both an anterior and posterior set of nostrils that did not communicate with the oral cavity. Additionally, a primitive piscine configuration of the maxillary nerve occurs in which it runs medial to the posterior nasal aperture among extant and extinct representatives of the lungfish. It has been displaced even further medially from its ancestral position by the migration of the posterior nostril into the oral cavity over lungfish evolution (Janvier, 2004).

Zhu and Ahlberg (2004) were the first to describe a genus (*Kennichthys*) that exhibited a morphology intermediate between that of fishes and tetrapods, in which the choanae were present at the junction of the maxilla and premaxilla. It evolved as a displaced posterior external nostril which was redirected ventrally from its primitive position on the snout to the lateral edge
Figure 2.5 Above is an Australian lungfish (*Neoceratodus forsteri*) exposing its oral cavity. Note that the choanae open ventrally into the hard palate. This is not a respiratory airway as the lungfish passes inspiratory air directly through its oral cavity. Rather, the nasal cavity houses specialized olfactory epithelia that function in aquatic environments. Photograph of specimen catalog # 55451, Group 7) from the Division of Ichthyology at the American Museum of Natural History, Collection of Fishes.
of the maxilla. These choanae are more laterally located than those of early tetrapods but clearly
differ from the primitive piscine morphology. Additionally, the maxillary nerve is located lateral
to the choanae, a synapomorphy with tetrapods and their osteolepiform relatives (Janvier, 2004).
The evidence suggests that the anatomical configuration of the tetrapod choanae (arguably the
earliest aspect of the nasopharyngeal boundaries to evolve) may have resembled *Kennichthys*,
first evolving from the standard posterior nostril bounding the piscine nasal sac and later
migrating to a position on the palate. The palatine choanae of early tetrapods also appears similar
to the condition seen during human embryologic growth, potentially serving as a resume of
evolutionary history (as per Crelin, 1983).

2.2.2 Nasopharyngeal anatomy of amphibians

The earliest land tetrapods were probably amphibians (Long and Gordon, 2004; Clack, 2006).
Modern amphibians are extremely specialized relative to the first land tetrapods, which
possessed dermal plates overlying the skull and lacked occipital condyles, among other primitive
traits expressed in common with their piscine ancestors (see Clack, 2006). Nonetheless, they
maintained choanae that communicate between the nasal cavity and oral cavity, which now
allowed them to pass air through the external nares and nasal cavity into the oral cavity via the
inferiorly-oriented choanae (Figure 2.6). Once air reached the oral cavity, they may used a
buccal pump system similar to modern anurans in which the inspired air is pumped downward
toward a nearly intraoral glottis by specialized pharyngeal muscles. There is thus no
nasopharyngeal airway among amphibians as they lack clear postnasal separation between the
airway and alimentary tract. The nasal cavity itself is an anteroposteriorly closed sac bounded by
the external nares superiorly and the choanae inferiorly in most amphibians (see Parsons, 1967 for a good review of nasal morphology among extant amphibians). The only known tetrapod to possess completely occluded choanae as part of its adult morphology is *Atretochoana eiselti*, a large lungless salamander from the cold, mountain habitats of the Andean highlands (Wilkinson and Nussbaum, 1997). It conducts respiration solely through specialized epithelia over its skin, much like other members of the Plethodontidae (the family of lungless salamanders).

---

**Figure 2.6 a)** Frontal view of a bullfrog (*Rana catesbeiana*) with the right anterior naris indicated by a black arrow.  **b)** Basal view of the same specimen with the right choana indicated by a black arrow.

Aside from a varying reliance on transcutaneous gas exchange, most amphibians breathe via a buccopharyngeal pump mechanism in which air is passed from the nostrils to the nasal cavity, through the choanae, and into the oral cavity where powerful muscles redirect its flow down the respiratory pathway (see classic description by de Jongh and Gans, 1969). During inspiration, specialized valves over the external nares are opened and the oral floor is depressed, creating negative pressure to draw air through the nasal cavity and into the oral cavity via the
ventrally-oriented choanae. This air rests in a depression on the floor of the buccopharyngeal cavity, while spent air from the lungs is released over it at high velocity, exiting through the external nares. Next, the valves of the external nares narrow (as shown by Jones, 1982) and the buccopharyngeal floor is raised, pushing the previously inspired air into the glottis, which opens directly into the lungs (de Jongh and Gans, 1969). An apneic phase ensues before the next cycle is commenced.

2.2.2.1 Evolutionary origins of the Eustachian tube

Among the features derived in the first amphibians for life on land were the tympanic membrane and cavity that allowed for improved audition. Clack (2002) argues that there were at least four independent evolutionary origins of the tympanic cavity among tetrapods. Others such as Laurin and Reisz (1997) propose even more. Among most early amphibian fossil crania, there exists an opening in the spiracular chamber of the temporal bone (which houses a portion of the internal carotid artery) that has been reconstructed as admitting a pharyngotympanic tube that communicated with the posterosuperior portion of the oral cavity (Clack, 1998). This configuration may still be observed among modern amphibians and is necessary for maintaining equilibrium of pressure between the tympanic cavity and external environment. The typical anuran Eustachian tube is broad and remains patent, its orifice posteriorly abutting a ventrally-oriented pterygoid element (Narins et al., 1988; Purgue, 1997).

Being a diverse taxon, a fair amount of amphibian species has evolved derived or specialized middle ear and Eustachian tube anatomy. Narins et al. (2001) describe a Central African frog species that uses the broad, stiff Eustachian tube to resonate vocalizations from the
larynx through to the middle ear and tympanic membrane, producing grossly observable vibrations. Other amphibians have lost the middle ear cavity and thus the Eustachian tube exists as small indentation on the posterolateral aspect of the oral cavity roof; this is likely a paedomorphic condition as it characterizes most anurans upon completion of metamorphosis from the tadpole stage of development (Vorobyeva and Smirnov, 1987). The Eustachian tube in its earliest expression was not related to the nasal cavity but rather opened into the alimentary tract. Its use in delimiting the anterior nasal and posterior pharyngeal portions of the nasopharyngeal boundaries of mammals (including humans) thus appear warranted from an additional line of evolutionary evidence.

2.2.3 Nasopharyngeal anatomy of reptiles

Among most reptiles, as in the amphibians, there is no nasopharyngeal space sensu stricto. Rather, the choanae end in the oral cavity (Figure 2.7), opening between the parasphenoid wings and epipterygoid bone at the roof of the alimentary tract (Hopson, 1966). The pterygoid plates are ventrally oriented and located far from the choanae, which lay anteriorly at the junction of the primary and secondary palate derivatives between the premaxilla and maxilla. As per Fuchs' (1908) classic description of reptilian nasal embryology, the nasopharyngal duct is defined as the posterior ending of a space overlying a well developed secondary palate as seen in the Crocodilia and mammals but not in most extant reptiles, which lack this structure. Parsons (1959), however, used the term more broadly to describe the area of the cavum nasi leading into the choanae in all reptiles.
Figure 2.7 a) Frontal view of a sea turtle (*Lepidochelys* sp.) with the enlarged choanal opening visible through the anterior naris (arrow on left choanal communication). b) A basal view of the same specimen illustrating the position of the choanae opening into the oral cavity (black arrow indicating the position of the left choana).

2.2.4 The evolution of the pterygoid complex and the rise of mammals

Among crossopterygian fishes, amphibians, and reptiles, the pterygoid elements partially compose the posterior portion of the palate and are tooth-bearing in some taxa. Primitively, the tetrapod pterygoid complex is composed of an ectopterygoid ventrally abutting the horizontal plates of the palatine bones (comprising the posterior-most extent of the alveolus), an epipterygoid articulating with the basisphenoid centrally and the wing of the parasphenoid on each side, and a main pterygoid element that composes the posterior half of the palate. This
The pseudo-temporalis is a single muscle in reptiles which includes elements of the medial and lateral pterygoid muscles, temporalis, and masseter that have become differentiated in mammals (see the classic description by Brock, 1939). This muscle has a portion of its origin on the pterygoid and ectopterygoid elements and inserts onto the mandibular ramus at the postdentary bones. Parrington and Westoll (1940) argued that, with the movement of the quadrate, articular, and angular elements superior and deep into the middle ear (believed to be the evolutionary precursors of the incus, malleus, and ectotympanic bones, respectively), the pterygoid complex has shifted position to become the lateral border of the now posteriorly oriented choanae. In their classic work, they use descriptions of transitional cynodonts and other mammal-like reptile fossils to argue cogently that the evolution of the anterior nasopharyngeal boundaries of mammals was part of a complex restructuring that affected several functional units within the middle ear, masticatory apparatus, and upper respiratory tract. Such changes likely accommodated greater auditory acuity, brain size, and thermoregulation as can be deduced by the longer secondary palate and, by extension, nasal cavity and conchae. Given the above evidence, it appears likely that the route of the cartilaginous Eustachian tube also underwent morphologic change with the shifting position of the medial pterygoid plate.

In gross appearance, the mammalian medial pterygoid plate appears simpler than the tri-partite pterygoid of other tetrapods. However, Fawcett (1905) first showed that this bone develops from an intramembranously ossifying site superiorly and an inferior site of intramembranous growth with an embedded chondral growth center (the primordium of the pterygoid hamulus) in human embryos. Gaupp (1908) soon after noted the persistence of two
separate pterygoid elements in *Echidna* development, which he termed the "sauger-pterygoid" or mammalian pterygoid superiorly and the "echidna-pterygoid" inferiorly. He argued that the former evolved from a portion of the parasphenoid ala and that the latter was derived from the reptilian pterygoid element. This assessment sparked a nearly century-long debate on the homology of the mammalian pterygoid complex. The opposing (and later consensus) view was presented by Broom (1914) who examined crania of *Ornithorhynchus* and several histological growth series of Edentate marsupials that exhibited separate superior and inferior medial pterygoid growth centers late into fetal development. After assessing the morphology of these extant monotremes and marsupials, he concluded that the articular relationships of the echidna pterygoid to the palatine bone and the mammalian pterygoid to the alisphenoid and basisphenoid were similar to their purported homologues among some cynodonts (which maintain separate pterygoid and ectopterygoid elements). It was thus concluded that the superior mammalian pterygoid element had evolved from the reptilian pterygoid and that the inferior echidna pterygoid likely evolved from the reptilian ectopterygoid. This line of paleontological evidence has also been supported by Parrington and Westoll (1940) who discussed the evolution of the pterygoid complex in osteolepiform fishes, early labyrinthodont amphibians, and mammal-like reptiles such as the cynodonts and other early synapsids.

Presley and Steel (1978) provide the most recent evidence supporting Broom's (1914) homology. They studied prenatal developmental series from a range of monotremes, marsupials, and eutherian mammals. All showed a clear distinction between pterygoid and ectopterygoid elements during earlier stages of development though only some taxa continued to express this bi-partite morphology after birth (*Ornithorhynchus, Tachyglossus*). All other taxa exhibited
fusion either before \((\text{Talpa, Erinaceus, Sorex, Meriones, Rattus})\) or after \((\text{Trichosurus, Didelphys, Roussettus, Hipposideros, Tadarida})\) ossification of each element.

Two lines of evidence are proposed by Presley and Steel (1978) to challenge the homology scheme of Gaupp (1908) that the dorsal and ventral pterygoid elements respectively derive from the reptilian parasphenoid and pterygoid. Presley and Steel (1978) show that both pterygoid elements develop from the first branchial arch via the upper jaw anlage, disassociating the dorsal pterygoid growth center from the interchondrally derived sphenoid bone. Further, they noted that among \text{Didelphis}, the one sampled mammalian taxon that preserves a primitive reptilian parasphenoid, the dorsal pterygoid element grows in a separate anatomical plane.

Richman et al. (2006) question the homology of the ventral pterygoid element with the reptilian ectopterygoid as the former ossifies endochondrally to become the pterygoid hamulus while the other undergoes intramembranous ossification. Yet this criticism was already addressed by de Beer (1929), Eloff (1950), and Presley and Steel (1978) who all argued that the hamular cartilage arises as a secondary structure within the mesenchyme of the ectopterygoid element in association with the tensor veli palatini (TVP) muscle. It acts as a sling for this muscle, redirecting conjoined contributions from its origins on the sphenoid or temporal bone and cartilaginous Eustachian tube (CET) despite considerable variation in bony attachment and the extent of its relationship with the CET among living mammals (e.g. Gannon et al., 1994; Maier, 2013). Over human embryologic growth, the tensor veli palatini muscle relates to the anlage of the pterygoid hamulus soon after differentiating from a common blastema with the medial pterygoid muscle during the seventh week (de la Cuadra Blanco et al., 2011). The appearance of a cartilaginous pterygoid hamulus thus appears related to the specialized function of the tensor veli palatini muscle.
2.2.5 Reconstructing the evolution of the mammalian nasopharynx and its boundaries

The evolution of the mammalian nasopharynx can arguably be tied to the appearance of the secondary palate present among the earliest Cynodonts. It has been argued that a transversal ligament spanning between the tubercles of the vomer and the vomerine processes of the maxillae on either side ventrally covered the choanae to create a ligamentous precursor of the secondary palate (Broom, 1936; Crompton, 1955; Brink, 1957; Tatarinov, 1963; Barghusen, 1986; Maier et al., 1996). Barghusen (1986) and Maier et al. (1996) argue that the development of this palatal precursor within the common ancestors of therocephalians and cynodonts was tied to the development of bony choanal crests to anchor fleshy choanal folds capable of separating the nasal cavity from the oral cavity. These choanal crests were believed to be the precursor of the osseous portion of the secondary palate (Barghusen, 1986). Maier et al. (1996) suggest that this was an adaptation to carnivory which allowed for the continued patency of the airway during deglutition of large meat boluses, which could not be reduced via mastication as no shearing or occluding postcanine dentition had yet evolved among early therocephalians and cynodonts.

It is suggested by Maier et al. (1996) that the velum of mammals is derived from an unossified remnant of the choanal folds and that the tensor veli palatini and levator veli palatini muscles that comprise the majority of its bulk have migrated from elsewhere. They argue that the tensor veli palatini (along with the tensor tympani, which are both innervated by the trigeminal nerve) are derived from a ventral expansion of the pterygoid muscle with its origin on a fossa bounding the posterior border of the choanal crest. This arrangement would necessitate the evolution of a neomorphic pterygoid hamulus cartilage not derived from the intamembranously
ossifying ectopterygoid moiety (thus agreeing with Eloff, 1950 and Presley and Steel, 1978). Maier et al. (1996) further suggest that the tensor veli palatini muscle is a derived trait among eutherians and marsupials as it is absent among monotremes (Eschweiler, 1899; Schulman, 1906) and diminutive among marsupials, potentially necessitating assistance from medial portions of the superior pharyngeal constrictor muscle to open the Eustachian tube orifice. Indeed muscular contributions from both the palatopharyngeus and lateral portions of the superior constrictor muscle have been observed in *Monodelphis* to extend to the torus tubarius (Maier et al., 1996). Conversely, Barghusen (1986) argued that the tensor veli palatini muscle was derived from a posterior portion of the medial pterygoid muscle as could be deduced from the morphology of cynodonts such as *Thrinaxodon*. Maier et al. (1996) disagree with his assessment as no explanation is provided on how the tensor veli palatini could have had its insertion redirected onto the velum from its original position on the postdentary mandible.

The levator veli palatini muscle, like the tensor veli palatini, may have first appeared among the most recent common ancestor of Eutherian mammals. Among both monotremes and marsupials, this muscle is undifferentiated from the palatopharyngeus (Edgeworth, 1935), and is innervated by the vagus and glossopharyngeal nerves via the pharyngeal plexus. It thus appears that the earliest possible expression of a distinct levator muscle of the velum was tied to a larger evolutionary trend toward differentiation of the pharyngeal musculature. One may further conclude that the velum, the floor of the eutherian nasopharynx, has composite evolutionary origins related to the secondary palate, pterygoid muscle, and pharyngeal musculature.

The highly specialized morphology of mammals may be defined by the presence of an elongated hard palate and velum and well defined pharyngeal constrictor musculature. The former trait likely evolved alongside a differentiated nasal cavity containing an olfactory recess.
(a probable adaptation for macrosmia, Smith and Rosie, 2006) separated from a nasopharyngeal duct inferiorly by a transverse ethmoidal lamina. Additionally, this specialized morphology may have evolved to allow more efficient suckling among neonates (Maier et al., 1996). Proper suckling is mediated by the induction of negative pressure in the oral cavity, which must be completely separated from the nasal cavity. Such separation is normally achieved via the passive action of the hard palate and active contraction of the velar and pharyngeal constrictor muscles, which can separate the nasopharynx from communication with the alimentary tract. The functional importance of this mechanism is demonstrated in cases of cleft palate infants who exhibit insufficient separation of the oral and nasal cavities, thus rendering normal suckling difficult (Choi et al., 1991; Reid et al., 2007).

Despite the presence of choanal crests and a secondary palate among therodonts, the choanae are ventrally oriented and the pterygoid plates do not appear to border the choanae laterally. It is not until the Triassic period among early anomodont mammals such as the dicynodonts that truly posteriorly-oriented choanae are observable. In Kombuisia, the choanae take on an elongated, funnel shaped appearance with the pterygoid element at the caudal end of a long process of the palatine bone (see figures within Frobisch, 2007). The choanae among early anomodonts are primarily bounded by the palatine bones as in the therodonts, however, the position of the pterygoid element in the former group may signify a transition to the choanal morphology of extant mammals (see Figure 2.8).
**Figure 2.8** Basal views of a red kangaroo (*Macropus rufus*; a) and whitetail deer (*Odocoileus virginianus*; b). Note the location of the choanae is posterior and superior to the hard palate, even among distantly related mammals. This creates a separation of oral and nasal cavities not present among reptiles.
2.2.6. The nasopharyngeal morphology of primates: Macrosmats versus microsmats

Among mammals, primates are a decidedly derived order in many aspects of cranial and postcranial anatomy. This may be reflected in the century-old debate on their proper classification and the traits that distinguish them from other euarchontans such as *Tupaia*. However, within the order Primates, strepsirhines exhibit primitive morphology in aspects of the face and upper respiratory tract related to olfactory acuity. The nasopharyngeal anatomy of the primates may thus be divided between the macrosmatic strepsirhines and the microsmatic haplorhines (Turner, 1891; Cave 1967b, 1973; Cartmill, 1970; Maier, 1983; Smith and Rossie, 2006). Though there is currently no reliable histological criterion for distinguishing macrosmatic primates from microsmatic ones (Smith et al., 2004, Smith and Bhatnagar, 2004), certain skeletal features of the nasal cavity tend to distinguish these two groups. Macrosmats often possess a greater number of ethmoturbinals that are horizontally arrayed and separated from respiratory airflow by a posterior transverse lamina or lamina transversalis posterior (Cave, 1973; Smith and Rossie, 2006) otherwise known as the “schlussplatte” of Zuckerkandl (1887). At the end of this recess lies the vertically oriented cribriform plate. A “nasopharyngeal duct” (Smith et al., 2007) is created in the space between the posterior transverse lamina and the hard palate, which ends in a vertically reduced (compared to haplorhines) choanal opening. The medial pterygoid plates usually take on an elongated, funnel-shaped appearance as in other non-primate mammals, which may be a structural consequence of a long, narrow rostrum and nasopharyngeal duct. These features are shared among most eutherians and suggest that both the earliest representatives of the order Primates and extant strepsirhines possess greater olfactory acuity than most haplorhines. However, some haplorhines have been shown to exhibit a high degree of olfactory
acuity, necessitating caution when inferring sensorial abilities from gross anatomy (Cartmill, 1970; Smith and Rossie, 2006).
Figure 2.9 Microsmatic primates exhibit choanae that are relatively tall and narrow, leading directly into the posterior nasal cavity (as in *Cercocebus torquatus*; Top image). Macrosmatic primates instead exhibit choanae that are vertically short and lead into a nasoharyngeal duct that is superiorly bounded by a transverse lamina (as in *Daubentonia madagascarensis*; Bottom image).
Relative to most generally macrosmatic strepsirhines, microsmatic haplorhines are characterized by a shorter hard palate and nasal cavity, a reduced lamina transversalis posterior, a less defined olfactory recess, fewer ethmoturbinals (usually two) and choanal apertures not bounded anteriorly by a nasopharyngeal duct (Maier, 1983; Smith and Rossie, 2006). Accompanying relative foreshortening of the rostrum and nasal cavity, the medial pterygoid plates reach laterally at a relatively obtuse angle with the posterior hard palate. The choanae take on a tall, narrow appearance and are variably angled anteroinferiorly (a condition approached in *Eulemur*, though its choanae are wider and more square-shaped than those of most haplorhines). This anatomical configuration may influence airflow dynamics by redirecting inspiratory air inferiorly, thus promoting laminar movement to the larynx by meeting the posterior pharyngeal wall at a more oblique angle.

Accompanying these traits is orbital convergence, frontation, and retraction under the forebrain, which characterizes anthropoids relative to other primates (discounting the derived orbital morphology of *Tarsius*). Ross and Ravosa (1993) argue that orbital convergence among haplorhines renders facial, nasal, orbital, and anterior cerebral morphology part of a single functional unit so that, when any of these structures undergoes morphologic change, they influence basicranial flexion to a greater degree than among the strepsirhines. They measured internal basicranial flexion (angle made at the intersection of the lines connecting the planum sphenoidale with the occipital clivus) from lateral plain-film radiographs of a diverse sample of non-human haplorine and strepsirhine primate crania. It was found that, among haplorhines, basicranial flexion was positively and significantly (p<0.05) correlated with angle of facial kyphosis (the angle made between the intersection of the lines connecting the palatal plane and the occipital clivus) and orbital axis orientation (angle made at the intersection of lines passing
through the midpoint of the orbital cavity and the occipital clivus). It was also shown to be negatively correlated with encephalization (the cube root of endocranial volume relative to length of the basicranial axis). The dimensions of the haplorhine nasopharynx, which is bordered superiorly by the sphenoid body between the spheno-ethmoid and spheno-occipital synchondroses, thus appear related to morphologic change in several functional anatomic units. This may account for the marked inferior curvature observed by Schreider and Raabe (1981) in the *Macaca* nasopharynx relative to that of *Rattus* and *Canis*, two macrosmatic mammalian genera.

2.2.7. The nasopharyngeal anatomy of humans

Several large-scale evolutionary changes have occurred in the human upper respiratory tract that distinguishes it from that of other haplorhines. In gross appearance, the adult human nasopharynx is anteroposteriorly and superoinferiorly reduced and mediolaterally wide. The velum does not interlock with an intranarial epiglottis as in most other mammals, thus the nasopharynx is no longer the sole passageway for inspired air to reach the lungs (see Figure 2.9). Evolution of a one-tube aerodigestive tract with a crossing alimentary tract and airway is likely related to changes in external basicranial flexion (Laitman et al., 1979; Laitman et al., 1982) which have been argued to share a functional relationship with laryngeal position (e.g. Laitman and Crelin, 1976; Laitman et al., 1979, 1982; Reidenberg and Laitman, 1991). This aspect of basicranial anatomy may also be functionally related to nasopharyngeal dimensions as anteroinferior inclination of the sphenoid body and basi-occipital element relative to each other could contribute to increasing nasopharyngeal height. Adult modern human nasopharynges are
tall and anteroposteriorly shallow, abutting a basicranial axis that is greatly flexed relative to infants.

**Figure 2.10** Most mammals such as Macaca (left) exhibit an epiglottis (E) that interlocks with the soft palate (S), lying within the nasopharynx (delimited by the blue rectangle). Adult *Homo sapiens* exhibits a low laryngeal position so that the epiglottis is far inferior to the soft palate and nasopharynx.

External basicranial flexion measured with exocranial landmarks is distinct from internal basicranial flexion, measured with endocranial landmarks. The latter has long been examined for its potential relationship with brain size (e.g. Biegert, 1963; Gould, 1977; Ross and Ravosa, 1993, Ross and Henneberg, 1995; Strait, 1999) or lack thereof (Jeffery and Spoor, 2004) as well as cranial vault shape (Huxley, 1867; Lieberman et al., 2000) and facial morphology (e.g. Virchow, 1857; Ranke, 1892; Ashton, 1957; Enlow, 1975; Ross and Ravosa, 1993; May and
Sheffer, 1999). Few have considered the potential relationships between external and internal basicranial flexion (as commented by Lieberman et al. 2000). These two aspects of basicranial morphology have been shown to distinguish modern human adults from other non-human primates (e.g. Ross and Henneberg, 1995; Spoor, 1997; Strait, 1999) but may be related to different aspects of the facial skeleton and cranial vault.

Given the central location of the nasopharynx, morphologic change of its boundaries may be functionally related to evolutionary change in other portions of the human upper respiratory tract. Trends of facial orthognathy and kyphosis and neurocranial globularity all appear related to changes in basicranial flexion (Bookstein et al., 2003), which likely occurred alongside anteroposterior reduction of the nasopharyngeal space. Spoor et al. (1999) suggest that, among modern humans, the midline length of the anterior sphenoid bone between sella and the posterior maxillary plane (distance from the midpoints of the maxillary tuberosities to the midpoint between the anterior-most points on the wings of the planum sphenoidale) on lateral radiographs was related to midfacial prognathism (measured as the minimum distance between nasion and foramen caecum). They concluded from a relative warps analysis of eight midsagittal landmarks that, relative to archaic Homo, the anterior sphenoid of modern humans was elongated and that midfacial length was reduced. They agree with the assessment of Lieberman (1998) that the anteroposterior dimensions of the human nasopharynx were reduced but argue against the direct role of anterior sphenoid shortening over the course of its evolution. It would seem that their measure of sphenoid length would indeed be only indirectly related to pharyngeal dimensions as it is an endocranial measure. The soft tissue dimensions of the nasopharyngeal boundaries more closely correspond to the distance between the choanae and the sphenoorbital synchondrosis (Takagi et al., 1962). Separate influences of pre- and post-sphenoid
growth on anteroposterior nasopharyngeal depth thus have yet to be studied in adequate detail. Michejda (1972) provided evidence suggesting that a reduced growth schedule at the human midsphenoidal synchondrosis (fused at birth) contributes to shorter relative length of the posterior sphenoid bone relative to *Macaca*, which continues to show growth in this region. Prolonged growth may account for the relatively elongated medullary clivus and posterior sphenoid body of non-human primates (Michejda, 1972).

Aside from anterior basicranial length, orientation of the posterior face (as estimated from the posterior maxillary plane) may potentially be related to anteroposterior nasopharyngeal depth. The position of the posterior maxilla as measured at pterygomaxillare (the inferior-most point on the pterygomaxillary fissure) has long been associated with anterior basicranial length as measurable from the nasion-sella chord (e.g. Brodie, 1941; Bjork, 1955). McCarthy and Lieberman (2001) presented evidence that the angles made between length of the cribriform plate (between foramen caecum and its posterior border) and planum sphenoidale length (measured between the tuberculum sellae and the posterior-most midline point on the cribriform plate) with their intersection at the posterior maxillary plane show a strong relationship. After studying lateral radiographs of a wide array of haplorhine and strepsirhine crania, they conclude that the anterior cranial base and face move as a single morphologic unit and that rotation of the "facial block" produced the anteroposteriorly constricted nasopharyngeal outline of modern humans. A similar conclusion was reached by Hayashi (2003) who measured interlandmark distances on CT scans of 45 Japanese modern human crania. He found that increasing anterior sphenoid length (measured endocranially) was related to rotation of the facial skeleton and anteroposterior positioning of the maxilla. The distinct nasopharyngeal anatomy of humans may thus have arisen from morphologic change in both facial orientation and the relative length of the
anterior basicranium, which appear to share a functional anatomic relationship. This study will test for such a relationship and its impact on nasopharyngeal dimensions using Procrustes-corrected exocranial landmark coordinate data.

Finally, the human Eustachian tube and its related musculature are distinct from those of other non-human primates. As outlined by Bluestone (2008), there are few data on the Eustachian tube anatomy of non-human hominoids but much data exists from captive populations of *Macaca*. He reports an absence of otitis media (middle ear infection) among the laboratory subjects of Doyle and Rood (1980) over a 30 year observation period and attributes this to anatomical differences that render them less susceptible than human infants and young children. The Eustachian tube of *Macaca* is comprised of a bony portion for nearly two thirds of its length whereas humans possess a much longer cartilaginous portion, which spans two thirds the distance between the nasopharynx and middle ear cavity (Cantekin et al., 1982). The tubes of macaques have additionally been reported as more rigid than those of humans, being better able to maintain patency (Doyle and Rood, 1980). Aside from tubal structure, the supporting musculature of humans is anatomically distinct as the area of insertion of the tensor veli palatini onto the tubal cartilage is much shorter and the levator veli palatini is in closer communication with the inferior aspect of the tubal cartilage along its length (Doyle and Rood, 1980).

2.2.7.1 Diversity among human populations

Population differences in the dimensions of the human nasopharyngeal boundaries may be the product of craniofacial adaptations to varying climatic conditions. Study of such variation spans at least as early as Topinard's (1885) description of mean choanal height among male
European, Asian, African, and Melanesian crania. However, analysis of population differences in choanal and overall nasopharyngeal dimensions has only intermittently been approached in previous studies, which instead focused on normal growth and anatomy within single populations. Bergland (1963) was the first to perform a detailed comparative analysis of bony nasopharyngeal anatomy between two populations. He gathered craniometric and cephalometric data from a sample of 159 Norwegian crania and 113 Lapp crania, concluding that the nasopharynges of the former group were deeper as a result of reduced basicranial flexion (between the nasion-sella-basion angles and the staphyion-hormion-basion angles). No significant (p<0.05) differences were found in nasopharyngeal volume (measured as half the product of basion-posterior nasal spine distance, nasopharyngeal height, and choanal width), which he suggested was functionally constrained. Brown (1973) later compared the nasopharyngeal dimensions of a sample of 100 aboriginal Australian crania to Bergland's (1963) Norwegian and Lapp samples and concluded that their nasopharynges were deeper and vertically shorter than either group. Average nasopharyngeal volume was determined as lying between the Norwegian and Lapp values. Finally, it was found by Kean and Houghton (1982) that their Polynesian cranial sample (n=60) possessed deeper and shorter nasopharynges than all three previously studied groups and was the only to exhibit significantly (p<0.05) greater volume.

Preston (1979) examined the nasopharyngeal morphology of living adults from the Lengua tribe of South America and San from Southwest Africa, comparing them to a British sample of adult males collected by Lavelle (1977). The Lengua and San were grouped to form a "pre-literate sample" for the purpose of measuring the amount of adenoid tonsilar tissue covering the nasopharynx. This was considered alongside reports of lower rates of allergies and upper respiratory tract inflammation among such pre-literate groups as compared to those inhabiting
industrialized societies (reviewed in Ricketts, 1968). All measurements were collected via lateral radiographs. Relative nasopharyngeal depth was measured as the basion-sella-posterior nasal spine angle while tonsilar tissue coverage was measured as the percentage of the vertical axis of the nasopharynx (between sphenobasion and its intersection of the posterior nasal spine-anterior arch of the atlas chord) covered in epithelium. However, the manner in which tonsilar tissue was discerned from lateral radiographs was not described. This technique is unreliable for soft tissue imaging and does not account for mediolateral structures when radiographs are taken laterally. Nevertheless, Preston (1979) described a relatively greater nasopharyngeal depth among the San while the Lenga exhibited absolutely greater vertical height. Both groups were found to differ in overall dimensions from the British sample with such differences attributed to normal patterns of "racial" morphology. Both pre-literate groups also exhibited relatively greater amounts of tonsilar tissue coverage despite the use of a suboptimal imaging technique and a lack of control populations of similar craniofacial morphology and ancestry living in an industrialized, Western cultural context.

Among the more recent detailed studies of population differences in upper respiratory tract morphology was Franciscus (1995), who analyzed the craniofacial morphology of a wide range of modern human groups and fossil hominins via caliper measurement. Among the measurements he collected were choanal width and height (as per Martin, 1928), from which a ratio of breadth/height was calculated. He concluded that modern and archaic Sub-Saharan African populations exhibited relatively wide choanae whereas modern and archaic Supra-Saharan groups (including Neanderthals) were characterized by relatively narrow choanae. These differences were suggested to be a product of the disparate respiratory demands of tropical and
temperate environments and were likely related to similar trends observed in piriform aperture dimensions (except for Neanderthals, which had extremely wide piriform apertures).

More recently, geometric morphometrics have been used in studying the nasal complex as a whole, including aspects of the bony nasopharyngeal boundaries. Rosas and Bastir (2002) described sex differences in the facial and basicranial morphology of a sample of male (n=52) and female (n=52) Portuguese crania. They found that, when the mean male and female landmark coordinate configurations were superimposed over each other (each being scaled over centroid size to preserve only differences in shape, not size) males appeared to exhibit relatively taller choanae, posterosuperior displacement of hormion, and greater basicranial flexion as can be inferred from the inferior position of basion. They argue that this creates a more capacious nasopharynx among males when viewed in norma lateralis. Their results were corroborated in a later study by Bastir et al. (2011) of sex differences in upper respiratory tract morphology among this and other samples from Europe, North America, West Africa, and Australia. Males of all sample populations appeared to exhibit relatively tall choanae and greater basicranial flexion than females when average landmark coordinate configurations were superimposed (as was done by Rosas and Bastir, 2002). They attributed their results to differing metabolic demands associated with dimorphism in overall body composition.

Among the most recent studies to investigate population differences in nasopharyngeal morphology was by Noback et al. (2011) who used a geographically diverse sample (n=100) of pooled sex to assess the effects of climate on the nasal complex. They conceptualized the bony nasopharyngeal boundaries as the posterior-most extension of the nasal cavity and hypothesized that it, along with dimensions of the piriform aperture and overall nasal cavity dimensions would vary among populations inhabiting different climates. A Generalized Procrustes Analysis was
used alongside several linear measurements synthesized from Procrustes-corrected landmark coordinate data. Their results indicated that choanal height increases among populations from cold climates and that nasopharyngeal depth is reduced (as inferred from a more anteriorly placed pharyngeal tubercle and posteriorly placed superior medial pterygoid plate borders). Populations from arid climates exhibited reduced choanal height relative to the height of the posterior portion of the nasal cavity while nasopharyngeal depth was decreased between hormion and the pharyngeal tubercle. It was suggested that both cold and dry climates would necessitate vertical expansion in the nasal cavity to increase surface area-to-volume ratio but that this trend occurs independent of nasopharyngeal shape under the influence of aridity. They posit that a separate growth of nasal cavity dimensions was made necessary by the need for increasing air turbidity. However, they did not discuss the possibility that decreased nasopharyngeal depth coupled with a more vertical orientation of the choanae among populations from cold, dry climates (as can be visualized from their wireframe models) could provide a further increase in postnasal turbidity as inspiratory airflow must traverse a more abrupt inferior curvature before reaching the larynx. Most recently, Evteev et al. (2014) derived similar results using the same method on a sample of Asian crania ranging in provenance from Northern China and Korea to Siberia.

The most recent geometric morphometric analysis of the osseous nasopharyngeal boundaries and its morphological relationships to related cranial structures has been by Bastir and Rosas (2013). They collected three-dimensional coordinate data from the facial skeleton, piriform aperture, endonasal structures, and nasopharyngeal bones. Their sample was composed of a growth series spanning from eruption of deciduous dentition to eruption of the full adult dentition. The latter adult sample was geographically diverse, including Europeans, Africans,
Australians, Inuit, and Fuegans. Bastir and Rosas (2013) correlated nasopharyngeal landmark values against those collected on the osseous anterior airway boundaries and midface (Escofier’s RV coefficient and partial least squares analysis) to assess modularity. Their results indicated that nasopharyngeal shape shares a strong relationship with midfacial shape but not anterior airway shape. This was interpreted as evidence against the presence of climatic adaptations in the choanal and overall nasopharyngeal dimensions, contra Noback et al (2011) and Evteev et al. (2014).

The one area of the nasopharynx that has yet to be investigated using 3D geometric morphometrics techniques is the path of the cartilaginous Eustachian tube as determined from osseous boundaries. Morphologic differences between human populations have rarely been described in detail. Doyle (1977) investigated the estimated dimensions of the cartilaginous Eustachian tube among a sample of Inuit (n=80), Native American (n=80), European American (n=80) and African American (n=80) dry crania. He aimed to describe anatomic features that may be related to high reported incidences of otitis media among the Inuit and some Native American groups. His results indicated that Inuits have absolutely longer cartilaginous Eustachian tubes between the orifice on the temporal bone and the posterior edge of the medial pterygoid plate. The Native American sample exhibited more horizontal orientation of the estimated Eustachian tube floor, a condition he argued was paedomorphic as it resembles the condition of infants and young children. Both Inuit and Native American crania exhibited a smaller absolute size of the Eustachian tube orifice on the temporal bone. These results are an important "first step" toward quantification of clinically important diversity in Eustachian tube anatomy. However, this study did not employ geometric morphometrics techniques which allow assessment of both relative and absolute dimensions.
2.2.7.2 Normal human development

2.2.7.2.1 Midline nasopharyngeal structures

Some of the earliest investigations of postnatal nasopharyngeal growth are from Disse (1889), Merkel (1890), Dwight and Rotch (1891), and Escat (1894) who used cadaveric specimens and small collections of dry crania to describe soft tissue changes (e.g. relative orientation of the cartilaginous Eustachian tube orifice, nasopharyngeal depth) and absolute growth changes in choanal dimensions, and relative vomeral orientation and position. They describe marked vertical growth of the choanae and nasopharyngeal space and correlate it to similar trends in nasal cavity and facial growth, stating that the choanae in infants appear broad compared to those of adults. Merkel (1890) and Escat (1894) also note that the vomer is obliquely angled in infants and that this orientation changes with increases in basicranial flexion and changes in facial dimensions into adolescence. However, among the earliest large, systematic studies of nasopharyngeal growth was by Keith and Campion (1922) who examined cranial growth series. They concluded that anteroposterior skull growth occurs largely at the sphen-o-occipital synchondrosis, which results in anterior displacement of the pterygoid plates and, subsequently, forward movement of the face. Todd and Tracy (1930) also argued that much growth occurs at this site. Such growth results in both posterior migration of the occipital bone and anterior displacement of facial elements, which acted to increase the anteroposterior depth of the nasopharynx in a growth series of African American crania. The former growth trend was observed to occur only up to seven years of age when full adult size was attained. Subsequent
increases in nasopharyngeal dimensions were attributed to anterior facial displacement.

Nonetheless, Diamond (1946) proposed that this forward growth of the maxillae does not occur as there is no posterior resistance from the medial pterygoid plates until adulthood. Rather, they are located medial and anterior to the maxillary tubercles throughout development. It is only after the growth of the posterior maxillae into the sphenomaxillary space that the medial pterygoid plates move laterally to meet the tuberosities. The majority of this growth may occur as early as the first year of life as Subtelny (1955) described for absolute bi-hamular width. Thus the hypothesis that the maxilla is pushed anteriorly at the pterygopalatine fissure during growth was rejected by Diamond (1946) who instead argued that there is posterior movement of the hard palate, as later described in studies of the external basicranial axis (see below).

After the development of the earliest cephalometric techniques by Broadbent (1931), many early studies of nasopharyngeal growth were conducted using lateral radiographs. Among the earliest was Rosenberger (1934) who conducted a longitudinal study of children from three months to five years of age. He argued that nasopharyngeal growth was achieved from anterior displacement of the hard palate related to growth of the sphenoid body whereas mediolateral growth is achieved by expansion of the greater wing of the sphenoid. Similar results were attained by Brodie (1941) who examined the Bolton growth series and studied the span between three months and eight years. The anteroposterior displacement of the point pterygomaxillare (representing the posterior maxilla) was stabilized with sella-nasion chord length but most of this growth occurred only until age 1.5 years. He also noted a continual vertical growth and inferior displacement of the palate with age, which resulted in greater vertical height of the nasopharyngeal space. King (1952) corroborated the findings of Brodie (1941) as anteroposterior growth between pterygomaxillare and the anterior border of the atlas occurred mostly within
early infancy. Vertical nasopharyngeal growth was, however, observed throughout puberty and attributed to vertical growth of the atlas and descent of the hard palate, mandible, and hyoid bone. This trend was also described by Bjork (1955) in a radiographic study of males at 12 and 20 years of age. He cited influence from appositional growth at the nasal floor and sutural growth in the upper face, regarded as coordinated growth rather than a case of the basicranium exerting direct mechanical influence upon the midface.

In his classic study, Bergland (1963) examined growth in the nasopharyngeal boundaries from lateral radiographs of dry crania rather than live patients. The depth, height, and width (at the choanae) of the nasopharynx were measured in a Norwegian and Lapp cranial sample spanning from 6 years to adulthood. Population differences were noted as the height of the nasopharynx was shown to undergo a significantly greater increase in the Norwegian sample over ontogeny. Nonetheless, the relatively long, low nasopharynges of Lapss and high, short nasopharynges of Norwegians encompassed roughly the same volume (as discussed above), which was shown to increase the most following nasopharyngeal height over both ontogenetic trajectories. A growth increase of 80% was observed between six years and adulthood with sustained increase in nasopharyngeal height. Also, as observed by Brodie (1941) and King (1952), there was minimal increase in anteroposterior nasopharyngeal depth within the Norwegian sample but well expressed growth change between consecutive growth stages among the Lapp Sample. This is likely related to the anteroposteriorly longer, vertically shorter sagittal nasopharyngeal profile of adult Lapss relative to Norwegians. The results suggested that not all of the growth trends observed in earlier cephalometric studies may apply to all human groups as population differences had been under-studied.
Growth trends in the relative position of hormion have long been studied (e.g., Merkel, 1890; Lang and Issing, 1989). Takagi (1964) used a developmental series of European and East Asian dry crania, accounting for differences between populations, and found that hormion moves posteriorly throughout development but undergoes its greatest growth change within infancy and early childhood (between birth and the period of eruption of the deciduous dentition), roughly corresponding to the cessation of growth in nasopharyngeal depth observed by Brodie (1941) and King (1952). However, posterior displacement of the vomer acts to decrease relative nasopharyngeal depth despite increases in absolute dimensions, which Preston et al. (2004) observed increasing with absolute nasopharyngeal height throughout a pubertal growth spurt. As noted by Bergland (1963) and corroborated in later studies (e.g. Laitman, 1977; May and Sheffer, 1999; Pagano et al. 2011), the plane of the vomer at the staphylion-hormion chord rotates posteriorly as the entire basicranial surface of the upper respiratory tract becomes more flexed. Such growth changes in the relative dimensions of the nasopharynx had long been under-investigated with most studies up until Takagi (1962) focusing on growth change in absolute measures.

Laitman (1977) employed a composite measure of external basicranial flexion to assess scale-free relative growth changes in the upper respiratory tract, including the nasopharyngeal boundaries. The positional relationships of landmarks at the midline axis of the hard palate, choanae, and basicranium were measured (i.e. prosthion, staphyion, hormion, sphenobasion, basion). He utilized several growth series spanning from infancy to adulthood and representing humans, non-human hominoids, and *Macaca*. Humans underwent marked flexion at all areas of the upper respiratory tract between birth and the eruption of the deciduous dentition, which roughly corresponds to the time at which the larynx descends and approaches the adult position.
The non-human primate series, on the other hand, exhibited some retro-flexion at the upper respiratory tract boundaries but remained stable over development. These results suggest that the upper respiratory tract (including the nasopharyngeal boundaries) may grow as a module and share functional relationships.

Jo (1980) studied growth changes in the nasopharyngeal boundaries of dry crania and calculated correlation coefficients for various measures. Her measure of nasopharyngeal volume (or “geometrisches volumen des epipharynx”) grew slowly, attaining nearly half its adult size at five years of age and 79% of adult size at 15 years. In contrast choanal height and breadth had achieved over half of the adult value by the first year of life and respectively grew to 93% and 94% of their final size by 15 years. Of all the measures taken, only internal basicranial flexion (“sphenoidalwinkel”) was significantly (p<0.05) correlated with age and was negatively correlated with choanal height. Nasopharynx volume was significantly (p<0.05) correlated with choanal height only, there being no strong relationship with basicranial flexion as shown by Bergland (1963).

Angular growth changes were also studied by May and Sheffer (1999) who measured internal basicranial flexion (IFA) and craniofacial flexion angle (CFA) on the midline skull as the basion-tuberculum sella-anterior cribriform plate angle and the basion-hormion-staphyllion angle, respectively. These were applied to a cross-sectional growth series of dry crania representing *Gorilla gorilla, Pan troglodytes*, and modern humans. IFA increased over *Gorilla* ontogeny while those of *Pan* and humans remained stable. CFA was seen to increase over growth among all three genera but decreased among humans. This growth change was mostly concentrated in the interval between birth and the eruption of the first permanent molars.
The same two angles were applied by Jeffery (2005) to the study of postmortem human fetuses ranging in age from 10 to 29 weeks of gestational age. Two triangles representing the sagittal profiles of the nasopharynx and oropharynx were calculated between the posterior nasal spine-hormion-anterior atlas arch and posterior nasal spine-hyoid bone-anterior atlas arch, respectively. Both angles increased or retroflexed with age but the external angle showed twice the amount of growth change. Nasopharyngeal area was shown to increase at a slower rate than oropharyngeal area. A ratio of the two measures showed consistent increase of the latter over the former with age, likely driven by rapid descent of the hyoid relative to the slower vertical growth of the choanae between hormion and the posterior nasal spine. These results suggest that, during the fetal stage of development, facial growth or some other functional influence is related to retroflexion of the basiranium and midline choanae (as suggested by the internal angle), producing a contrary growth trend to the one seen in postnatal development (Laitman, 1977; Reidenberg and Laitman, 1991).

More recent studies have employed 3D geometric morphometrics techniques in studying cranial growth trends. These techniques allow for standardization of size and optimal visualization of relative growth changes. They may also be ideal for more detailed study of the functional interaction between the osseous nasopharyngeal boundaries and other aspects of the upper respiratory tract and face. Issues of cranial modularity and the functional relationships of human facial, basicranial, and neurocranial growth are now being considered (e.g. Bookstein et al., 2003; von Cramon-Taubadel, 2011) in both developmental and evolutionary contexts (see review by Lawing and Polly, 2010). However, few of these have focused on the growth of the osseous nasopharyngeal boundaries.
2.2.7.2.2 The cartilaginous Eustachian tube

Studies by Suzuki et al. (1998), Ishijima et al. (2000), and Takasaki et al. (2009) examined CET growth via three-dimensional computerized reconstructions of histological sections from the Sando Collection (University of Pittsburgh School of Medicine, Pittsburgh). Sections were taken in an axial plane relative to the long axis of the CET from temporal bone specimens removed at autopsy. The interval from birth to senility is represented here. It was found that adults exhibit a gradual decrease of CET area between its pharyngeal and osseous orifices whereas in children, the area is uniformly small over 80% of its length, abruptly widening close to the nasopharyngeal orifice. CET volume (total and within the medial and lateral laminar cartilages) is also lowest in infants and is positively correlated with age (Takasaki et al., 2009) as the ratio between CET length and length of the osseous Eustachian tube decreases from 8:1 in infants to 4:1 in adults (Ishijima, 2000). Curvature of the CET was also calculated; it was measured as the distance between the center of the CET lumen on every fourth slide and the main axis of the CET between its osseous orifice and the pharyngeal orifice. Adults had a CET that was inferiorly and laterally deviated from its main axis while infants and young children exhibited little curvature along its axis (Ishijima, 2000).

Despite the ubiquity of middle ear disease among infants and young children, growth of the cartilaginous Eustachian tube (CET) remains to be studied in adequate detail. It is widely known that infants are born with short, horizontally oriented CET’s, often patulous or "floppy" relative to the adult condition. It has been hypothesized that the shorter, more horizontal tube allows for easier access to the middle ear by pathogenic microorganisms. When combined with weaker tubal dilator muscles and an immature immune system, the CET may become a frequent
route for upper respiratory tract infections to travel from the nasal cavity and sinuses (see the excellent discussions in Bluestone, 2005a,b).

Doyle (1977) was among the first to estimate the dimensions of the CET from dry crania. He observed populations differences among adult crania. Doyle and Swarts (2010) later adapted this technique to the study of a growth series representing the interval between three and four years of age \((n=10)\) and adulthood \((\text{above } 18 \text{ years}, n=10)\). They collected 10 separate measurements of choanal width, estimated CET length, estimated tensor veli palatini muscle (TVP) length, choanal height, and anteroposterior basicranial length on each specimen and synthesized nine others from these measures. Significant \((p<0.05)\) differences were found in the absolute dimensions of the choanal boundaries and estimated CET and TVP dimensions. While there were growth differences observed in the projected deviation of the TVP muscle from the CET, none were found in CET angulation relative to the basicranial surface or parasagittal plane. They conclude that it is differences in the TVP muscular anatomy and absolute CET length that are likely responsible for the high rates of pediatric otitis media. Despite being an important first step in the examination of CET growth in relation to osseous nasopharyngeal boundaries, relative CET dimensions and the condition of infants were not adequately addressed. The current study will be the first to use three-dimensional geometric morphometrics techniques in quantifying relative CET dimensions and their functional relationships with other nasopharyngeal structures between birth and adulthood.
2.3 Early study of the nasopharyngeal boundaries

The nasopharynx has been understudied relative to neighboring anatomical regions. Its boundaries are derived embryologically from portions of both the digestive and respiratory tract. Indeed, the nasopharynx is a highly complex, integrated space whose function, development, and evolution still remain unclear after nearly 5000 years of study.

Historically, most work on the nasopharynx was done with a focus on other, related structures. It has most often been mentioned in relation to the middle ear (via the Eustachian tube) in ancient texts. One of the earliest mentions of the nasopharynx can be placed to Ebers’ Papyrus, a medical text dating to 1550 B.C.E. (but whose content was copied from a text dating to approximately 2600 B.C.E.) found in Luxor, Egypt. It describes the “breath of life” as passing through the right ear and the “last breath” as passing through the left, which suggests an understanding of the Eustachian tube. The Edwin Smith papyrus (written circa 1600 BCE but whose content may be from 2600-3000 B.C.E.) further suggests the ancient Egyptians had a detailed knowledge of the anatomical boundaries of the nasopharynx. Within this document, fractures of the sphenoid body (and possibly the choanae) are described as untreatable and as the cause of bleeding through the ear and loss of speech. Mummified remains have been found with holes drilled through the sphenoid body for brain removal, a less common alternative to incision of the cribiform plate (see Pahor, 1992a,b,c for a good review of ENT medicine in ancient Egypt).

The nasopharynx is again indirectly mentioned circa 400 B.C.E. by Hippocrates of Cos who described the surgical excision of nasopharyngeal fibromas (Acuna, 1956). Also at this
time, Alcmaeon of Sparta discussed Eustachian tube function in goats (Singer, 1925), proposing that it allows for respiration through both the ears and nose. Aristotle of Stagira agreed that there was a passage for communication between the middle and inner ear and an area adjacent to the palate (a position later echoed by Vesalius in 1543) and explicitly identified this communicative pathway (what would later be termed the Eustachian or pharyngotympanic tube). However, he denied the ear any direct role in respiration.

Aristotle was arguably the first to discuss the nasopharynx in the context of normal function, considering it part of the nose and stating that the fauces is the roof of the pharynx (or upper alimentary tract). He also made the first official description of the nasopharynx as "…the aperture formed by the nostrils into the mouth—an aperture through which, when men, in drinking, inhale any of the liquid, this liquid finds its way out through the nostrils" (Thompson, 1910: 16-17; see his classic translation of Aristotle’s *Historia Animalum*). Aristotle did little in the way of explicitly stating the boundaries of the nasopharynx, describing only the palatopharyngeal arch and uvula. Galen (175 C.E.) later expounded on the respiratory function of the nasopharynx, describing it as a bend between the nose and larynx. He proposed that the nasopharynx may aide in the warming of cold, inspiratory air and in the filtering of inspired particulate matter but did not directly name it or identify its boundaries in the eleventh book of his *De Usu Partium Humani*. He ascribed a prominent role to the uvula, viewing it as the primary body to which particulate matter is attracted and diverted from entry into the trachea. Galen argues that, without the uvula, filtration of inspiratory air is hampered and can lead to fatal chilling of the lungs.

Until the 15th century, all Western anatomical studies (including those of Aristotle and Galen) were performed via the dissection of non-human animals as the use of human cadavers.
was prohibited (Singer, 1957). Thus these ancient anatomists had no means of visualizing the morphology or spatial relationships of the human nasopharyngeal boundaries. However, at approximately 1200-600 B.C.E., human dissection was performed in India, forming one of the bases for a rich medical tradition called Ayurveda. For the first time in history, human cadavers were openly utilized to study anatomy (predating the works of Herophilus and Erasistratus circa 300 B.C.E.). However, human dissection (termed Avagharshana) via incision of structures was not practiced until the European renaissance (first published protocol by Mondino de Luzzi in his *Anathomia Mundini*, 1478). Instead, cadavers were prepared by covering them with Kusa grass and placing them in a thatch container, which was immersed in a river for three days. When ready, successive layers of skin and muscle were removed by rubbing with a brush (Bishagratna, 1911). This technique leaves minute nerves and blood vessels undisturbed but does not allow for optimal visualization of more complex musculoskeletal structures comprising the nasopharyngeal boundaries.

The best known text of this era (approximately 800-500 B.C.E.) is the *Sushruta Samhita* of the sage Acharya Sushruta (see translation by Bhishagratna, 1911). In it, he stated that the hard palate consisted of a single bony element and that the soft palate was composed of only two muscles. Though not explicitly stated, this is the first recorded description of the components of the nasopharyngeal floor. He further discussed the routes of communication between the nasal cavity and other cranial organs via the nasolacrimal duct and Eustachian tube. Sushruta warned that improper usage of a “sneha” or oily snuff medicine blown through the nose and ejected through the mouth could result in damage to the eyes, ears, nose, and tongue. This caveat suggests an understanding of the basic anatomy of the nasopharyngeal boundaries.
It was not until Vesalius (1543) that knowledge of the skeletal and soft tissue boundaries of the nasopharynx improved. He described the entire osseous frame of the anterior and lateral nasopharyngeal walls, introducing the term choanae (from the Greek “choane” or χοάνη), processus pterygoideus, and formally describing the cuneiform bone (sphenoid or wedge-shaped; he preferred the Latin cuneiform over the Greek “sphenoid” or σφηνοειδής) of the head in his seminal work *De Humani Corporis Fabrica*. Vesalius (1543) described the choanae in one of his figures as nasal foramina divided by a septum and stretching from the nose to the throat cavity. He continues that these spaces have many functions but are primarily for normal respiration and for vocalization. This suggests that Vesalius (1543) adopted the same concept of the postnasal space as his predecessors and would have disagreed with the inclusion of the nasopharynx into a tripartite pharyngeal space.

The Eustachian tube was also discussed by Vesalius (1543) who expounded on Aristotle's description and provided an illustration of the communicating tube traversing the distance between the palate and middle ear. He also provided an experiment to test for its function, in which one takes air into the mouth and attempts to redirect it into the ears. This results in the hearing of wind or a sound redolent of a whirlpool. Despite his understanding of the extent of communication provided by the Eustachian tube, Vesalius (1543) never described the basic structure of the Eustachian tube or its muscular relationships.

Bartolomeo Eustachi (also known as Eustachius) was a contemporary of Vesalius and had made substantial contributions to the study of human anatomy. Aside from his identification of the cochlea, optic and abducens nerves, thoracic duct, and adrenal glands, he was also the first to describe the pharyngeal constrictor muscles and the structure and full course of the tube that now bears his name. In the *Epistola de Auditus Organis*, Eustachius (1564) lists an osseous and
cartilaginous portion of the Eustachian tube, the former being lined with a mucous membrane continuous with the one lining the nasal cavity. The muscular slips of the tensor veli palatini that close the pharyngeal Eustachian tube orifice were called a sphincter but not explicitly named (reviewed in Bluestone, 2005). Despite the great importance of his research, the bulk of Eustachius's work was not published until 1714, 140 years after his death.

Antonia Maria Valsalva (published posthumously in 1741; see translation by Mangano, 1930) was the first to fully describe the muscular relationship between the soft palate and Eustachian tube and the anatomy of the constrictor muscles of the pharynx in his “Teatise on the Human Ear” (Canalis, 1990). He identified the full extent of the tensor veli palatini muscle and the palatoglossus and palatopharyngeus muscles. Valsalva argued that the Eustachian tube functioned as a conductor of sound, allowing for waves entering via the vibrations of the tympanic membrane to exit the middle ear. The Valsalva maneuver, which bears his name, was originally intended to dilate the Eustachian tube and restore its function. It was at this point in history that the muscular boundaries of the nasopharynx were first clearly defined.

The last components of the nasopharyngeal boundaries to be identified were its tonsils. According to Chauveau (1910), the first description of the pharyngeal tonsil was by Schneider (1862) who, in the same publication, was among the first to successfully argue that nasal catarrh does not originate in the Pituitary gland (an idea first reported by Galen; Garrison, 1917). Luschka (1884) first described the tubal tonsils and argued that the pharyngeal tonsil is not embryologically or functionally related to the drainage of the pituitary gland. However, the true physiology of these two tonsils was first described by Waldeyer-Hartz (1884) who linked their function with that of the palatine tonsils as part of “Waldeyer’s Tonsilar Ring,” which is arranged around the airways to protect against external contaminants. It was at this time that the
anatomic boundaries of the nasopharynx were fully described. Yet, after nearly 5,000 years of study, the normal growth and functional morphology of this region in humans and non-human primates has yet to be rigorously investigated.

2.4 Clinical relevance for studying the morphology of the nasopharyngeal boundaries

Upper respiratory tract disease is the most commonly reported pediatric clinical condition (Merrill and Owens, 2007). Given the central location of the nasopharynx, it may act to spread infections from the nasal cavity to the middle ear and mastoid air cells (via the Eustachian tube) or inferiorly to the larynx. Many such cases may also arise from pathology of the nasopharyngeal boundaries, including the Eustachian tube. Indeed frequencies as high as 30% have been reported in some populations for otitis media related to Eustachian tube dysfunction (Kaplan et al., 1973; Tschopp, 1977; Brody, 1965). Other conditions such as cleft palate (Harville et al., 2007), choanal atresia and stenosis (Ramsden et al., 2009; Corrales and Koltai, 2009), and adenoid tonsillitis (Subtelny, 1954; Huang and Giannoni, 2001) can deter normal breathing and feeding by altering the surface morphology and function of the nasopharynx and adjacent structures. Among adults, nasopharyngeal carcinoma is a life-threatening condition whose etiology is still, as yet, not fully understood. Data on the normal functional morphology and growth of the nasopharyngeal boundaries are thus of great clinical importance. Below is a discussion of some clinical conditions of the nasopharyngeal boundaries.
2.4.1 Eustachian tube dimensions and otitis media

Otitis media is among the most common diseases involving the nasopharynx; approximately 29% to 50% of all pediatric upper respiratory tract infections develop into acute otitis media (Heikkinen and Ruuskanen, 1994; Koivunen et al., 1999; Revai et al., 2007). There appears to be a peak of incidence within the first two years of life (Teele et al., 1989; Alho et al., 1991; Dewey et al., 1995; Paradise et al, 1997; Bentdal et al., 2007; Mandel et al., 2008) with infants aged between six and 12 postnatal months exhibiting the highest rates (Dewey, 1995; Bentdal et al., 2007). These early episodes of otitis media may often be followed by other respiratory infections later in childhood while repeated episodes of otitis media become less common (Bentdal et al., 2007).

Several anatomic differences between adults and infants have been cited in studies of otitis media etiology. They include shorter absolute cartilaginous Eustachian tube (CET) length (Sadler-Kimes et al., 1989; Ishijima et al., 2000; Doyle and Swarts, 2010), more horizontal orientation of the tube (Proctor, 1973; Doyle and Swarts, 2010; Pagano et al., 2012), smaller lumen area and volume (Kitajiri et al., 1987; Suzuki et al., 1998; Ishijima et al., 2002), and a relatively wider Ostmann's fat pad (Orita et al., 2003). Also, histological differences have been noted, such as increased cartilage cell density (Yamaguchi et al., 1990) and fewer elastins at the hinge (superior junction of the medial and lateral CET laminae; Matsune et al., 1993), creating a more patulous CET that may resist passive closure at its roof (see Bluestone, 2005a).

Bluestone (2005b) suggests that the anatomic and histologic factors predisposing infants to otitis media are related to our evolutionary past. As the human birth canal is relatively narrow and the brain is relatively large, infants must be born relatively early for an animal of our body
mass. Martin (1990) estimates that humans should be expected to have a 21 month-long gestational period but that we evolved to become secondarily altricial. As a result, the immune capabilities and sensory systems of infants are immature. The CET’s of infants may exhibit functional differences from the adult condition as a result of this altricial birth. It is over the first two years of postnatal growth that brain size, orbit size, and laryngeal position begin to approximate the adult condition. Just as well, the CET undergoes a substantial amount of growth change during this time (Pagano et al., 2012), when infants are the most susceptible to developing otitis media.

Among human populations, some have been found to be at greater risk than others for the development of otitis media (Bluestone, 2005a; Daly et al., 2010). These may reflect any number of epigenetic or genetic factors. Homoe et al. (1996) presented evidence that Greenland Inuits, a contemporary population characterized by high frequency of otitis media, were far less likely to develop infectious middle ear disease before European colonization in 1721. Environmental and cultural factors were cited for the rise of otitis media within this population. However, the opposite was found among Native Americans of Arizona where both ancient and modern populations show high rates of otitis media (Titche et al., 1981).

2.4.2 Cleft Palate

Cleft palate arises from a defect in which the mesenchyma of the primary, secondary, or both palatal precursors fail to fuse with the nasal septum in the midline. Those arising from failure of primary palate fusion result in clefts anterior to the incisive foramen while those resulting from non-fusion of the secondary palate can extend from the incisive palate through to
the uvula. It can occur unilaterally or bilaterally and be associated with cleft lip. This condition may also occur alongside various other deformities and can be caused by genetic and environmental factors. Cleft palate as expressed alone or with cleft lip occurs at a rate of roughly 1-2 out of 1000 live births among most populations, making it a common birth defect (Vanderas, 1987; Moore and Persaud, 2003; Calzolari et al., 2004).

Clefting of the secondary palate can cause velopharyngeal insufficiency and interfere with suckling (Choi et al., 1991; Reid et al., 2007) and normal speech (Tian et al., 2010) related to malformation of the soft palate. The levator veli palatini may develop with anomalous locations for its origin and insertion (Perry, 2007). The tensor veli palatini has been observed inserting onto a variety of abnormal locations such as a laterally shifted palatine aponeurosis (Koch et al., 1998), maxillary tubercle and inferior end of the medial pterygoid plate (Abe et al., 2004), or sometimes onto the medial pterygoid hamulus itself (Fara and Dvorak, 1970) with disruption of its insertion onto the pharyngeal orifice of the Eustachian tube. These can often lead to otitis media with effusion and related complications such as cholesteatoma (Abe et al., 2004). When the tensor veli palatini muscle can no longer dilate the Eustachian tube orifice, the action of the levator veli palatini muscle which underlies the cartilaginous portion of the tube constricts it (Doyle et al., 1980; Bluestone and Swarts, 2010). Ventilation of the middle ear is thus prevented.

Previous studies have found that children and adults with cleft palate tend to exhibit reduced maxillary height but normal amounts of basicranial flexion (e.g. Satoh et al., 1999; Smahel and Mullerova, 1992). In one study, Satoh et al. (1999) found no disturbances in growth schedule or the functional morphologic relationships between structures of the face and basicranium among patients with unrepaired unilateral cleft lip and palate (Satoh et al., 1999).
However, this and previous studies were performed from lateral plain film radiographs without adequate means of assessing shape differences independent of scale.

2.4.3 Nasopharyngeal carcinoma

The earliest reported case of nasopharyngeal carcinoma may date back at least as far as the work of Durand-Fardel (1837; cited by Muir, 1983). However, nasopharyngeal carcinoma has likely been present among human groups for as long as 5,000 years as can be inferred from lesions on the nasopharyngeal boundaries of a cranium dating to the beginning of Egypt's Old Kingdom (Wells, 1963). Similar pathology was described on two crania from medieval Britain (Wells, 1964). It may thus be considered a disease with a long history.

Among most global populations, cancers of the nasopharynx are a rare condition occurring at a rate of less than 1 per 100,000 persons per year (Parkin et al., 1997) with 84,000 cases reported globally and 51,600 reported deaths (Jemal et al., 2011). Relative prevalence has traditionally been much higher among some ethnic groups, especially Cantonese (with Guangdong Province marked by one of the highest rates), Taiwanese, and Malays (Muir, 1983; Yu and Yuan, 2002; Wei et al., 2010; Jia and Qin, 2012) and Greenland Inuits (Albeck et al., 1992). Viral activity combined with environmental irritants and genetic predisposition has been cited as potential causative factors for the development of nasopharyngeal carcinomas in these groups (Hildesheim and Wang, 2012). Early exposure to preserved foods such as salted fish have been linked to higher rates of nasopharyngeal carcinoma (Ho, 1971) and have been found to contain chemical agents that act as carcinogens, promoting activity of the Epstein-Barr virus (e.g. Tannenbaum et al., 1985; Shao et al., 1988; Poirier et al., 1989; see Zheng et al, 1994 who tested
this hypothesis on a rat model). Interestingly, immigrants from South China living in nations with lower rates of nasopharyngeal carcinomas retain increased prevalence relative to other sympatric ethnic groups, though these are reduced from the rates observed in South China (Parkin et al., 1997). This suggests the involvement of some heritable trait in susceptibility.

The potential roles of many external carcinogenic agents in promoting nasopharyngeal carcinoma have been assessed (Hildesheim and Wang, 2012; Jia and Qin, 2012). However, few have investigated the possibility that there may be differences in the nasopharyngeal morphology of high-risk populations that render them more susceptible to developing nasopharyngeal carcinoma. The lateral pharyngeal recess (of Rosenmuller) is the most common site for the initial development of nasopharyngeal cancers, which initially arise from keratinizing squamous epithelia and can often involve the torus tubarius (Fu, 1987; Hoe, 1989; Yamashiro and de Souza, 2007). This may be due to variation in depth (e.g., Manusco et al., 1980; Yamashiro and de Souza, 2007), which can promote accumulation of environmental irritants that trigger Epstein-Barr virus activity (Hildesheim and Wang, 2012).

2.4.4 Sleep apnea

Apneic sleep disorders were first studied by Gastaut et al. (1965) who observed them in relation to Pickwickian disorders (named after a fictitious Charles Dickens character; first defined by Osler, 1916) in which patients were typically obese, experienced unexpected daytime sleepiness, and waking apneic episodes. Osler (1916) determined that these also occurred during nighttime sleep and that they were disruptive, potentially accounting for observed patterns of
hypersomnolence. Other work by Duron et al. (1967), Lugaresi et al. (1968), and Kuhlo et al. (1969) extended this work to non-obese patients in which apneas were not visible during daytime activity. They determined that persistent sleep apnea may be related to cardiovascular and other health issues left untreated.

Obstructive sleep apnea is a common condition affecting 2-5% of adults (Kubin, 2009). Apnea is defined as a cessation of respiratory airflow in the upper respiratory tract lasting at least 10 seconds (Guilleminault et al., 1975) appearing at least 30 times during REM and non-REM sleep over seven hours (Guilleminault et al., 1976). Upper airway obstructions can occur at several sites (Rama et al., 2002) but the most common is at the uvula and posterior edge of the soft palate (Isono et al., 1997; Kubin, 2009), which can be pressed against the posterior pharyngeal wall by the tongue. OSA prevalence in humans has been argued to be a consequence of the evolution of the supralaryngeal vocal tract, characterized by an equal proportion of its horizontal and vertical segments (Davidson, 2003; Davidson et al., 2005). This configuration pushes the tongue into a relatively posterior location where it is in closer contact with the uvula in supine position. Papaxanthos (in preparation) stresses the importance of paramedian shortening of the posterior soft palate edge around a well expressed uvula, which provides an alternative route for airflow in cases of midline airway blockage (explained in the "in-out contact hypothesis"). He argues that, in the presence of redundant mucosa and submucosal fat deposits, these paramedian channels become constricted and result in an apneic episode.
2.5 CONCLUSIONS

The component structures of the nasopharynx are of composite developmental and evolutionary origins, rendering precise definition difficult. From the origins of the earliest lobe-finned fish to the appearance of the first anatomically modern humans, the nasopharynx has undergone many largescale restructurings. Indeed, such changes are recapitulated during pre- and postnatal development. This process has resulted in the situation of the nasopharynx among several functional units within the head (e.g., nasal cavity, oral cavity, middle ear) with many of their diseases involving nasopharyngeal structures. These may be seen as consequences of the long and complex evolutionary that have shaped the nasopharynx [see similar arguments by Bluestone (2008) and Bluestone et al. (2012)].

Given the vital importance of the nasopharynx in a wide range of functions such as respiration, speech, and middle ear aeration, it is curious that few evolutionary studies have focused on this region exclusively, despite incorporating many of its boundaries in studies of the nasal cavity, oral cavity, vocal tract, basicranium, and middle ear. This dissertation aimed to fill gaps in understanding of the development, functional morphology, and evolutionary change of the human and non-human primate nasopharynx. The breadth of this topic required separate analyses of osseous morphology, soft tissue histology, interaction with local climate over development, and evolutionary differences between humans and Neanderthals, our closest extinct relatives. The results generated in this dissertation may offer a novel perspective on some controversial topics within human evolution.
3. Geometric morphometric analysis of the osseous nasopharyngeal boundaries: Testing for functional morphologic relationships with adjacent cranial structures

3.1 Aim of the chapter

This chapter will analyze morphological relationships among the osseous nasopharyngeal boundaries, facial skeleton, and basicranium. To this end, three hypotheses will be tested: 1) nasopharyngeal width is greater among individuals with shorter facial length (orthognathy) and inferiorly oriented faces (facial kyphosis); 2) Individuals with greater basicranial flexion will exhibit nasopharynges that are vertically taller and anteroposteriorly shorter; 3) The cartilaginous Eustachian tube tract of humans grows from a short, horizontal morphology among infants to a long, vertically oriented morphology among adults. Additionally, a survey of human variation and the morphology of individuals with developmental defects (i.e., cleft palate, hydrocephalus, microencephaly) will be analyzed within the respective contexts of climatic adaptation and disease etiology.

3.2 Background on previous morphometric studies of the nasopharyngeal boundaries in relation to adjacent structures.

The nasopharyngeal boundaries are centrally located among the nasal cavity, orbital cavity, oral cavity and oropharynx, external basicranium, cervical vertebrae, and middle ear, likely sharing a functional morphologic relationship with all of these structures. Evolutionary and developmental change in nasopharyngeal morphology would thus impact respiration, olfaction, deglutition, vocal resonance, middle ear aeration, and audition. As such, many studies have covered aspects
of the nasopharyngeal boundaries without focusing directly on their functional integration with other cranial structures. Below is a discussion of these previous studies.

3.2.1 Studies of basicranial flexion

Basicranial flexion may directly influence the dimensions of the nasopharyngeal space as the basicranial axis is its roof. Flexion at both the internal basicranial axis and external basicranial axis (measured using internal and external landmarks, respectively) has been studied. However, the relationship between these two variables has yet to be examined (as noted by Lieberman et al, 2000).

Internal basicranial flexion

Internal basicranial flexion has had a long history of study, spanning possibly as far as Leonardo da Vinci (1452-1519) who illustrated linear axes on a drawing of a mid-sagittally sectioned cranium corresponding to the nasion-sella distance and a perpendicular axis running approximately at the site of the sphenoid-occipital synchondrosis (but Finlay [1980] described this second axis as not corresponding to any skeletal landmarks). However, Virchow (1857), Landzert (1866), and Huxley (1867) were among the earliest to explicitly quantify flexion of the internal basicranial axis. In his classic work on the relationship between the nasion-ethmoidale-basion angle and cranial vault shape, Huxley (1867) concluded that basicranial and neurocranial shape were not functionally related as two crania deemed extremely brachycephalic and mekistrocephalic exhibited similar values for this angle. It was later modified by Duckworth (1904) to the nasion-pituitary point-basion angle, which Cameron (1925, 1927) used in
comparing facial and basicranial dimensions of different human populations and hominoid genera. Growth changes in basicranial flexion were also assessed among non-human hominoids by Ashton (1957) and Krogman (1930, 1931), who measured the nasion-sella-basion angle on radiographs rather than sectioned crania, as in the previous studies.

Aside from studies of comparative morphology, basicranial flexion also became widely studied by orthodontists as part of the suite of cranial growth changes that occur alongside dental malocclusions. Beginning with the specialized apparatus of Broadbent (1931) to collect cephalometric data from lateral head radiographs, many classic studies such as Keith and Campion (1922), Brodie (1941, 1955), and Bjork (1955) examined the interaction of facial and basicranial structures over development, including growth change in basicranial flexion. Others such as Rosenberger (1934), King (1952), and Bergland (1963) focused on areas of the upper respiratory tract and nasopharynx. Some of these early cephalometric studies (e.g. Schuller, 1929; Ricketts 1954; Brader, 1957) even covered cleft palate and other rarer developmental abnormalities of the nasopharyngeal boundaries (see Schulter [1977] for a review of earlier studies on the basicranial axis).

Other studies have tested the spatial packing hypothesis (Biegert, 1963; Gould, 1977) that the greater amounts of internal basicranial flexion characterizing some taxa (e.g., humans, non-human hominoids) relative to other less encephalized primates is an adaptation to creating a greater amount of endocranial volume to accommodate a larger brain. Ross and Ravosa (1993) directly tested this hypothesis by correlating basicranial flexion (measured as the angle made by the intersection of the planes of the clivus occipitale and planum sphenoidale) with the index of relative encephalization (cube root of endocranial volume/internal basicranial length; IRE) among non-human primates. After calculating these on a series of lateral radiographs, they found
that there was a strong and significant ($P<0.05$) correlation among hominoids, catarrhines, platyrrhines, and the total primate sample. However, the correlation was not found to be significant ($P<0.05$) among strepsirrhines.

Ross and Henneberg (1995) applied the angle of basicranial flexion used by Ross and Ravosa (1993) to a similarly expansive series of non-human primates and both contemporary human and fossil hominin crania. They concluded that humans and archaic *Homo* exhibit less basicranial flexion than predicted from regression against IRE (likely constrained by the nasopharynx, which would otherwise become occluded with excessive basicranial flexion). However, Spoor (1997) argued that use of the planum sphenoidale did not encompass the entire basicranial axis. He instead calculated midline flexion at the foramen caecum-sella-basion angle from mid-sagittal CT images, arguing that the midline length of the cribriform plate marked the true anterior boundary of the basicranial axis and was a more reliable measure. While the cribriform plate is derived from cartilaginous precursors and developmentally a component of the growing internal basicranial axis (de Beer, 1937), it comprises a portion of the nasal capsule (van der Klaauw, 1952) and exhibits a closer functional relationship with the facial skeleton at its anterior extent (Moss, 1958). The anterior boundary serves as the functional limit of the midline anterior cranial fossa only in groups such as the hominoids with horizontally oriented cribriform plates. This point has been understood at least since Topinard (1885) distinguished the orientation of the midline cribriform plate in humans and other mammals. Thus use of the foramen caecum in comparison of internal basicranial flexion angles among haplorhine primates and strepsirrhines that possess a vertically oriented cribriform plate and skeletal adaptations to olfactory acuity may not be ideal.
Spoor (1997) found that modern humans did exhibit expected amounts of basicranial flexion relative to encephalization as did the Sts 5 specimen. However, the KNM-WT 17000, OH 5, and Sangiran 17 specimens show greater amounts of basicranial flexion than predicted by encephalization. He concludes that the functional demands of locomotion may be responsible for the observed morphologic patterns. Adaptations to bipedal posture may thus have been a greater factor than encephalization in the evolution of increased internal basicranial flexion among hominins.

Strait (1999) raised the possibility that body mass may also play an indirect role among the primates in determining internal basicranial flexion. Among 87 non-human primate species, he found that relative basicranial length scaled with strong negative allometry against body mass, which was likely related to proportionally smaller non-cortical brain elements that have lesser metabolic demands in larger animals (as per Kleiber's law). Thus, with the denominator of IRE being the basicranial axis, larger animals may require increased basicranial flexion to accommodate a shorter basicranial length, related to smaller non-cortical brain.

Evidence against the spatial packing relationship was produced by Jeffery and Spoor (2002) who studied a sample of 46 formalin-preserved fetuses via high resolution MRI. Midsagittal slices were taken from each radiograph and measured with 10 landmarks on the basicranium and midface. Gestational age was estimated by bi-parietal diameter, which has yielded a sample ranging from 10 to 29 weeks of age. The general trend observed was that the cranial base retroflexes or flattens with age/size as the midface protrudes anterosuperiorly and brain size increased. It appears that facial growth also exerts influence over internal basicranial flexion and must be considered alongside encephalization.
Recent studies suggest that both brain size and facial size act to influence internal basicranial flexion, as shown by Hallgrimsson et al. (2007) and Lieberman et al. (2008) who worked with experimental mice. Three strains of mutants were used as models for a short basicranial axis, an elongated basicranial axis, and increased brain size. Basicranial flexion was quantified using the ventral angle of foramen caecum-midsphenoidal synchondrosis-basion. It was found that relative brain size (calculated without the brain stem) and facial dimensions comprised the majority of sample variance in internal basicranial flexion. The former was responsible for a larger proportion than facial size which, among primates with greater variation in relative brain size than the inbred murine sample, may often comprise a greater proportion of interspecific variation in internal basicranial flexion. When considering the influence of both extreme encephalization and orthognathy on internal basicranial flexion among humans, both of these factors must be accounted.

Bastir et al. (2010) combined geometric morphometrics of lateral radiographs and mid-sagittal CT images with multiple regression analysis to distinguish the effects of brain size (its cube root) and several measures of facial size on internal basicranial flexion. They tested the hypotheses that brain size accounts for the greater amounts of basicranial flexion characterizing hominins relative to other primate groups and that variation in facial dimensions among equivalently encephalized hominin species produces the relatively non-flexed basicrania of Neanderthals and archaic Homo as opposed to basicranially flexed, orthognathic modern humans. Multiple regression analysis and thin plate spline analysis both show that as brain size increases, the angles made from the projected intersections among the spheno-occipital clivus, presphenoid plane, and cribriform plate all decrease. Increasing facial size led rotation of the basicranium away from the face and slight flexion of its axis. This is convincing evidence that
both brain size and facial morphology act to influence internal basicranial flexion among the primates, especially highly encephalized, orthognathic *Homo sapiens*. Bastir et al. (2010) corroborated the results of Biegert (1957) who supported a similar model for internal basicranial flexion in which relative brain and facial size interacted to increase or decrease flexion, respectively.

Alongside facial morphology and brain size, the functional demands of bipedal locomotion and the anterior placement of foramen magnum (Daubenton, 1764; Bolk, 1909; Schultz, 1955; Ahern, 2005) should be considered another likely source of influence over internal basicranial flexion. Trends toward facial orthognathy began well before increased encephalization came to characterize the genus *Homo* (e.g. Rak, 1983; Kimbel et al, 2004). This may reflect both dietary shift from ancestral hominoids as well as an adaptation to bipedal locomotion as it reduces the cranial mass balanced anterior to the foramen magnum and cervical vertebrae, on which the head pivots (see Schultz, 1955). The dimensions of the nasopharynx may also be intimately tied to these changes as Bosma (1963) and Moss and Salentijn (1969) agree that its functional demands influence head posture in response to the vital need for maintaining airway patency. The nasopharynx may thus have been one of several functional matrices interacting with internal basicranial flexion and other cranial adaptations to bipedal locomotion over human evolution.

**External basicranial flexion**

Most studies of external basicranial flexion have focused on the dimensions of the supralaryngeal vocal tract. As discussed by Escat (1894) and Takagi et al. (1962), this directly
influences the dimensions of the nasopharynx. The relationship between external basicranial flexion and soft tissue morphology of the pharyngeal boundaries was seldom directly discussed before Laitman and Crelin (1976), Laitman (1977), and Laitman et al. (1979, 1982) quantified several external measures of midline flexion in growth series representing human and non-human hominoids, *Macaca*, and fossil hominins. They found that non-human primates and human infants exhibit both non-flexed basicranial axes and a high placement of the larynx, creating a separation of the airway and alimentary tract. Adult humans, however, are basicranially flexed with low placement of the larynx and a "one-tube aerodigestive tract" in which the airway and alimentary tract share space. This configuration renders humans more susceptible to choking, aspiration of vomitus in cases of gastroesophageal reflux, and sleep apnea (caused by downward displacement of the tongue; Davidson et al., 2005).

Reidenberg and Laitman (1991) directly tested the relationship between external bascranial flexion and laryngeal position. Neonate rats were subjected to three treatments: surgical ablation of the spheno-occipital synchondrosis (SOS; which produces premature synostosis and increased basicranial flexion), the surgical exposure of the SOS without ablation (shams), and no surgical intervention. It was shown through the calculation of angular relationships that individuals subjected to surgical treatment experienced significant laryngeal descent as there was much flexion at the SOS. Normal controls rats maintained the high position characteristic of their species along with flat basicrania. Individuals from the sham groups showed moderate descent of the larynx, suggesting that the disturbance of muscle attachments as well as basicranial flexion can lead to laryngeal descent.

The functional relationship between external basicranial flexion and laryngeal placement was called into question by Lieberman and McCarthy (1999). They tested whether laryngeal
descent in humans continued after the majority of external basicranial flexion had been completed after approximately two years of age (as previously reported; e.g. Laitman, 1977). Laterally-oriented radiographs from the Denver Growth Study were measured for vertical height of the vocal folds, five separate measures of internal and external basicranial flexion, and several measures of the face and lateral mandible. It was determined that the majority of basicranial flexion occurred between three and six years of age and that the vocal folds of the larynx continued their descent well after this interval, offering evidence against the functional relationship between flexion at the sphen-no-occipital synchondrosis and laryngeal placement. However, radiography is not an optimal technique for visualizing soft tissue structures (such as the larynx), as they are not dense enough to appear radio-opaque. The amounts of KVP (kilovolt potential, a measure of image refinement) were not specified. Furthermore, bilateral mandibular landmarks are rendered unreliable since there was no true control for parallax. The one tantalizing detail from this study is that the external basicranial flexion angle hormion-sphenobasion-basion was correlated against several internal basicranial flexion angles and showed a weak relationship. This point requires future study so that the functional relationship between encephalization (producing internal basicranial flexion) and pharyngeal growth (related to external basicranial flexion) may be better understood.

3.2.2 Facial orientation and kyphosis

Facial kyphosis is usually defined as the ventral deflection of the facial skeleton relative to the neurocranium. While rotation of the facial skeleton was discussed as early as the mid-nineteenth century (e.g. Virchow, 1857; Baer and Lucae, 1861), it was Hofer (1952) who formally defined
“klinorhynchie” as the rostroventral rotation of the nasal floor/hard palate relative to the basicranial axis and “airorhynchie” as rostrodorsal rotation. He noted that the positioning of the foramen magnum is variable among klinorhynch and airohynch crania as animal models of the former variety exhibit basicrania that appear more flexed while the latter appeared non-flexed or retro-flexed. He quantified this variable as the intersection of the plane of the dorsal hard palate and the plane of the occipital clivus using both avian and mammalian models. Starck (1953) further discussed this concept using a wide range of primate and non-primate mammalian models. These were followed by Biegert (1957) who devised a model in which both brain size and facial size influenced basicranial flexion. He argued that, as facial size decreases relative to brain size, the basicranium becomes more flexed. Later supporting evidence would come from thin-plate spine analysis of primate crania (Bastir et al., 2010) and from work on genetically altered, encephalized mutant mice (Lieberman et al., 2008).

Ross and Ravosa (1993) have argued against a relationship between palatal orientation and internal basicranial flexion, instead finding one between the latter and orbital orientation. Studies by Bookstein et al. (2003) and Bastir et al. (2010) further investigated this issue with thin plate spline analysis of lateral radiographs and described facial size in both the vertical and horizontal dimensions rather than relying on a single measure (e.g. palate length). They found that overall facial size only shares a relationship with certain measures of basicranial flexion (i.e. intersection of the planum ethmoidale and occipital clivus). In samples of archaic Homo, a modern human growth series (Bookstein et al., 2003), and non-human primates, they describe a resulting morphospace in which the basicranial axis rotates ventrally away from the face as it becomes relatively larger in non-human primates and archaic Homo relative to adult modern humans. Nonetheless, Bookstein et al. (2003) find no facial-basicranial reorientation in the
human growth series, instead observing growth changes in facial dimensions between three
months of age and adulthood. These results may suggest that the functional demands of
increased facial size may necessitate reorientation between it and the anterior basicranium, thus
simultaneously decreasing facial kyphosis and increasing the depth of the nasopharynx.

Perhaps the most direct discussion of the interaction of facial orientation and
nasopharyngeal dimensions was by McCarthy and Lieberman (2001). They quantified the
"posterior maxillary plane," which is defined in norma lateralis as the vertical line between the
posterior maxillary tubercle and the anterior-most point on the greater wing of the sphenoid (as
determined by Enlow and Azuma, 1975) on a wide sample of lateral radiographs from human
and non-human primate crania. It was found that the respective angles of the anterior cranial base
(sella-posterior cribiform plate measure) and the anterior cranial floor (sella-foramen caecum
measure) with the posterior maxillary plane correlate well with each other. McCarthy and
Lieberman (2001) concluded that these comprise a "facial block" whose rotation over growth and
evolution may act to influence nasopharyngeal dimensions. However, they did not collect any
measures of the osseous nasopharyngeal boundaries nor did they directly test this hypothesis.
The functional relationship between the maxillary tubercles and the medial pterygoid plates
were, however, tested by Subtelny (1955) in normal human infants and those with cleft palate.
He found strong and significant ($P<0.01$) correlations between posterior maxillary width and bi-
hamular width, suggesting a functional integration of nasopharyngeal boundaries with facial
orientation (and likely width) at the pterygomaxillary junction.

There exists some functional evidence to suggest that rotation of such a facial block may
indeed occur at the spheno-ethmoid junction, encompassing the cribiform plate. Hoyte (1976)
jected a sample of rabbits, rats, and guinea pigs with alizarin red dye and sacrificed them at
various postnatal ages so that a cross-sectional view of ethmoidal growth could be established. It was found that growth at the cribriform plate and sphenoid-ethmoid synchondrosis continued after birth in rabbits (but not the other animal models) and thus contributes to their comparatively kyphosed faces. The sphenoid-ethmoid complex may arguably serve as the junction between the neurocranium and nasal complex, with sustained growth at this intersection influencing facial and basicranial growth and angulation. Nonetheless, these trends were not quantified. This study will be among the first to directly test for three-dimensional, functional morphologic relationships between aspects of facial orientation and nasopharyngeal and facial dimensions via quantification and qualitative assessment via wireframe models.

3.2.3 Vertical Facial growth and choanal dimensions

It may be argued that vertical facial growth is related to choanal height. In particular, the functional role of the vomer (which divides the left and right choanae) in vertical and anteroposterior facial growth has been a source of controversy. Scott (1953, 1959, 1963) argued that the vomer acted to push adjacent boundaries of the nasal capsule over fetal and postnatal growth, causing expansion along its sutural boundaries. These were hypothesized to result in superoinferior and anteroposterior expansion of the midface. Experimental studies by Wexler and Sarnat (1961, 1965) and Sarnat and Wexler (1966) were perceived to have corroborated this hypothesis. They subjected rabbits to varying amounts of vomeral ablation ranging from dislocation to complete removal. The resulting deformities varied in severity with the amount of vomeral removal and were described as the interruption of expansive growth at the vomer. Moss et al. (1968) interpret these results instead as the interruption of the vomeral pillar, which braces
the nasal cavity while expansion is related to the functional demands of maintaining patency for respiration. These functional demands promote increased growth and modeling of surrounding bony tissues in an epigenetic process, rather than genetically regulated growth at sutural surfaces. They call attention to the uninterrupted midfacial morphology that develops in spite of vomeral dislocation. They replicated this experiment on growing rats and found that, in cases of complete vomeral removal, the maxilla and premaxilla continue to grow normally while the vector of midfacial growth is abnormally redirected in the presence of nasal cavity "collapse." Thus vertical and anteroposterior midfacial growth is not achieved by vertical forcing from the vomer.

3.3 Hypotheses

This chapter tests the hypothesis that the osseous nasopharyngeal limits are morphologically related to facial length and kyphosis and to external basicranial flexion. Being at the center of several functional anatomical units, it may interact with all of these structures and morphologic changes therein. The following are corollary hypotheses that, if accepted, will support the broader hypothesis outlined here.

Hypothesis #1: Humans over all periods of postnatal ontogeny possess greater relative external basicranial (nasopharyngeal) width compared to other anthropoid species because of facial orthognathy and kyphosis.
*Homo sapiens* and its extinct relatives may be defined by the greatest amounts of facial kyphosis (Ross and Henneberg, 1995; Spoor, 1997) and orthognathy (Dean and Wood, 1981; Lynch et al., 1996; Kimbel and Rak, 2004) relative to any other primate group. These aspects of human anatomy render an ideal anatomic model for testing the relationship between facial morphology and the dimensions of the nasopharyngal boundaries. The comparative non-human primate sample ranges from models of extreme facial prognathism (*Papio*) to models of moderate prognathism (*Pan, Cercocebus*).

**Hypothesis #2:** The nasopharynges of basicranially flexed modern human adults are antero-posteriorly short and vertically high, growing from a long, low shape in infants.

Human adults and infants serve as ideal anatomic models for the functional morphologic relationship between external basicranial flexion and naspharyngeal dimensions as prognathism remains constant over human development relative to most other mammals (Hofer, 1952; Starck, 1953; Enlow, 1975; May and Sheffer, 1999). Human adults exhibit marked amounts of basicranial flexion relative to infants, who possess a flatter osseous upper respiratory tract roof similar to the non-human primates.

**Hypothesis #3:** Human infants are characterized by a relatively shorter cartilaginous Eustachian tube, which is horizontally oriented relative to the adult condition.

As reviewed above (see section 2.5.1), human infants and young children suffer from high rates of otitis media whereas adults seldom exhibit this condition. It has been widely argued that the
morphology of the cartilaginous Eustachian tube (CET) is responsible for this phenomenon as infectious agents originating from the nasal cavity and paranasal sinuses gain easier access to the middle ear cavity via the short, horizontally oriented CET's of infants and young children (Bluestone, 2005). This study will estimate the relative size and position of the CET from osseous landmarks and describe growth change in its scaled length and orientation between birth and adulthood.

3.4 Materials and Methods

3.4.1 Materials

In testing for growth changes and functional morphologic relationships among structures comprising the osseous nasopharyngeal boundaries, human and non-human anthropoid primates were utilized as anatomical models. Anthropoids lack a nasopharyngeal duct and other, concomitant skeletal adaptations for olfactory acuity (though some exhibit great olfactory acuity). Thus they all possess nasopharyngeal boundaries comprised of homologous skeletal elements, making them ideal for testing hypotheses on the functional morphology of these structures. Multiple species were sampled within each genus but were pooled as this study is concerned with large-scale morphological and growth changes in upper respiratory tract structures. A similar approach has been used in previous studies of external basicranial flexion and osseous upper respiratory tract morphology (e.g. Laitman et al. 1979, 1982; Pagano et al., 2010).
Three-dimensional landmark coordinate data were collected on several samples of dry crania representing these groups. They were gathered from several large collections (See Table 3.1) and assigned developmental stages by dental eruption to create growth series. Growth was divided into Stage 1 (newborn, before the eruption of the deciduous dentition), Stage 2 (eruption of the deciduous dentition only), Stage 3 (time of eruption of the M1), Stage 4 (time of eruption of the M2), and Stage 5 (time of eruption of the M3). This system of growth stages is adapted from Hellman (1927) and Ashton (1957) as they were shown to roughly correspond to periods of facial and basicranial growth among human and non-human hominoid genera, respectively (e.g. Laitman, 1977). The sample is comprised of separate growth series for each of the sampled genera so that differences in the developmental trajectories of osseous nasopharyngeal boundary morphology may be contrasted among them.

Among the anthropoids, both hominoids and papionins are characterized by aspects of cranial anatomy that make them ideal for testing several hypotheses on the functional integration of nasopharyngeal boundaries with other upper respiratory tract and facial structures.

Table 3.1 Sample sizes arranged by genus and developmental stage. A total sample of 1336 crania were used.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo</td>
<td>28</td>
<td>127</td>
<td>73</td>
<td>49</td>
<td>298</td>
</tr>
<tr>
<td>Pan</td>
<td>15</td>
<td>29</td>
<td>29</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Gorilla</td>
<td>13</td>
<td>20</td>
<td>24</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Pongo</td>
<td>5</td>
<td>20</td>
<td>21</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Hylobates</td>
<td>9</td>
<td>26</td>
<td>21</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Papio</td>
<td>12</td>
<td>24</td>
<td>14</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Macaca</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Cercocebus</td>
<td>5</td>
<td>23</td>
<td>23</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Lophocebus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
</tr>
</tbody>
</table>
Homo sapiens

Humans are of interest to this study not only for clinical issues that impact nasopharyngeal function, but also as anatomic models for extreme orthognathy and basicranial flexion. Humans are the most orthognathic primates, possibly as a result of adaptations to speech (e.g. Laitman et al., 1979, 1982), locomotion (Schultz, 1955), and encephalization (Weidenreich, 1941). They also undergo extreme amounts of growth change in the upper respiratory tract (e.g. Laitman, 1977; Vorperian et al., 1999, 2005), including the cartilaginous Eustachian tube (Bluestone, 2005), while the vertical dimension of the facial skeleton experience marked growth change (Enlow, 1975). These all lead to a remodeling of the upper airways whose comprising structures may all share a functional morphologic relationship.

Non-human hominoids (Pan, Gorilla, Pongo, Hylobates)

The greater and lesser apes serve as unique anatomical models for the osseous nasopharyngeal upper respiratory tract boundaries as they possess greater basicranial flexion relative to other non-human anthropoids (Ross and Henneberg, 1995; McCarthy, 2001), which may be a function of increased body mass (Strait, 1999). They also share a close evolutionary relationship with humans, allowing for the assessment of phylogenetic polarity.

Specimens included in this study represented Pan troglodytes, Gorilla gorilla, Pongo pygmaeus, Hylobates agilis, Hylobates hoolock, Hylobates lar, Hylobates mulleri, and Hylobates symphalangus.
Papionins (*Macaca, Cercocebus, Lophocebus, Papio*)

The papionins will be used as models for pronounced facial prognathism and airorhynchy. They range from moderately long faces (*Macaca, Cercocebus, Lophocebus*) to extremely long faces (*Papio*; see Collard and O’Higgins [2001] for a review of evolutionary relationships and phylogenetic polarity of facial length among papionin genera). It has been suggested that this extreme facial prognathism is the end result of intense intrasexual selection (Harris, 2000; Harris, 2002). In turn, developmental studies have suggested that prognathism may be related to multiple allometric growth vectors (Gould, 1966; Gould, 1971; Cheverud, 1982; Shea, 2000; Collard and O'Higgins, 2001; Singleton, 2002; Leigh, 2006).

Specimens included in this study represented *Macaca fascicularis, Macaca mulatta, Cercocebus agilis, Cercocebus torquatus, Lophocebus albigena, Papio anubis, Papio cynocephalus, Papio hamadryas.*

**Samples**

The human sample was from the American Museum of Natural History (New York) and the Spencer R. Atkinson Collection, University of the Pacific School of Dentistry (San Francisco). The former collection consisted of adults from several geographic regions, representing multiple human populations (see Table 3.2). These were meant to model separate climatic stresses and frequencies of upper respiratory tract disease [see Marquez et al. (in press) who used this sample to represent human populations from cold and warm climates]. Most of these populations are pre-industrial and lacked access to modern means of transportation. Gene
flow and mobility is thus presumed to have been limited for most of these groups relative to modern populations, rendering a more reliable test of climatic variables.

Human growth changes were assessed in the Atkinson Collection, which includes many well preserved infant and child crania. The collection is comprised of individuals from Mexico, Peru, India, China, Europe, and the United States with others of undetermined provenance (n=264; Dechant, 2000). Also present were adult and sub-adult crania presenting with deformities such as hydrocephalus and cleft palate. These were included in the analysis for the purpose of assessing the functional malleability of the upper airways sensu Moss et al. (1968).

Despite variation among human populations in craniofacial ontogeny (e.g., Strand Vidarsdottir and Cobb, 2004; Smith et al., 2007), this study used a growth series comprised of individuals from multiple populations. The focus of this work is the analysis of growth trends that characterize humans as a species. All human groups are distinct from other non-human hominoids in exhibiting extreme orthognathy and external basicranial flexion. Yet, the developmental impact of these conditions on the morphology of the nasopharyngeal boundaries has been understudied.

Table 3.2 Sample sizes for Stage 5 human adults arranged by population and sex.

<table>
<thead>
<tr>
<th>Population</th>
<th>Males</th>
<th>Females</th>
<th>Unknown Sex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>19</td>
<td>13</td>
<td>37</td>
<td>69</td>
</tr>
<tr>
<td>Aleutian Islands</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>China</td>
<td>37</td>
<td>8</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>South East Asia</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Australia</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>25</td>
<td>6</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>India</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>North Africa</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>West Africa</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>East Africa</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>67</td>
<td>61</td>
<td>298</td>
</tr>
</tbody>
</table>
The non-human primate sample was collected from the American Museum of Natural History (New York), the National Museum of Natural History (Washington, D.C.), the Cleveland Museum of Natural History (Cleveland, Ohio), and the Museum of Comparative Zoology (Cambridge, Massachusetts). These do not include any Stage 1 specimens, instead extending from Stage 2 to adulthood. No Stage 1 individuals representing any non-human hominoid genus were available in any of the above collections. As the human sample does include Stage 1 crania, a greater proportion of its developmental trajectory is represented here than among the other primates.

3.4.2 Sexual dimorphism

Prior to data analysis, tests of sexual dimorphism were performed for each of the linear measures (see Methods below) to determine whether adult samples should be segregated by sex rather than pooled. A series of Student's t-tests were performed between male and female adults from each genus (see Table 3.3). These variables were considered in subsequent testing for growth changes and, when dimorphism was present, Stage 5 males and females were segregated.

Gorilla was the only hominoid to exhibit sexual dimorphism, which was in choanal height (greater in males; \( P<0.05 \)) and bi-ovale width (greater in females; \( P<0.05 \)). The papionins, however, exhibited substantially greater sexual dimorphism with Papio being the most dimorphic genus. Males possessed significantly \( (P<0.05) \) greater relative choanal height, relative nasal height, relative palate length, and external basicranial flexion. Females had significantly \( (P<0.05) \) greater relative choanal width and index, relative bi-ovale width, and relative bi-carotid width. Female Macaca exhibited significantly \( (P<0.05) \) greater relative choanal width and nasopharyngeal roof depth while males exhibited greater nasion-nasospinale height, and basi-
occipital length. Female *Cercocebus* had significantly (*P*<0.05) greater relative distance between the left and right anteromedial apices of the quadrate area of the temporal bone, suggesting a wider basicranium. *Lophocebus* was the only papionin genus with no significant (*P*<0.05) sex differences in any of the nasopharyngeal or upper respiratory tract dimensions.

**Table 3.3** Analysis of differences between sexes for univariate measures. Note: “*P*; Male” or “*P*; Female” indicate that the respective male or female mean is greater when there is a significant (*P*<0.05) difference. Bolded text indicates significance (*P*<0.05).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Gorilla</th>
<th>Macaca</th>
<th>Cercocebus</th>
<th>Papio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choanal Width</td>
<td><em>P</em>=0.864</td>
<td><em>P</em>=0.013; Female</td>
<td><em>P</em>=0.683</td>
<td><em>P</em>=0.003; Female</td>
</tr>
<tr>
<td>Choanal Height</td>
<td><em>P</em>=0.022; Male</td>
<td><em>P</em>=0.661</td>
<td><em>P</em>=0.517</td>
<td><em>P</em>=0.056</td>
</tr>
<tr>
<td>Bi-Ovale Width</td>
<td><em>P</em>=0.024; Female</td>
<td><em>P</em>=0.249</td>
<td><em>P</em>=0.301</td>
<td><em>P</em>=0.021; Male</td>
</tr>
<tr>
<td>Bi-Carotid Width</td>
<td><em>P</em>=0.951</td>
<td><em>P</em>=0.024; Female</td>
<td><em>P</em>=0.925</td>
<td><em>P</em>=0.065</td>
</tr>
<tr>
<td>Bi-Jugale Width</td>
<td><em>P</em>=0.817</td>
<td><em>P</em>=0.315</td>
<td><em>P</em>=0.553</td>
<td><em>P</em>=0.002; Female</td>
</tr>
<tr>
<td>Palate Length</td>
<td><em>P</em>=0.437</td>
<td><em>P</em>=0.103</td>
<td><em>P</em>=0.084</td>
<td><em>P</em>=0.012; Male</td>
</tr>
<tr>
<td>Nasopharyngeal roof depth</td>
<td><em>P</em>=0.568</td>
<td><em>P</em>=0.048; Female</td>
<td><em>P</em>=0.612</td>
<td><em>P</em>=0.465</td>
</tr>
<tr>
<td>Choanal Index</td>
<td><em>P</em>=0.253</td>
<td><em>P</em>=0.0631</td>
<td><em>P</em>=0.631</td>
<td><em>P</em>=0.009; Female</td>
</tr>
<tr>
<td>Angle of external basicranial flexion</td>
<td><em>P</em>=0.153</td>
<td><em>P</em>=0.556</td>
<td><em>P</em>=0.545</td>
<td><em>P</em>=0.044; Female</td>
</tr>
<tr>
<td>Bi-Petrous Apex Width</td>
<td><em>P</em>=0.77</td>
<td><em>P</em>=0.565</td>
<td><em>P</em>=0.00098; Female</td>
<td><em>P</em>=0.063</td>
</tr>
</tbody>
</table>
3.4.3 Methods

Landmarks

A landmark may be defined as any point on a biological form that is reliably measured on each specimen in the same way and maintains some degree of homology (see below) among all specimens within a sample. For this chapter, a total of 38 landmarks were used, 18 being standardized craniometric points (e.g., Martin 1928). The others were collected along the external surfaces of the splanchnocranium and basicranium and were chosen to outline both the nasopharyngeal boundaries and other structures such as the foramen magnum, basicranial axis, hard palate, piriform aperture, and bony orbits. An additional three bilateral landmarks were collected only on the human growth series as they were created for use in measures of growth change in the orbits, facial width, and cartilaginous Eustachian tube dimensions (see Synthesized Measures below).

Table 3.4 Landmarks used in the geometric morphometrics analyses. Note: *landmarks used only in the study of human development.

<table>
<thead>
<tr>
<th>Upper Respiratory Tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Prosthion (endoprosthion)</td>
</tr>
<tr>
<td>2) Intersection of the horizontal plates of the palatine bones with the palatine processes of the maxillae in the midline</td>
</tr>
<tr>
<td>3) Staphyion</td>
</tr>
<tr>
<td>4) Hormion</td>
</tr>
<tr>
<td>5) Sphenobasion</td>
</tr>
<tr>
<td>6) The point indicating the midline position of the pharyngeal tubercle (one-third of the distance from exobasion to hormion)</td>
</tr>
<tr>
<td>7) Basion (exobasion)</td>
</tr>
<tr>
<td>8) Left most posteroinferior point on the alveolar process of the maxilla (typically at the maxillary tubercle)</td>
</tr>
<tr>
<td>9) Right most most posteroinferior point on the alveolar process of the maxilla (typically</td>
</tr>
</tbody>
</table>
10) Left superior-most point on the posterior margin of the medial pterygoid plate
11) Right superior-most point on the posterior margin of the medial pterygoid plate
12) Left inferior-most point on the posterior margin of the pterygoid plate (just superior to the base of the medial pterygoid hamulus)
13) Right inferior-most point on the posterior margin of the pterygoid plate (just superior to the base of the medial pterygoid hamulus)
14) Left lateral-most midpoint point on the choanal margin (the medial pterygoid plate)
15) Right lateral-most midpoint point on the choanal margin (the vertical midpoint on the posterior medial pterygoid plate edge)
16) Left posterior-most point on the rim of the foramen ovale
17) Right posterior-most point on the rim of the foramen ovale
18) Left anteromedial-most point on the petrous temporal (its apex)
19) Right anteromedial-most point on the petrous temporal (its apex)
20) Left anteromedial-most point on the external rim of the carotid canal
21) Right anteromedial-most point on the external rim of the carotid canal

**Cranial Vault**

22) Left porion
23) Right porion
24) Opisthion

**Face**

25) Nasospinale
26) Rhinion
27) Nasion
28) Glabella
29) Left zygomaxillare (the most inferior point on the zygomaticomaxillary suture)
30) Right zygomaxillare (the most inferior point on the zygomaticomaxillary suture)
31) Left lateral-most point on the rim of the nasal aperture
32) Right lateral-most point on the rim of the nasal aperture
33) Left jugale (the point in the greatest depth of the notch between the temporal and frontal processes of the zygomatic bone)
34) Right jugale (the point in the greatest depth of the notch between the temporal and frontal processes of the zygomatic bone)
35) Left ectoconchion (most lateral point on the orbital margin)
36) Right ectoconchion (most lateral point on the orbital margin)
37) Left medial-most point on the orbital rim
38) Right medial-most point on the orbital rim

**Landmark coordinates collected only on humans**
(denoted by "*" on Figure 3.1)

39) *Left posterior border of canine tooth at the alveolus
40) *Right posterior border of canine tooth at the alveolus
<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>*Left inferior-most point on optic foramen rim</td>
</tr>
<tr>
<td>42</td>
<td>*Right inferior-most point on optic foramen rim</td>
</tr>
<tr>
<td>43</td>
<td>*Left Eustachian point (just inferior to the osseous Eustachian tube orifice rim at the junction of the sphenoid spine and tympanic plate of the temporal bone)</td>
</tr>
<tr>
<td>44</td>
<td>*Right Eustachian point (just inferior to the osseous Eustachian tube orifice rim at the junction of the sphenoid spine and tympanic plate of the temporal bone)</td>
</tr>
</tbody>
</table>
Figure 3.1a Basal landmarks used in the study of hominoids and papionins. Note the * indicates landmark coordinates collected solely on the human sample.
**Figure 3.1b** Facial landmarks used in the study of hominoids and papionins. Note that * indicates landmark coordinates collected solely on the human sample.
Degrees of homology

Landmark coordinates were used to quantify morphological differences among genera and growth stages and between sexes. Their locations were chosen to represent points of homology. In the evolutionary sense (historical homology; vanValen, 1982), homology denotes a structural likeness originating from shared inheritance from a common ancestor. Developmental or continuous homology (vanValen, 1982) within an individual refers to morphological similarity arising from growth via the same developmental process. The two definitions can, at times, grade into each other as the developmental origins of like structures among separate individuals may be inherited from a common ancestor (as in comparisons of growth trajectories among separate taxa). Thus, homology in the broader sense informs selection of landmarks in morphometric studies.

Within 3D geometric morphometrics, landmarks are ranked by degrees of homology. They are divided into three categories (e.g. Bookstein, 1993; O'Higgins, 2000):

Type I landmarks are constricted in all three dimensions and are of the greatest homology (e.g., the intersection of two sutures on a cranium).

Type II landmarks are geometrically reproducible and functionally similar though they may not be of strict developmental or evolutionary homology (e.g. the tip of a tooth or a wing).
Type III landmarks are the least constrained in that their coordinates are deficient in at least one dimension. That is, they may be localizable to a two-dimensional outline but not to an exact three-dimensional point (e.g., the tip of a raised bump or protuberance).

It was the aim of this study to include as many Type I landmarks as possible. However, Type II landmarks are also used to denote the functional boundaries of irregularly shaped structures such as the choanal boundaries or basicranial axis.

**Measurement technique**

The morphology of the osseous nasopharyngeal boundaries was quantified using 3D landmark coordinates and digitized with a Microscribe G2 Digitizer, a portable device comprised of a stylus at the end of a robotic arm with three joints. This arm has attached a counterweight and is pivoted on a rotating base. A network of optical encoders inside each joint of the robotic arm communicates with a microchip in the base of the instrument to send signals denoting the angle of rotation and flexion/extension at each joint. These are then used to calculate the position of the stylus tip in three-dimensional space to assign X, Y, and Z coordinates. These coordinates are registered via a foot pedal which signals the device to save a given stylus position (encoded as a 3D coordinate) into a computer file (Immersion Corporation, 2007).
Geometric Morphometrics Analysis

The digitized coordinates underwent superimposition and scaling (Procrustes fitting) using the program *Morphologika2* (Version 2.5, O’Higgins and Jones, 2006). Procrustes fitting involves the registration of a series of landmark configurations (defined by their X, Y, and Z coordinates) by optimally superimposing and scaling. In the former step, translational and rotational differences (i.e., artifacts of positional variation from the digitization process) are eliminated by spatially fixing all forms relative to all others. This is done by finding the positional configuration that entails the smallest sum of squared distances between homologous landmarks. Thus, a form space is created in which size differences and shape variation are preserved. The next step involves scaling the superimposed forms using centroid size as a metric. The centroid size is calculated as the square root of the sum of squared Euclidean distances from each landmark to the centroid (the mean of the landmark coordinates). It is through this final step that size differences between all specimens are removed so that only shape variation remains, creating a shape space.

All superimposed and scaled sets of landmark coordinate data underwent principal components analysis (PCA) after GPA, a technique shown to have high statistical power in practical applications (Rohlf, 2000) and is useful in assessment of overall shape diversity among multiple groups. Each resulting principal component is an eigenvector of the covariance matrix of total shape variance. The resulting PCA plot is a tangential projection from a multi-dimensional (Kendall's) shape space (see Rohlf, 1996; Slice, 2001). The first principal component encompasses the greatest proportion of total shape variance, with each consecutive principal component representing orthogonal vectors of progressively smaller amounts. Each
point on the PCA plot has a coordinate value on each of these axes, representing an individual’s relative value in each vector of deviation from the mean of the Procrustes-corrected landmark coordinates (O’Higgins and Jones, 1998, Mitteroecker and Gunz, 2009). The distribution of shape differences across each principal component can be visualized via point clouds, wireframes (lines connecting separate landmarks to give an outline of the overall structures measured), or thin plate splines (a series of rectangular plates, each spanning between four landmarks at each respective plate corner). Wireframes were chosen as the upper respiratory tract structures examined are of complicated morphology that would otherwise be obscured by point clouds or thin plate splines.

Univariate measures

PCA has great utility both in visualizing shape differences between groups and summarizing overall shape variance within a comparative sample. Yet, despite these advantages, PCA is primarily a technique of qualitative assessment of morphological diversity between and within groups as finer shape differences (e.g. relative facial length, basicranial flexion) may only be described from landmark arrangements and are not typically quantified. Thus data analysis in Morphologika is limited to visualization of shape space and concomitant shape changes in landmark coordinate sets over principal components axes, which are graphically displayed (e.g. wireframe models). For quantitative analysis it was thus necessary to calculate linear distances from the Procrustes-fitted (i.e. scale-free) landmark coordinate data that was used to generate principal components plots. The computer program SAS (Statistical Analysis System; SAS
Institute Inc.) was used to calculate these linear measures. The formula used to calculate the distance between two landmarks was:

\[
\sqrt{(X_1-X_2)^2 + (Y_1-Y_2)^2 + (Z_1-Z_2)^2}
\]

The derivation of linear measures from Procrustes-corrected landmark configurations holds two advantages over absolute distance measured with calipers. The first is the ability to directly compare length between any two homologous points on a scaled set of landmark coordinates. With manual caliper measurement, one must subsequently scale a given linear measure over another so that the influence of size may be mitigated. This is a disadvantage as the correlation between ratio and scaling variables is greater than zero, thus scaling with ratios introduces further size variation (e.g., Atchley et al., 1976; Jasienski and Bazzaz, 2009). The calculation of distances from Procrustes-corrected landmark coordinates, however, eliminates the need to scale over any single dimension because the centroid size (the metric used for scaling) is equally uncorrelated with all dimensions (e.g. Rohlf and Marcus, 1993; Adams et al., 2004; Mitteroecker and Gunz, 2009). A second advantage of using landmark coordinate data is that it allows for the synthesis of new measures between previously registered landmark locations. The implementation of new measures with calipers would require revisit of each specimen, a time-consuming endeavor.

After calculating distances, subsequent calculations of surface area, indices, volumes, and angular measurements were performed. Angular measurements were calculated from linear distances using the law of cosines:

\[
\cos(\gamma) = (a^2 + b^2 - c^2)/2ab
\]
Observer Error

Data collection was performed primarily by the author (ASP) but a portion of the data was also collected by a Master’s student who was trained by ASP in the protocol used for the current study. This was done so that a sufficient sample could be gathered in the limited time allotted for collection. To assess interobserver error, 12 human crania were digitized by the trained graduate student and by ASP on separate days. Procrustes distances between these two sets of measures collected from each specimen were calculated using Morphologika2 so that overall shape difference could be assessed. Most were small (≤0.0772) with only one pair of observations exhibiting an abnormally large distance (0.115871), likely as a result of measurement error. These distances were then compared to the Procrustes distances between different specimens in a separate human pilot sample (n=12) digitized by ASP. The interobserver distances were larger than only six (4.545%) of the 132 inter-specimen distances from this second pilot sample. Thus, interobserver error was considered low enough to justify pooling of data from both observers.

After all data collection was completed, a post hoc test of interobserver error was used (adapted from Terhune et al., 2007). Procrustes distances between each pair of specimens from the total hominoid sample (n=854) were calculated. This entailed a total of 264,130 separate Procrustes distances. When compared to this larger set of inter-specimen distances, the interobserver Procrustes distances were larger than only 1.28724% of the values. The results of this post hoc test thus confirmed that interobserver error was sufficiently low.

Intraobserver error was assessed on every 25 specimens by running a PCA on the Procrustes-corrected data and using a plot with PC 1 comprising the X axis and PC 2 comprising
the Y axis. If any of the individuals exhibited an extreme distance from congeners on the PC plot (exceeding twice the greatest distance within the cluster) or if landmark order appeared switched from visualization of a numbered point cloud diagram, that specimen was re-measured. Both observers employed this method. Re-measurement became less frequent as they became more familiar with the protocol.

**Measuring choanal dimensions** - Most studies (e.g., Lang and Baumeister, 1982; Franciscus and Long, 1991; Franciscus, 2003; Marquez and Laitman, 2008) used Martin’s (1928) protocol as a guideline to measure choanal width and height. As outlined by Martin (1928), choanal width should be measured by the greatest distance between the medial pterygoid plates, and choanal height by the distance from the posterior border of the horizontal plate of the palatine bone to the base of the pterygoid process on the right and left sides. Although endpoints for these distances are not exactly specified, these descriptions are suitable for studies using calipers. Hence, some variation in their relative locations among specimens would not confound such an analysis. The Microscribe used in this study is designed to collect fixed points rather than linear measures of distance. The exact position of these fixed points must be tightly constrained to preserve homology between specimens.

To measure choanal height and width, fixed points were used along the medial pterygoid plates and vomeral articulations. The line joining staphylion and hormion is parallel to the main direction of the medial pterygoid plates and is thus a reliable proxy for choanal height that is not influenced by choanal asymmetry. This measure is also used to represent the angle of inclination of the choanal plane relative to the basicranial axis, calculated between the measures staphylion-hormion and hormion-sphenobasion. The latter was chosen as it exhibits little to no flexion
relative to the hormion-basion distance and represents the shallowest anteroposterior depth of the nasopharynx (see below).

Figure 3.2 A midsagittal section through a *Macaca mulatta* cranium. Note: S=staphylion; H=hormion; Sb=sphenobasion. The S-H chord was used to measure choanal height while the H-Sb chord was used for measuring nasopharyngeal roof depth in the anteroposterior dimension. The latter measure represents the external portion of the sphenoid body midline. The midline orientation of the choanae was measured via the S-H-Sb angle.
Choanal width was measured using the midpoints between the superior- and inferior-most points on the left and right posterior medial pterygoid plate margins. These midpoints were thus measured midway between the superior border of the medial pterygoid hamulus inferiorly and tubercle superiorly. The resulting bilateral line was typically located in a slightly inferior position relative to the inferior border of the scaphoid fossa among humans and non-human hominoids.

An index was calculated for the choanae to provide a single value for its relative shape. This index was calculated as:

\[
\text{Choanal Index} = \left( \frac{\text{width}}{\text{height}} \right) \times 100
\]

**Nasopharyngeal roof depth** - The shallowest anteroposterior depth of the nasopharynx appears to be at its roof between the approximate locations of hormion and sphenobasion. Depth increases inferiorly as the posterior vomeral border curves anteroinferiorly. Maximum nasopharyngeal depth on dry crania is difficult to quantify directly on dry crania as this measure is bounded posteriorly by the superior pharyngeal constrictor muscle (PCM) in life. Depth is, however, estimable directly at the nasopharyngeal roof where it is limited posteriorly by the superior attachment of the PCM raphe. While this muscle has its origin at the basal (or pharyngeal) tubercle on the basioccipital element, its epithelium-covered pharyngeal surface corresponds to the approximate position of the sphen-o-occipital synchondrosis in life. Takagi et al., 1962 demonstrated that the functional posterior limit of the nasopharyngeal roof can be defined by the position of the sphen-o-occipital synchondrosis in adult and infant humans as well as several non-primate mammals including a calf, rabbit, rat, guinea pig, and opossum. This study uses the hormion-sphenobasion measure to estimate nasopharyngeal roof depth.
Maximum nasopharyngeal depth- The distance between staphylion and sphenobasion was used to represent maximum depth. This measure is of the greatest estimable dimension, comprising both depth and width.

Minimum nasopharyngeal height, vomeral depth, and inferior nasopharyngeal (floor) depth- Relative depth of the posterior vomeral edge was calculated by subtracting the value of the staphylion-hormion-sphenobasion angle from $180^\circ$ so that the height of the right triangle made with midline choanal height or staphylion-hormion length as its hypotenuse could be calculated. The height of this triangle was considered the minimum nasopharyngeal height as it represents the vertical distance between the nasopharyngeal roof (at hormion) and the horizontal level at which the central tendon of the soft palate attaches to the posterior hard palate. The inverted base of this vomeral triangle was considered the horizontal depth of the posterior vomeral edge. These two measures corrected for the sometimes variable orientation of the choanae so that a more accurate assessment of nasopharyngeal height may be used.

Nasopharyngeal volume- The volume of the nasopharynx was calculated by multiplying choanal area by the staphylion-sphenobasion length. The latter was selected to account for the inferiorly curved orientation of the soft palate, which adds greater vertical dimension inferior to the level of staphylion.

Cartilaginous Eustachian tube orientation and length- The cartilaginous Eustachian tube can be seen extending from its orifice on the temporal bone to a position in which its external lip
overlaps the medial pterygoid plates. The extent of this path is visible on the dry cranium (Bluestone, 2006; Proctor, 1967; Graves and Edwards, 1944). The inferior border of the Eustachian tube orifice (made between the osseous and cartilaginous parts) is intersected by the sulcus tubaris, a ridge between the petrous temporal element and the sphenoid spine. Doyle (1977) and Doyle and Swarts (2010) derived a measure for the estimated length of the cartilaginous Eustachian tube between its orifice on the temporal bone and the tubercle of the medial pterygoid plate (roughly at the middle of its posterior margin). They also calculated its orientation using the angle made between the estimated axis of the cartilaginous Eustachian tube and the posterior margin of the medial pterygoid plate (between the hamulus and the superior-most portion of the medial pterygoid plate posterior edge). However, the exact location around the osseous Eustachian tube from which distance to the medial pterygoid tubercle was measured was never specified; their method is not easily reproducible with coordinate-based methods.

This study utilizes the inferior-most point on the sulcus tubaris to denote the location of the osseous Eustachian tube orifice (here termed the Eustachian point). This spatial configuration (of intersection of the osseous Eustachian tube orifices by the sulcus tubaris) was present in all human and archaic Homo sapiens crania (see Introduction section of Chapter 6). However, in non-human primate crania, the sulcus made between the petrous temporal and sphenoid spine was never seen to coincide with the Eustachian tube orifice. Instead, the orifice was bounded inferiorly by a (relative to humans) enlarged protuberance which serves as an origin of the tensor veli palatini muscle and a portion of the levator veli palatini muscle (Owen, 1849; Sonntag, 1923, 1924; Cave, 1979; Dean, 1982). Much variation existed in the positioning, orientation, and robustness of this protuberance (see Ahern [1998] for a discussion of its occurrence among Pliocene fossil hominins]. Gorilla exhibited larger, more voluminous protuberances while those
in *Pan* and *Pongo* were comparatively gracile. These observations are in agreement with Owen (1849) and Dean (1982) who also noted an enlarged "Eustachian process" in *Gorilla* relative to other hominoids. The location of the Eustachian point in the genus *Homo* thus appears autapomorphic in light of its absence among all non-human primate specimens used in this study. It also justifies the exclusion of non-human primates from analyses of growth change in Eustachian tube length and orientation as there exists no homologous or adequately reproducible landmark location for the Eustachian point.

The location of the nasopharyngeal orifice of the cartilaginous Eustachian tube was estimated at the lateral edge of the ipsilateral choana. The floor of this orifice was estimated at a point just superior to the medial pterygoid hamulus. Thus the distance between this point and the Eustachian point (which lies at the inferior end of the osseous Eustachian tube orifice) corresponds to the floor of the cartilaginous Eustachian tube. Several measures of Eustachian tube orientation were quantified as the angle made from the respective distances between the posterior medial pterygoid plate edge and the respective lines connecting the Eustachian point to the superior-, middle-, and inferior-most points on the medial pterygoid plate posterior edge.
Figure 3.3 The position of the Eustachian point used in this study is demonstrated above. It is constrained by the meeting of the sulcus tubarius with the osseous Eustachian tube orifice. Only humans exhibited the coincidence of the sphenoid spine, tympanic bone, and osseous Eustachian tube orifice. The Eustachian point was thus used to estimate the lateral-most point on the cartilaginous Eustachian tube. The above specimen is a human child of approximately 12-17 years of age (Stage 4 of this study).

External basicranial flexion- Basicranial flexion has long been studied in relation to brain size, laryngeal placement, and orientation of the splanchnocranium (see Background section of this chapter). Functional constraints on nasopharyngeal morphology may also influence patterns of basicranial flexion as rotation of the basioccipital element at the spheno-occipital synchondrosis (here used to denote the location of the posterior wall) could impact the orientation and shape of
the nasopharyngeal roof. The angle between the hormion-sphenobasion and sphenobasion-basion lines were used to quantify external basicranial flexion.

Facial Kyphosis- Facial kyphosis is defined as the rotation of the splanchnocranium relative to the neurocranium (Hofer, 1952; Starck, 1953; Ross and Ravosa, 1993). This study defines facial kyphosis relative to the basicranial axis as it may share a functional morphologic relationship with the nasopharyngeal boundaries. It is quantified here as the prosthion-hormion-sphenobasion angle. The landmark sphenobasion was located midway between the posterior boundaries of the left and right foramina ovale, making it determinable even when the spheno-occipital synchondrosis was obliterated (see description by Laitman, 1979,1982).

Piriform aperture dimensions- The piriform aperture rim was measured at rhinion (superior-most midline point), nasospinale (inferior-most mifline point), and the left and right lateral-most points. Area was calculated by multiplying height (the rhinion-nasospinale line) by mediolateral width. Though this technique does not capture the exact dimensions of the aperture, it is a reproducible estimate. Nasal index was calculated as:

\[ \text{Index} = \frac{\text{width}}{\text{height}} \times 100 \]

This variable provides a value for the relative shape of the piriform aperture and has long been used (e.g., Thompson and Buxton, 1923; Davies, 1932; Weiner, 1954; Franciscus, 1995).
**Statistical Analyses**

Several statistical techniques were used to compare age groups, adult representatives of each genus, and the growth trajectories of each genus for the morphology of specific upper respiratory tract structures. Further, Pearson Correlation Coefficients were calculated for each of the synthesized measures on the pooled sample to discern functional morphologic relationships.

**t-tests with unadjusted Bonferroni correction**

This study is centered on growth, sexual dimorphism, and evolutionary change in the nasopharyngeal boundaries. They were quantified via ANOVA of each univariate measure and, when the result was significant ($P<0.05$), multiple pairwise comparisons were performed via t-tests. With each additional comparison, the $\alpha$ or chance of committing a Type I error (rejection of a true null hypothesis) increases. When any event is repeated, it increases the likelihood of any single outcome. The Bonferroni method corrects for this increased error by dividing the desired level of significance by the number of comparisons. Use of the Bonferroni correction ensures that each of the t-tests within a series of comparisons will have the intended confidence interval. An unadjusted Bonferroni correction (hereafter referred to as “Bonferroni correction”) was selected over other tests such as the Tukey-Kramer, which accounts for family-wise error with greater power, as the unadjusted Bonferroni formula uses a more conservative estimate of $\alpha$ [but see Holland and Copenhaver (1988) and Rice (1989) for less conservative alternatives to the unadjusted Bonferroni correction]. It can thus provide a more stringent test for large scale morphological relationships as investigated in this study.
Product-moment (Pearson) correlations were also calculated among all of the measures synthesized from landmark coordinate data. Significance was set at $P \leq 0.05$ and a strong correlation was considered at least $r \geq 0.6$. Correlation tests were performed among each growth series and the pooled sample using mean values for each growth stage within each genus to avoid inflating the degrees of freedom. Resulting $P$-values of these multiple comparisons were also corrected using the unadjusted Bonferroni method.

**Identifying evolutionary and developmental changes**

Pairwise t-tests were performed to compare each of the independent variables among all eight genera, yielding separate t-tests of evolutionary differences between adults of each genus. Infants of all genera were also compared in a separate set of t-tests. Growth changes, on the other hand, were assessed by performing t-tests between all stages within each genus. A significant difference between two contiguous growth stages was regarded as a rapid growth change while a significant difference between two distant growth stages not observable among contiguous stages was considered a slow or gradual growth change. Each variable was thus assigned a positive, negative, or neutral growth trajectory. These trajectories were compared among genera to identify evolutionary differences in the respective processes by which each adult configuration is attained over development.
Pearson Correlation Coefficients

Product-moment (Pearson) correlations were calculated among a subset of the measures synthesized from landmark coordinate data to test for relationships between nasopharyngeal width and facial length/kyphosis (as per Hypothesis #1) and between basicranial flexion and choanal height and depth (as per Hypothesis #2). In testing for Hypothesis #1, measures of nasopharyngeal width (choanal width, bi-ovale width, bi-carotid width, bilateral width between petrous apices) were correlated against measures of facial length (staphylion-prosthion length, hormion-rhinion length), facial width (bi-jugale width), and the angle of facial kyphosis for Correlation Analysis #1. The measure of facial width was included to compare the strength of its relationship with nasopharyngeal width to the respective relationships of facial length and facial kyphosis to nasopharyngeal width. In testing for Hypothesis #2, measures of anteroposterior depth (hormion-sphenobasion length, hormion-basion length, vomeral depth, nasopharyngeal floor depth, choanal orientation, and staphylion-sphenobasion distance) and vertical height (choanal height, choanal index, and minimum nasopharyngeal height) of the nasopharynx were correlated against measures of basicranial flexion for Correlation Analysis #2.

Significance was adjusted via the Bonferroni method so that Correlation Analysis #1 with its 12 orthogonal comparisons set significance at \( P<0.00417 \). Correlation Analysis #2 included 17 orthogonal comparisons and set significance at \( P<0.00294 \). A strong correlation was considered at least \( r=0.6 \). Both Correlation Analyses were performed among mean values for each growth stage within each genus to prevent inflation of the degrees of freedom.
3.5 Results

3.5.1 Principal Components Analysis

A Principal Components Analysis of the pooled growth series revealed that developmental changes accounted for a large proportion of total shape variance. The first principal component (PC1) accounted for roughly 50% of all shape variance and it was manifested in decreasing palate length, inferior displacement of basion (which suggests external basicranial flexion), and increased choanal height while moving from the negative to positive ends of this axis. All genera exhibited a gradient from Stages 2-5 with earlier growth stages plotting higher on PC1. Also, PC1 separated humans from all other non-human primates with only two Stage 2 *Gorilla* individuals overlapping on the outer edge of the adult human range of variation. PC 2 accounted for roughly 13% of total shape variance with increasing aiorrhynchy, basicranial retroflexion, basicranial length, and obtuse angulation of the choanae when moving from the negative to positive ends of this axis. It roughly separated the orthognathic and prognathic non-human primates with *Hylobates* lying at the highest end of the axis and *Papio* remaining at the lowest position. PC3 accounted for roughly 12% of all shape variance and specimens at the negative end of this axis exhibited basicranial flexion at the spheno-occipital synchondrosis, greater anteroposterior basi-sphenoid length (a measure of minimum nasopharyngeal roof depth), and relatively decreased midline choanal height. Humans between Stages 3-5 and great apes were completely separated from all *Hylobates* and papionins on this axis. Interestingly, Stage 1&2 humans extensively overlapped *Hylobates* in this shape vector,
suggesting their basicrania exhibit a similar relative length and that their osseous URT’s exhibited similarly low vertical height (i.e. short choanae and piriform apertures).

When removing landmarks not related to the URT and re-running the PCA (Table 3.5), the relative positions of the individuals on the plot do not change. PC1 accounted 49.9% of shape variance while PC2 accounted 12.8%. The landmarks that appear to be driving the analysis are prosthion, nasion, the lateral-most point on the piriform aperture, and the point at the apex of the quadrate temporal bone surface as they exhibit the greatest change in position along PC1. The entire face and basicranium appears elongated at the negative end of the first principal component (the *Papio* cluster) and anteroposteriorly reduced at the positive end (the human cluster). PC2 primarily influenced facial kyphosis and basisphenoid length with those at the negative end of this axis (humans and *Papio*) exhibiting marked facial kyphosis relative to human newborns and other non-human hominoids.
Table 3.5 Reduced set of landmarks used in PCA of upper respiratory tract landmark coordinate data.

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prosthion</td>
</tr>
<tr>
<td>2</td>
<td>Intersection of the horizontal plates of the palatine bones with the palatine processes of the maxillae in the midline</td>
</tr>
<tr>
<td>3</td>
<td>Staphylion</td>
</tr>
<tr>
<td>4</td>
<td>Hormion</td>
</tr>
<tr>
<td>5</td>
<td>Sphenobasion</td>
</tr>
<tr>
<td>6</td>
<td>The point indicating the midline position of the pharyngeal tubercle: one-third of the distance from exo-basion to hormion</td>
</tr>
<tr>
<td>7</td>
<td>Basion (endo-basion)</td>
</tr>
<tr>
<td>8</td>
<td>Left most posteroinferior point on the alveolar process of the maxilla</td>
</tr>
<tr>
<td>9</td>
<td>Left superior-most point on the posterior margin of the medial pterygoid plate</td>
</tr>
<tr>
<td>10</td>
<td>Left inferior-most point on the posterior margin of the pterygoid plate</td>
</tr>
<tr>
<td>11</td>
<td>Left lateral-most midpoint point on the choanal margin</td>
</tr>
<tr>
<td>12</td>
<td>Left anteromedial-most point on the petrous pyramid (petrous temporal)</td>
</tr>
<tr>
<td>13</td>
<td>Nasospinale</td>
</tr>
<tr>
<td>14</td>
<td>Rhinion</td>
</tr>
<tr>
<td>15</td>
<td>Nasion</td>
</tr>
<tr>
<td>16</td>
<td>Glabella</td>
</tr>
<tr>
<td>17</td>
<td>Left lateral-most point on the rim of the nasal aperture</td>
</tr>
</tbody>
</table>
Figure 3.4 A mid-sagittally sectioned human cranium with a wireframe model superimposed.

Wireframe models are used in the current study to visually represent the dimensions of the hard palate, choanae, and midline basicranial axis. Adapted from a figure provided courtesy of Jeffrey T. Laitman.
Figure 3.5 Principal components plot of multivariate shape variation within the sample along PC1 and PC2. Note: *Homo, Pan, Gorilla, Pongo, Hylobates, Macaca, Cercocebus, Papio, Lophocebus*; 1=Stage 1, 2= Stage 2, 3= Stage 3, 4= Stage 4, 5= Stage 5 adult of unknown sex, ♂= adult male; ♀= adult female. Humans tended to form their own cluster at the positive end of the axis representing PC1. Interestingly, the short palates and basicrania of humans appears closest to the condition of infant *Pan* and *Macaca*. Also note that human newborns (Stage 1) are concentrated even farther along the PC1 axis. Conversely, adult male *Papio* tend to occupy the extreme negative end of PC1, suggesting that hard palate length may play a large role in the distribution of overall shape variation in the upper respiratory tract and face.
Figure 3.6 Principal components plot of multivariate shape variation within the sample along PC1 and PC3. Note: Homo, Pan, Gorilla, Pongo, Hylobates, Macaca, Cercocetus, Papio, Lophocebus; 1=Stage 1, 2= Stage 2, 3= Stage 3, 4= Stage 4, 5= Stage 5 adult of unknown sex, ♂= adult male; ♀= adult female. Shape change along PC3 largely entailed shape differences in the vertical dimension with great apes and adult humans exhibiting greater height in overall URT proportions than Stage 1&2 humans, Hylobates, and the papionins.
Figure 3.7 Principal components plot of multivariate shape variation within the sample using a reduced set of landmarks plotted along PC1 and PC2. Note: *Homo, Pan, Gorilla, Pongo, Hylobates, Macaca, Cercocebus, Papio, Lophocebus*; 1=Stage 1, 2= Stage 2, 3= Stage 3, 4= Stage 4, 5= Stage 5 adult of unknown sex, ♂= adult male; ♀= adult female. Along PC1, the basicranium and face become elongated with humans and *Papio* occupying opposite ends of the axis. PC2 is related to vertical height as the choanae and maxilla appear taller at the positive end of the axis.
Figure 3.8 Wireframe models representing growth from infancy (Stage 1) to adulthood. Papio and Cercocebus are an exception as they also exhibit developmental increase in facial kyphosis, while humans undergo flexion (inferior rotation) of the basicranium and facial skeleton over development while most other non-human primates exhibit the opposite pattern of facial and basicranial retroflexion (superior rotation) of the basicranium and facial skeleton over development. Note that humans undergo flexion of the inferior basicranium and super-rotation of the superior basicranium while most other non-human primates exhibit the opposite pattern of facial and basicranial retroflexion (superior rotation) of the basicranium and facial skeleton over development.
Principal Components Analysis of *Homo*

On a principal components plot of Procrustes-corrected data, age stages were separated along the first principal component (PC1), which accounted for roughly 35% of all sample variance. The second principal component (PC2) accounted for approximately 9% of sample variance and distinguished only newborns and fetal crania from all others. Newborns were found to settle into their own cluster without any overlap with later stages. Moving from the positive to negative end of the PC1 axis, the choanae grew taller and narrower and became more vertically oriented, the basicranial axis exhibited flexion at the spheno-occipital synchondrosis, palate length increased, and the maxillary alveoli descended inferiorly relative to the midline of the hard palate. Crania representing Stages 2-5 grade into each other with much overlap between consecutive growth stages. Notably, the maxillary alveolae continue to descend over time and some slight shortening in the relative length of the basi-sphenoid element (between hormion and sphenobasion) is visible. However, these growth trends are not as pronounced as the ones occurring between birth and the eruption of the first deciduous dentition.
**Figure 3.9** The above principal components plot represents shape diversity within the human developmental series. Note that the only separate cluster is of the newborns (Stage 1). This indicates that substantial shape change occurs during the transition between Stages 1-2. Individuals with hydrocephalus (red circle) and microencephaly (red triangle) still exhibited relatively normal upper respiratory tract proportions. The two adult crania with unilateral cleft palate (red square) also fell within the Stage 5 cluster, signifying that their overall facial and basicranial shape was normal despite a defect in the structure of the hard palate and choanal boundaries.
Figure 3.10 Wireframes representing average Stage 1-5 humans all superimposed at the point hormion. Marked growth change is visible between Stages 1-2 as basion moves inferiorly and the basisphenoid becomes anteroposteriorly shorter. Relatively small growth changes in the basicranial axis continued into Stage 5 along with increasing facial kyphosis and palate length.

3.5.2 Developmental patterns

Growth trends in *Homo* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests was set at $P<0.005$ as there were 10 orthogonal pairwise comparisons among growth stages 1-5. Stage 1 humans exhibit choanae that are relatively shorter than adults (shorter hormion-staphylion length) but are of the same relative width. Choanal growth change in humans thus occurs in the vertical dimension and appears concentrated around the time of eruption of the first deciduous teeth (i.e., the transition from Stage 1 to Stage 2) with smaller but significant growth changes continuing up until adulthood (Figure 3.11a). This trajectory also characterized choanal index (Figure 3.11b) confirming that the choanae do indeed grow taller and narrower early in life with more subtle increases in relative height thereafter. Furthermore, the angle of choanal orientation exhibited slow but significant growth change between Stages 1-5 (Figure 3.11c).
Bi-ovale width exhibited a growth trajectory similar to that of relative choanal shape in which large-scale growth change occurred around the time of eruption of the first deciduous dentition. This dimension was greater in Stage 1 crania, presumably a result of greater relative breadth of the nasopharynx and overall basicranium (Figure 3.11d). Bi-carotid width undergoes the opposite trend in which there is a substantial increase in its relative dimension around the eruption of the first deciduous dentition (Figure 3.11e). The relative distance between the petrous apices (Figure 3.11f) also gradually increases between Stages 1-3.

**Figure 3.11** Box-whisker plots of significant (P=0.005 as per the Bonferroni formula) growth changes among *Homo*. This *P*-value is made lower than the Bonferroni *P*-values for the other genera by the inclusion of Stage 1. For each plot, the X axes represents growth stage. In the cases of (a) and (d)-(f), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (c) represents angular values. Significant growth changes were found in all of these measures. *P*-values listed inside each plot indicate the *P*-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
The measure of total basicranial axis length (hormion-basion distance) exhibited significant and marked growth changes between Stages 1-2 and Stages 2-3, when it reached adult proportions at the approximate time of eruption of the first permanent maxillary molar (Figure 3.12a). Despite a lack of significance, Stage 2-5 crania all exhibited more acute mean values for the hormion-sphenobasion-basion angle than Stage 1 crania. The nasopharyngeal roof depth also experienced the same growth pattern up until Stage 3 but continued experiencing gradual shortening until adulthood (Figure 3.12b). However, nasopharyngeal floor depth experienced only subtle growth between Stages 2-5 as it became relatively smaller but with no significant difference between Stages 1 and 5 (Figure 3.12c). This may be related to the similar growth schedule of the angle of choanal orientation, which gradually rotated posteriorly between infancy
(Stage 1) and adulthood with no significant differences among consecutive developmental stages.

Relative midline palate length and facial kyphosis both exhibited significant change between Stages 1-2 and Stages 3-4, comprising two separate growth spurts (Figures 3.12d and 3.12e). Palate length increased and the angle of facial kyphosis became more acute with increased age. Facial width exhibited a more complicated trajectory as relative bi-jugale width significantly decreased between Stages 1-2 and gradually increased between Stages 2-5 with no significant difference between the Stage 1 and Stage 5 crania (Figure 3.12f). There was also no difference between Stage 1 and Stage 5 crania in relative nasal width but there was a marked difference in nasal height, which (like choanal index) reached adult proportions at Stage 2.
Figure 3.12 Box-whisker plots of significant ($P=0.005$ as per the Bonferroni formula) growth changes among *Homo*. This $P$-value is made lower than the Bonferroni $P$-values for the other genera by the inclusion of Stage 1. For each plot, the X axes represents growth stage. In the cases of (a)-(d) and (f), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (e) represents angular values. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Growth trends in *Pan* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. Significant decrease was found in nasal index (indicating narrowing of the piriform aperture) between Stages 2-5 but with no significant change between intermediate growth stages (Figures 3.13a). Bi-jugale width exhibits a more rapid pace of growth, increasing between Stage 4 and Stage 5 (Figure 3.13b). Vomeral and overall nasopharyngeal floor depth also decreased significantly between Stage 4 and Stage 5 (Figure 3.13c), apparently as a result of the posterior movement of the posterior maxillary tubercles. There was also a significant increase in the posterior maxillary width between Stages 3-5 (Figure 3.13c). Overall, the palate appears to be growing wider and longer posteriorly with anteroposterior reduction in the postnasal airway.
Figure 3.13 Box-whisker plots of significant ($P=0.00833$ as per the Bonferroni formula) growth changes among Pan. For each plot, the X axes represent growth stages. In the cases of (a), (c), and (d), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (b) represents an index, which is also a relative measure. Palatal growth most distinguished the Pan developmental pattern with broadening and posterior growth (as can be seen in decreasing nasopharyngeal floor depth); this value decreased gradually between stages 2-5. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25$^{th}$ percentile, median value, 75$^{th}$ percentile, and maximum value. These plots were generated using the program SAS.
Growth trends in *Gorilla* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. Relative choanal width decreased significantly between eruption of the deciduous dentition and adulthood with no significant differences between intermediate growth stages (*Figure 3.14a*). This implies a gradual change that occurs over a long period of developmental time rather than between consecutive growth stages, between which significant differences are not detectible. There were no significant growth changes in relative choanal height or angulation when using a pooled adult sample. However, adult males exhibited significantly taller choanae than adult females in the pre hoc test (see Table 3.3), but this difference was not significant after Bonferroni adjustment. The basicranium narrowed over development with relative bi-carotid width decreasing significantly between Stages 2-3 and gradually between Stages 3-5 (*Figure 3.14b*) while relative bi-ovale width decreased between all consecutive stages except the junction of Stages 3-4 (time of eruption of the second permanent maxillary molars; *Figure 3.14c*). Despite the presence of sexual dimorphism in this measure during the pre hoc test (see Methods and Table 3.3), there were no significant differences between adult males and adult females after Bonferroni adjustment. However, only Stage 5 males exhibited significantly narrower bi-ovale width than Stage 4 crania as the females did not significantly differ from the latter in this measure. Relative palate length remained unchanged between Stages 2-4 and abruptly increased between Stages 4-5, suggesting a late growth spurt (*Figure 3.14d*). Growth change in facial kyphosis was gradual but significant between infancy and adulthood with no significant differences among consecutive growth stages (*Figure 3.14e*).
**Figure 3.14** Box-whisker plots of significant [as per the Bonferroni formula: $P=0.00833$ for (a)-(b) and (d)-(e); $P=0.005$ for (c)] growth changes among *Gorilla*. For each plot, the X axes represent growth stages. In the cases of (a)-(d), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (e) represents an angle. Overall, the upper respiratory tract grows longer and narrower between Stages 2-5 with large scale growth changes occurring later between Stages 4-5. *Gorilla* is also the only hominoid genus to exhibit dimorphism in any of the linear measures during a pre hoc test (see Methods). However, these sex differences were not significant after Bonferroni adjustment. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Significance for the Bonferroni-adjusted t-tests was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. No significant growth changes were found in relative choanal width, height, or angulation in *Pongo*. Hormion-basion length and nasopharyngeal roof depth (between hormion and sphenobasion) both increased gradually and significantly between Stages 3-5, there being no significant differences between Stages 3-4 or Stages 4-5 (Figures 3.15a and 3.15b). Also occurring between Stages 3 and 5 was narrowing of the relative bi-ovale and bi-carotid widths (Figures 3.15c and 3.15d).
Figure 3.15 Box-whisker plots of significant ($P=0.00833$ as per the Bonferroni formula) growth changes among *Pongo*. For each plot, the X axis represents growth stages while the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. Development in *Pongo* is marked by narrowing and elongation of the basicranium. *P*-values listed inside each plot indicate the *P*-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Growth trends in *Hylobates* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. Relative choanal width and angulation did not change significantly in this genus. Stage 4 crania exhibited taller choanae than Stage 2 crania but an ANOVA of this measure did not yield a significant $P$-value ($P=0.0532$; Figure 3.16a). Instead, an ANOVA of nasal height was significant ($P=0.0053$; Figure 3.16b) with the only significant difference occurring between Stage 2 and Stage 5 crania. Also occurring at the same growth rate was hard palate elongation (Figure 3.16c). The one variable to exhibit rapid growth was bi-ovale width, which increased significantly between Stage 4 and Stage 5 crania (Figure 3.16d).
Figure 3.16 Box-whisker plots of significant ($P=0.00833$ as per the Bonferroni formula) growth changes among *Hylobates* from pairwise t-tests. For each plot, the X axes represent growth stages while the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. Development in *Hylobates* is marked by palatal elongation, increasing nasal height, and basicranial narrowing between the foramina ovale. Despite a nonsignificant ANOVA, choanal height grew significantly between Stages 2-4 when analyzed via pairwise t-tests. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Growth trends in *Macaca* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests for most univariate measures was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. However, when sexual dimorphism existed in a given measure (see Materials above), significance was set at $P<0.005$ as there were ten orthogonal comparisons between Stages 2-4 and Stage 5 Males and Females. Relative choanal width and height remained constant over development until late growth change between Stages 4-5 when the choanae grew narrower and taller (Figure 3.17a-b). Midline angulation decreased (i.e., became more obtuse) between Stage 3 and Stage 5 with no growth changes between intermediate stages (Figure 3.17c). Relative bi-ovale width increased significantly between Stages 2-3 but subsequently decreased between Stages 4-5 to become significantly narrower than at Stage 2 (Figure 3.17d). There was also late growth change in relative bi-carotid width, which narrowed significantly between Stages 4-5 but not at any other time over development (Figure 3.17e). All measures of anteroposterior length also exhibited similar patterns of growth in which significant increases occurred only between Stage 4 and Stage 5. With respect to relative nasopharyngeal roof depth, adult males exhibited significantly smaller values than Stage 2 and Stage 3 individuals who were not significantly different from each other or adult females (Figure 3.17f). This suggests a longer growth trajectory for males whereas females reach adult proportions by Stage 3. Only facial kyphosis remained unchanged throughout development.
Figure 3.17 Box-whisker plots of significant [as per the Bonferroni formula: $P=0.00833$ for (b)-(d); $P=0.005$ for (a), (e), and (f)] growth changes among Macaca. For each plot, the X axes represent growth stages. In the cases of (a)-(b) and (d)-(f), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (c) represents an angle. Male ontogeny includes prolonged periods of choanal narrowing and shortening of the basisphenoid relative to females who reach adult proportions in these measures by Stage 4. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Growth trends in *Cercocebus* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. There were no significant growth changes in relative choanal width, area, or midline angulation. There was, however, a late increase between Stages 4-5 in relative choanal height and a decrease in choanal index (*Figure 3.18a-b*). Both relative bi-ovale and bi-carotid width also decrease significantly between Stages 4-5 as does facial kyphosis (*Figure 3.18c-d*). Palate length also increases between Stages 4-5 (*Figure 3.18e*) but not the hormion-rhinion length. Thus, the floor of the nasal cavity undergoes developmentally late expansion while the superior boundary of the respiratory portion of the nasal cavity remains the same length.
Figure 3.18 Box-whisker plots of significant ($P=0.00833$ as per the Bonferroni formula) growth changes among *Cercocebus*. For each plot, the X axes represent growth stages. In the cases of (a)-(b) and (d)-(f), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (c) represents an angle, which is also a relative measure. Growth change occurred in elongation and narrowing of the URT but without the marked dimorphism of some other papionin genera. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Growth trends in *Papio* as deduced from univariate measures

Significance for the Bonferroni-adjusted t-tests was set at $P<0.0083$ as there were six orthogonal comparisons between Stages 2-5. All relative dimensions remained unchanged until a late growth spurt between Stages 4 and Stage 5. During this time, relative choanal width and area (but not height or angulation) underwent significant decrease as did relative bi-ovale and bi-carotid widths (Figure 3.19a-d). Yet, among these measures, Stage 5 females were not significantly different from Stage 4 crania in choanal width and bi-ovale width, suggesting that females achieve adult proportions earlier in development and that males continue to exhibit morphological changes into Stage 5. These changes are likely related to the extremely long hard palates of Stage 5 males, which were significantly longer than those of Stage 5 females and Stage 4 crania (Figure 3.19e). There was no such dimorphism of nasal cavity length between hormion and rhinion despite significantly greater values among the pooled Stage 5 sample than Stage 4 crania (Figure 3.19f).
**Figure 3.19** Box-whisker plots of significant [as per the Bonferroni formula: $P=0.005$ for (a), (c), and (e); $P=0.00833$ for (b), (d), and (f)] growth changes among *Papio*. For each plot, the X axes represent growth stages. The Y axes represent units of Procrustes-corrected length and are thus relative dimensions. Stage 5 *Papio* males have the greatest palate length among all the papionins, which may be related to its marked nasopharyngeal narrowing. *Papio* is also the only genus to exhibit adult dimorphism in palate length. *P*-values listed inside each plot indicate the *P*-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
3.5.3 Intergeneric variation among adults

A total of nine genera were compared, yielding 36 orthogonal comparisons for measures that were not significantly ($P<0.05$) different among males and females from any genus. This resulted in a Bonferroni-adjusted $P$-value of $P=0.00139$. For measures that were dimorphic, males and females were segregated, leading to 171 orthogonal comparisons among 18 groups (thus, $P=0.00029$). Humans were found to have the significantly greatest relative choanal width and area among all the sampled genera but were nested among the great apes in choanal height. They possessed a choanal index greater than every other genus except *Hylobates*, which exhibited the significantly highest value for this variable but had the significantly lowest choanal area. Humans were intermediate in midline choanal angulation between the great apes (who exhibited less flexion) and papionins (who exhibited greater flexion) with no significant differences from *Hylobates* or *Lophocebus*. Humans also possessed significantly greater relative nasopharyngeal volume than all other genera except *Pan*, from which they did not significantly differ.

Aside from choanal dimensions, the pooled sex human sample was also significantly different from all other genera in exhibiting the shortest relative palate length and most acute angle of basicranial flexion at the spheno-occipital synchondrosis. They also had a relative nasopharyngeal roof depth (hormion-sphenobasoin distance) that was significantly smaller than all genera except for *Gorilla*, from which they did not significantly differ. One other feature distinguishing humans was relative midline length of the basicranial axis (hormion-basion length), which was significantly smaller than in any other genus.
The significantly greatest relative bi-ovale and bi-carotid widths were in humans. Male *Papio* and male *Gorilla* had the significantly smallest bi-ovale widths and thus the narrowest basicrania among the genera sampled except for a lack of significant difference from each other. Bi-carotid width was also narrowest among these genera in pooled sex samples. Surprisingly, humans were found to be intermediate between hominoids and papionins in the angle of facial kyphosis used here. The great apes exhibited larger angles while the papionins had smaller angles. Also, male *Papio* possessed significantly greater basicranial flexion than all non-human hominoids except male *Pan*, from which it was not significantly different. Given male *Papio* also has the significantly (*P*<0.05) greatest palate length and some of the greatest relative nasal cavity heights (both at the choanae and piriform aperture), it may require greater relative basicranial flexion and a shorter basicranial axis between hormion and basion (which was significantly shorter than all genera except *Homo* and *Gorilla*) to support the extra weight of its extremely prognathic and tall midface.

3.5.4 Intergeneric variation among Stage 2 crania

There were a total of eight genera for which Stage 2 crania were available for study, thus 36 orthogonal comparisons were made for each univariate measure. This resulted in a Bonferroni-adjusted *P*-vale of *P*=0.00139.

By the time of eruption of the deciduous dentition, humans already possess significantly greater relative choanal width than any of the other sampled primate genera. They take on a broad, short appearance and have a choanal index (a measure of width relative to height) that is larger than all genera except for *Hylobates*, which exhibits a markedly and significantly higher
value. Nonetheless, both relative choanal width and relative height are significantly greater in human infants as is overall choanal area. Interestingly, Pan and Pongo exhibit significantly less relative choanal width and area than humans but exceed all other sampled genera.

The extremely prognathic Papio did not significantly differ in relative choanal width from any other papionin genus or from Gorilla but exhibited narrower choanae relative to the remainder of hominoid genera. Its choanae take on a tall, narrow appearance and have a significantly lower choanal index than all other hominoid and papionin genera except Gorilla, from which it does not significantly differ. The choanae of Papio have a significantly greater relative area than all other papionin genera sampled but do not significantly differ from Gorilla. Conversely, Hylobates had significantly smaller choanal area than all other sampled genera. This seemingly autapomorphous condition may relate to an, as of yet, unexplored aspect of Hylobates physiology and energy dynamics.

Humans have significantly wider relative bi-ovale and bi-carotid width than any other genus while Papio had a significantly narrower width than Homo, Pan, and Cercocebus. Bi-carotid width was also narrower in Hylobates than in Gorilla and Macaca with no other significant differences in either measure among the sampled genera. These measures appear to be relatively conservative traits among anthropoid primates.

By the time of eruption of the deciduous dentition, human infants already exhibit significantly greater basicranial flexion and shorter relative palate length. They were, however, nested among other non-human primate genera in relative nasopharyngeal roof depth (between hormion and sphenobasion), relative nasopharyngeal volume, relative midline basicranial length (between hormion and basion), relative superior nasal cavity length (hormion-rhinion distance), relative subnasal prognathism, and facial kyphosis. Overall, fewer of the distinguishing
characteristics of the adult hominoid and papionin face and upper respiratory tract are present among the infants.

3.5.5 Univariate measures distinguishing human adults and newborns from non-human hominoids

Significance for the measures comparing growth stages among all genera was set at $P<0.00125$ (for 40 pairwise comparisons of each linear measure at $\alpha=0.05$, as per the Bonferroni formula). Measures that significantly distinguished humans from all other hominoids included greater bi-jugale widths, choanal widths, and bi-ovale widths (Figure 3.20a-c). Thus humans were distinguishable by wider faces, choanae, and basicrania, respectively. Stage 1 humans also exhibited significantly shorter palate length than every other developmental stage of every genus (Figure 3.20d). Nonetheless, human adults were distinguished from Stage 1 crania and all other non-human hominoids by significantly greater width between the left and right carotid canals and shorter hormion-basion length (Figure 3.20e-f).
Figure 3.20 Box-whisker plots of measures that significantly (P<0.00125 as per Bonferroni adjustment of 40 orthogonal comparisons) distinguish all humans (a)-(c), human newborns (d) or human adults (e)-(f) from non-human hominoids. The measure of total basicranial length between hormion and basion (f) was also used as a measure of external basicranial flexion as these two landmarks constitute the endpoints of the basisphenoid and basioccipital elements, respectively. Only adult humans exhibited sufficiently short hormion-basion distance to infer marked external basicranial flexion. Yet, humans of all growth stages exhibited wider faces and nasopharynges than non-human hominoids. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. The X axes represent growth stages within each genus while the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
There were some relative dimensions in which adult humans were significantly different from Stage 1 humans but not Stage 2 apes (Figure 3.21). These included facial kyphosis, palate length, angle of choanal orientation, and width between the left and right petrous apex. Also, adult humans exhibited a significantly lower relative nasopharyngeal roof depth (hormion-sphenobasion distance) than all other groups except adult *Gorilla*. The adult human condition may thus be defined by a unique combination of the above features rather than significant differences in each dimension.
Figure 3.21 Box-whisker plots of measures that are significantly (P<0.00125 as per Bonferroni adjustment) different between newborn and adult humans but do not distinguish human adults from non-human hominoids. Humans and non-human hominoids overlap the least in the hormion-sphenobasion-basion angle. When this measure is considered alongside extremely short relative hormion-basion distance (see Figure 3.19), human adults appear to exhibit marked external basicranial flexion. P-values listed inside each plot indicate the P-value for that respective ANOVA. The X axes of each plot represents growth stages within each genus while the Y axes of (c) and (e) represent units of Procrustes-corrected length and are thus relative dimensions. For plots (a), (b), and (d), the Y axes represent angles. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
3.5.6 Measuring variability with coefficient of variation

The coefficient of variation (CV) was calculated for all of the measures among the sample of mean values for each growth stage within each genus (Table 3.6). It was found that the angle of external basicranial flexion (angle made between the hormion-sphenobasion and sphenobasion-basion lines) was the least variable (CV = 3.858% when expressed in radians) while relative bi-jugale width (CV = 5.963%), relative bi-porion width (CV = 5.724%), angle of facial kyphosis (CV = 7.802% when expressed in radians), relative length of the respiratory portion of the nasal cavity (hormion-rhinion distance; CV = 9.334%), and relative maximum nasopharyngeal depth (the staphylion-sphenobasion distance; CV = 7.24%) were also among the least variable. The most variable traits were relative nasopharyngeal roof depth (hormion-sphenobasion distance; CV = 30.353%) and relative nasopharyngeal volume (CV = 27.593%). It appears from the data that, despite great variation in the nasopharyngeal roof depth, lower amounts of variation in maximum depth and moderately low amounts of variation in midline choanal angulation (CV = 11.991%) may render some aspect of airflow dynamics constant among morphologically disparate groups.
Table 3.6 Coefficients of variation for univariate measures used in this study in the total hominoid sample. Note that angles were converted into radians as per Schmall and Finkel (1909).

<table>
<thead>
<tr>
<th>Univariate Measure</th>
<th>Coefficient of Variation (in percentages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>External basicranial flexion angle in radians (hormion-sphenobasion-basion)</td>
<td>3.858%</td>
</tr>
<tr>
<td>Bi-Porion Width</td>
<td>5.724%</td>
</tr>
<tr>
<td>Bi-Jugale Width</td>
<td>5.963%</td>
</tr>
<tr>
<td>Maximum Nasopharyngeal Depth (staphylion-sphenobasion distance)</td>
<td>7.24%</td>
</tr>
<tr>
<td>Maxillary Width (between the maxillary tubercles)</td>
<td>8.5%</td>
</tr>
<tr>
<td>Facial Kyphosis angle in radians (prosthion-hormion-sphenobasion)</td>
<td>9.156%</td>
</tr>
<tr>
<td>Hormion-Rhinion length</td>
<td>9.334%</td>
</tr>
<tr>
<td>Nasal Height (rhinion-nasospinale)</td>
<td>9.342%</td>
</tr>
<tr>
<td>Choanal Orientation angle in radians (staphylion-hormion-sphenobasion)</td>
<td>10.442%</td>
</tr>
<tr>
<td>Bi-Carotid Width</td>
<td>10.855%</td>
</tr>
<tr>
<td>Bi-Ovale Width</td>
<td>12.069%</td>
</tr>
<tr>
<td>Nasal Width</td>
<td>12.642%</td>
</tr>
<tr>
<td>Choanal Width</td>
<td>13.126%</td>
</tr>
<tr>
<td>Hormion-Basion length</td>
<td>13.482%</td>
</tr>
<tr>
<td>Palate Length</td>
<td>15.086%</td>
</tr>
<tr>
<td>Bi-Petrous Apex Width</td>
<td>16.22%</td>
</tr>
<tr>
<td>Choanal Height</td>
<td>19.816%</td>
</tr>
<tr>
<td>Choanal Area</td>
<td>24.49%</td>
</tr>
<tr>
<td>Nasopharyngeal Volume</td>
<td>27.593%</td>
</tr>
<tr>
<td>Nasal Index</td>
<td>27.813%</td>
</tr>
<tr>
<td>Nasopharyngeal Roof Depth</td>
<td>30.352%</td>
</tr>
</tbody>
</table>

3.5.7 Correlations

Hypothesis 1 states that greater nasopharyngeal width among human adults and newborns are related to extreme orthognathy and well expressed facial kyphosis. Correlation Analysis #1 tested these relationships with a Bonferroni-adjusted series of product-moment correlation coefficients (significance was set at $P<0.00417$ as per the Bonferroni formula). It was found that palate length was strongly and significantly correlated with choanal width ($r=-0.76$, $P<0.0001$),
bi-ovale width \( (r=-0.883, P<0.0001) \), and bi-carotid width \( (r=-0.709, P<0.0001) \). A moderate but significant \( (r=-0.53, P=0.0013) \) correlation was found between palate length and the bilateral distance between the petrous apices. Bi-jugale width was also strongly and significantly correlated with choanal width \( (r=0.736, P<0.0001) \), bi-ovale width \( (r=0.613, P=0.0001) \), bi-carotid width \( (r=0.667, P<0.0001) \), and bilateral width between the petrous apices \( (r=0.646, P<0.0001) \). The angle of facial kyphosis was not significantly correlated with any measure of nasopharyngeal or basicranial width.

**Table 3.7** Multiple correlations performed on the total sample of hominoids and papionins in testing Hypothesis 1. Note that significant correlations are bolded and that all \( P \)-values are Bonferroni corrected so that, for 12 separate comparisons, significance was set at \( P=0.00417 \).

<table>
<thead>
<tr>
<th></th>
<th>Palate Length</th>
<th>Bi-Jugale Width</th>
<th>Facial Kyphosis Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choanal Width</td>
<td>( r=-0.829 )</td>
<td>( r=0.736 )</td>
<td>( r=0.075 )</td>
</tr>
<tr>
<td></td>
<td>( P&lt;0.0001 )</td>
<td>( P&lt;0.0001 )</td>
<td>( P=0.67 )</td>
</tr>
<tr>
<td>Bi-Ovale Width</td>
<td>( r=-0.883 )</td>
<td>( r=0.61 )</td>
<td>( r=-0.347 )</td>
</tr>
<tr>
<td></td>
<td>( P&lt;0.0001 )</td>
<td>( P=0.0001 )</td>
<td>( P=0.044 )</td>
</tr>
<tr>
<td>Bi-Carotid Width</td>
<td>( r=-0.709 )</td>
<td>( r=0.67 )</td>
<td>( r=-0.103 )</td>
</tr>
<tr>
<td></td>
<td>( P&lt;0.0001 )</td>
<td>( P&lt;0.0001 )</td>
<td>( P=0.56 )</td>
</tr>
<tr>
<td>Bilateral Width Between Petrous Apices</td>
<td>( r=-0.53 )</td>
<td>( r=0.646 )</td>
<td>( r=0.309 )</td>
</tr>
<tr>
<td></td>
<td>( P=0.0013 )</td>
<td>( P&lt;0.0001 )</td>
<td>( P=0.08 )</td>
</tr>
</tbody>
</table>
Correlation Analysis #1 was performed a second time using only the hominoid sample (significance set at $P=0.00417$ as per the Bonferroni formula), palate length shared strong and significant ($P<0.0001$) negative correlations with choanal width ($r=-0.89$), bi-ovale width ($r=-0.94$), and bi-carotid width ($r=-0.7; P=0.00046$). Bi-jugale width was also strongly and significantly correlated with choanal width ($r=0.75; P=0.0001$), bi-ovale width ($r=0.65; P=0.0013$), and bi-carotid width ($r=0.76; P<0.0001$). Facial kyphosis was strongly and significantly correlated with choanal width ($r=-0.76; P<0.0001$), bi-ovale width ($r=-0.73; P=0.00019$), and bi-carotid width ($r=-0.7; P=0.00041$) but was not significantly correlated with the angle of choanal orientation ($r=0.59; P=0.049$). Nasal width was strongly and significantly ($P<0.0008$) positively correlated with choanal height ($r=0.67$) and choanal angulation ($P=0.69$).

**Table 3.8** Multiple correlations performed on the hominoid sample in testing Hypothesis 1. Note that significant correlations are bolded and that all $P$-values are Bonferroni corrected so that, for 20 separate comparisons, significance was set at $P=0.0025$.

<table>
<thead>
<tr>
<th></th>
<th>Choanal Width</th>
<th>Choanal Orientation</th>
<th>Bi-Ovale Width</th>
<th>Bi-Carotid Width</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Angle of Facial Kyphosis</strong></td>
<td>$r=-0.76$</td>
<td>$r=0.59$</td>
<td>$r=-0.73$</td>
<td>$r=-0.7$</td>
</tr>
<tr>
<td>$P=0.00007$</td>
<td>$P=0.49$</td>
<td>$P=0.0002$</td>
<td>$P=0.00041$</td>
<td></td>
</tr>
<tr>
<td><strong>Palate Length</strong></td>
<td>$r=-0.89$</td>
<td>$r=0.49$</td>
<td>$r=-0.94$</td>
<td>$r=-0.7$</td>
</tr>
<tr>
<td>$P&lt;0.00001$</td>
<td>$P=0.02359$</td>
<td>$P&lt;0.00001$</td>
<td>$P=0.00046$</td>
<td></td>
</tr>
<tr>
<td><strong>Bi-Jugale Width</strong></td>
<td>$r=0.75$</td>
<td>$r=0.26$</td>
<td>$r=0.65$</td>
<td>$r=0.76$</td>
</tr>
<tr>
<td>$P=0.0001$</td>
<td>$P=0.26$</td>
<td>$P=0.0013$</td>
<td>$P=0.000034$</td>
<td></td>
</tr>
</tbody>
</table>
Hypothesis 2 states a morphological relationship between external basicranial flexion and nasopharyngeal height and anteroposterior depth. Correlation Analysis #2 tested for these relationships in a separate set of Bonferroni-adjusted correlation analyses (Correlation Analysis #2; significance was set at $P=0.0028$). The hormion-sphenobasion-basion angle was strongly and significantly correlated with maximum nasopharyngeal depth (staphyion-sphenobasion distance; $r=-0.722$, $P<0.001$) and the angle of choanal orientation ($r=0.565$, $P=0.0005$). The hormion-basion distance (a measure of external basicranial flexion) was strongly and significantly correlated with nasopharyngeal roof depth ($r=0.94$, $P<0.0001$), vomer depth ($r=-0.764$, $P<0.0001$), choanal height ($r=-0.829$, $P<0.0001$), choanal index ($r=-0.64$, $P<0.0001$), minimum nasopharyngeal height ($r=-0.743$, $P<0.0001$), and the angle of choanal orientation (staphyion-hormion-sphenobasion angle; $r=-0.497$, $P=0.0028$). Surprisingly, nasopharyngeal floor depth was not significantly correlated with either measure of external basicranial flexion despite both of its component measures, nasopharyngeal roof depth and vomeral depth (of which it is the sum) both exhibiting significant correlation with hormion-basion depth.
Table 3.9 Multiple correlations performed in testing Hypothesis 2. Note that significant correlations are bolded and that all $P$-values are Bonferroni corrected so that, for 17 separate comparisons, significance was set at $P=0.0028$.

<table>
<thead>
<tr>
<th></th>
<th>External Basicranial Flexion Angle (hormion-sphenobasion-basion)</th>
<th>Hormion-Basion Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal roof depth</td>
<td>$r=0.478; P=0.0042$</td>
<td>$r=0.94; P&lt;0.0001$</td>
</tr>
<tr>
<td>Hormion-Basion Distance</td>
<td>$r=0.203; P=0.25$</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>Vomer Depth</td>
<td>$r=-0.476; P=0.0045$</td>
<td>$r=-0.764; P&lt;0.0001$</td>
</tr>
<tr>
<td>Nasopharyngeal Floor Depth</td>
<td>$r=0.32; P=0.0625$</td>
<td>$r=0.153; P=0.39$</td>
</tr>
<tr>
<td>Maximum nasopharyngeal depth</td>
<td>$r=-0.72; P&lt;0.0001$</td>
<td>$r=0.104; P=0.56$</td>
</tr>
<tr>
<td>depth (Staphylion-sphenobasion distance)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choanal Orientation Angle</td>
<td>$r=0.565; P=0.0005$</td>
<td>$r=-0.497; P=0.0028$</td>
</tr>
<tr>
<td>Choanal Height</td>
<td>$r=-0.348; P=0.04$</td>
<td>$r=-0.83; P&lt;0.0001$</td>
</tr>
<tr>
<td>Choanal Index</td>
<td>$r=0.152; P=0.39$</td>
<td>$r=-0.64; P&lt;0.0001$</td>
</tr>
<tr>
<td>Minimum Nasopharyngeal Height</td>
<td>$r=-0.29; P=0.1$</td>
<td>$r=-0.743; P&lt;0.0001$</td>
</tr>
</tbody>
</table>

When Correlation Analysis #2 was performed on just the hominoid sample (significance set at $P=0.01$ as per the Bonferroni formula), the basicranial flexion angle was moderately ($r=0.58$) but significantly ($P=0.0064$) correlated with nasopharyngeal depth at its roof and strongly correlated with anteroposterior depth at the nasopharyngeal floor ($r=0.6; P=0.0039$). Basicranial axis length between hormion and basion was also strongly correlated with depth at the nasopharyngeal floor.
Anthony S. Pagano

(r=0.70; \( P=0.0004 \)). The strong correlation between nasopharyngeal roof and floor depth (r=0.98; \( P<0.0001 \)), was likely a result of the hormion-sphenobasion distance comprising a portion of the hormion-basion length. Relative shortening of the basicranial axis may thus be related to the appearance of external basicranial flexion when visualized via wireframes after GPA as hormion and basion may be located closer together.

**Table 3.10** Correlations performed on the hominoid sample in testing Hypothesis 2. Note that all correlations are significant, even with a Bonferroni-corrected \( P \)-value of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>Nasopharyngeal Roof Depth</th>
<th>Nasopharyngeal Floor Depth</th>
<th>Total Basicranial Length (Hormion-Basion Length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basicranial Flexion Angle</td>
<td>r=0.58 ( P=0.0064 )</td>
<td>r=0.6 ( P=0.0039 )</td>
<td>r=0.69 ( P=0.00053 )</td>
</tr>
<tr>
<td>Total Basicranial Length (Hormion-Basion Length)</td>
<td>r=0.98 ( P&lt;0.000000001 )</td>
<td>r=0.70 ( P=0.0004 )</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

3.5.8 Growth in estimated Eustachian tube dimensions

The dimensions of the cartilaginous Eustachian tube (CET) were estimated using several measures between the meeting point of the sphenoid spine and petrous temporal bone (Eustachian point; chosen for reproducibility) and the posterior medial pterygoid plate border at the hamulus (point just superior to it), midpoint, and superior-most point. All bilateral measures were estimated on both the left and right sides and the mean was used. Significance was set at \( P=0.005 \) as per the Bonferroni formula.
It was found that relative distance between the Eustachian point and the midpoint of the medial pterygoid plate posterior border increased gradually between Stages 2-5 (Figure 3.22a) while respective relative CET lengths from the hamulus and from the superior-most medial pterygoid margin underwent substantial growth changes between Stages 1-2 with little to no change thereafter (Figure 3.22b-c). The distance to the hamulus increased while the distance to the superior-most point on the medial pterygoid plate edge increased. Growth change in CET angulation was also largely constrained to Stages 1-2. These angles grew more acute between Stages 1-2 but underwent no further significant growth change (Figure 3.22d). These all occurred at the same time that the medial pterygoid plates undergo substantial and significant relative increases in height (Figure 3.22e).
Figure 3.22 Box-whisker plots of significant ($P=0.005$ as per the Bonferroni formula) growth changes in estimated dimensions and angulation of the cartilaginous Eustachian tube of humans. For each plot, the X axes represent growth stages. In the cases of (a)-(c) and (e), the Y axes represent units of Procrustes-corrected length and are thus relative dimensions. The Y axis of (d) represents angular values. The transition between Stages 1-2 appears to a time of major growth change in cartilaginous Eustachian tube dimensions. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. Asterisks between growth stages represent the presence of a significant difference identified during post hoc testing. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, $25^{th}$ percentile, median value, $75^{th}$ percentile, and maximum value. These plots were generated using the program SAS.
3.5.9 Description of variation among humans

Correlations

Among humans, nasopharyngeal dimensions showed only weak correlations with facial dimensions. In particular, relative choanal height was weakly correlated with relative midfacial height at the nasion-nasospinale line ($r=0.31732$, $P=0.0002$) and relative piriform aperture area ($r=0.2339$, $P=0.0061$). Both relative choanal height and relative height at the medial pterygoid plate posterior edge (between its superior-most extent and the point just superior to the hamulus) were weakly correlated with the relative nasospinale-prosthion length ($r=0.17643$ and $r=0.23058$, respectively; $P<0.05$). These were also weakly and significantly ($P<0.05$) correlated with relative palate length (between prosthion and staphyion) as was choanal width and nasopharyngeal roof depth. Choanal area bore a weak but significant ($r=0.16919$, $P=0.0489$) correlation with piriform aperture height. Finally, choanal index showed a weak but significant ($r=-0.18386$, $P=.0321$) negative correlation with piriform aperture area.

3.5.9.1 Nasopharyngeal morphology of individuals with cleft palate, microencephaly and hydrocephalus

In quantifying differences between pathological specimens and the normal adult control sample, Z scores were calculated for relative choanal width, height, index, and angle of orientation, relative nasopharyngeal roof depth, cartilaginous Eustachian tube angulation and relative length, basicranial flexion, and facial kyphosis. One individual with cleft palate
exhibited relative choanal width that was 2.262 standard deviations above the normal adult human mean when measured at the midpoint of the medial pterygoid plate posterior edge. This resulted in a choanal index that was 4.383 standard deviations above the normal adult mean. When relative choanal width is measured at the superior and inferior-most points on the medial pterygoid plate edge, this individual was 3.645 and 5.642 standard deviations above the normal adult mean, respectively. Another individual with cleft palate was 2.187 and 4.63 standard deviations above the normal adult means for relative choanal width measured at the superior- and inferior-most portions of the medial pterygoid plate edge, respectively. The especially wide distance represented by the latter measure may be related to the great frequency of non-contact between the pterygoid hamulus and the tensor veli palatini muscle tendon among cleft palate patients (see review in Perry, 2007). Additionally, relative choanal height was 2.133 standard deviations below the normal adult mean in one of the cleft palate individuals while both crania exhibited greater medial pterygoid plate height (2.225-2.785 standard deviations above the normal adult mean) and nasopharyngeal roof depth (2.149-2.389 standard deviations above the normal adult mean). One of the cleft palate individuals also exhibited a relatively small distance between the bony Eustachian tube orifice and the superior-most point on the medial pterygoid plate posterior boundary, likely related to its relatively great choanal width.

The one microencephalic cranium sampled exhibits relatively long palate length (2.222 standard deviations above the normal adult mean) and a narrow piriform aperture (2.949 standard deviations below the normal adult mean). All measures of nasopharyngeal dimensions from this individual were well within the normal adult range. However, an adult cranium with hydrocephalus exhibited relatively low choanal height (-2.433 standard deviations below the normal adult mean), which resulted in a relatively high choanal index (2.266 standard deviations
above the normal adult mean). It was well nested among the normal crania in all other measures of nasopharyngeal and cartilaginous Eustachian tube morphology. A separate hydrocephalic cranium at Stage 4 (roughly 12 years of age) also exhibited relative nasopharyngeal dimensions that were well nested among similarly aged normal crania.
### Table 3.11 Description of measures used in Table 3.12

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChoanW</td>
<td>Choanal width measured between the left and right midpoints on the posterior medial pterygoid plate edge</td>
</tr>
<tr>
<td>ChoanHt</td>
<td>Choanal height measured between staphylion and hormion</td>
</tr>
<tr>
<td>IndChoan</td>
<td>Width between the posterior-most points on the left and right foramen ovale rims</td>
</tr>
<tr>
<td>CHPang</td>
<td>Choanal orientation as measured by the staphylion-hormion-sphenobasion angle</td>
</tr>
<tr>
<td>InfChW</td>
<td>Choanal width measured between the inferior-most points of the left and right posterior medial pterygoid plate edges (at the superior boundary of the hamular base)</td>
</tr>
<tr>
<td>SupChW</td>
<td>Choanal width measured between the superior-most points of the left and right posterior medial pterygoid plate edges</td>
</tr>
<tr>
<td>AvPterHt</td>
<td>Distance between the superior- and inferior-most points on the posterior medial pterygoid plate edge</td>
</tr>
<tr>
<td>PalL</td>
<td>Distance between prosthion and staphylion (palate length)</td>
</tr>
<tr>
<td>NasW</td>
<td>Maximum width of the piriform aperture</td>
</tr>
<tr>
<td>SphenL</td>
<td>Hormion-Sphenobasion distance (basisphenoid length)</td>
</tr>
<tr>
<td>AvEusFlr</td>
<td>Mean of the left and right distances between the osseous Eustachian tube orifice and the inferior-most point on the posterior medial</td>
</tr>
</tbody>
</table>
pterygoid plate edge, just superior to the base of the hamulus. This is a measure of the cartilaginous Eustachian tube floor length.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AvEust</td>
<td>Mean of the left and right distances between the osseous Eustachian tube orifice and the midpoint of the posterior medial pterygoid plate edge</td>
</tr>
<tr>
<td>AvEust2</td>
<td>Mean of the left and right distances between the osseous Eustachian tube orifice and the superior-most point on the posterior medial pterygoid plate edge</td>
</tr>
<tr>
<td>Euang</td>
<td>Mean of the left and right angles made between the chord connecting the osseous Eustachian tube orifice to the midpoint on the posterior medial pterygoid plate edge and the chord connecting the superior- and midpoints on the posterior medial pterygoid plate edge. This is a measure of the orientation of the orifice relative to medial pterygoid plate height.</td>
</tr>
<tr>
<td>ESupang</td>
<td>Mean of the left and right angles made between the chord connecting the osseous Eustachian tube orifice to the midpoint on the posterior medial pterygoid plate edge and the chord connecting the osseous Eustachian tube orifice to the superior-most point on the posterior medial pterygoid plate edge. This is a measure of cartilaginous Eustachian tube length.</td>
</tr>
<tr>
<td>EuFlrang</td>
<td>Mean of the left and right angles made between the chords connecting the osseous Eustachian tube orifice to the inferior-most point on the posterior medial pterygoid plate edge and the chord connecting the</td>
</tr>
</tbody>
</table>
inferior-most and midpoints on the posterior medial pterygoid plate edge. This is a measure of cartilaginous Eustachian tube floor orientation relative to the vertical axis of the posterior edge of the medial pterygoid plate.

| CBAang | External basicranial flexion as measured by the angle hormion-sphenobasion-basion |
Table 3.12  Z-scores of pathological individuals plotted against the normal human range.

<table>
<thead>
<tr>
<th>ID</th>
<th>stage</th>
<th>ChoanW</th>
<th>ChoanHt</th>
<th>IndChoan</th>
<th>CHPang</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_31 (cleft palate)</td>
<td>5</td>
<td>2.261</td>
<td>-2.132</td>
<td>4.383</td>
<td>0.562</td>
</tr>
<tr>
<td>E_32 (cleft palate)</td>
<td>5</td>
<td>1.441</td>
<td>-0.339</td>
<td>1.476</td>
<td>-0.56</td>
</tr>
<tr>
<td>E_30 (microencephaly)</td>
<td>5</td>
<td>-0.588</td>
<td>0.696</td>
<td>-0.984</td>
<td>0.321</td>
</tr>
<tr>
<td>E_28 (hydrocephalus)</td>
<td>5</td>
<td>0.024</td>
<td>-2.433</td>
<td>2.266</td>
<td>0.992</td>
</tr>
<tr>
<td>E_29 (hydrocephalus)</td>
<td>4</td>
<td>-1.714</td>
<td>-1.122</td>
<td>-0.571</td>
<td>0.166</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>stage</th>
<th>InfChW</th>
<th>SupChW</th>
<th>AvPterHt</th>
<th>PalL</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_31 (cleft palate)</td>
<td>5</td>
<td>5.642</td>
<td>3.645</td>
<td>2.225</td>
<td>-0.61</td>
</tr>
<tr>
<td>E_32 (cleft palate)</td>
<td>5</td>
<td>4.63</td>
<td>2.187</td>
<td>2.785</td>
<td>0.255</td>
</tr>
<tr>
<td>E_30 (microencephaly)</td>
<td>5</td>
<td>-0.25</td>
<td>0.234</td>
<td>1.549</td>
<td>2.222</td>
</tr>
<tr>
<td>E_28 (hydrocephalus)</td>
<td>5</td>
<td>2.06</td>
<td>0.876</td>
<td>1.918</td>
<td>-0.436</td>
</tr>
<tr>
<td>E_29 (hydrocephalus)</td>
<td>4</td>
<td>-1.626</td>
<td>-1.226</td>
<td>0.695</td>
<td>-2.181</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>stage</th>
<th>NasW</th>
<th>SphenL</th>
<th>AvEusFlr</th>
<th>AvEust</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_31 (cleft palate)</td>
<td>5</td>
<td>2.384</td>
<td>2.149</td>
<td>0.716</td>
<td>0.027</td>
</tr>
<tr>
<td>E_32 (cleft palate)</td>
<td>5</td>
<td>1.834</td>
<td>2.389</td>
<td>-0.048</td>
<td>-0.9</td>
</tr>
<tr>
<td>E_30 (microencephaly)</td>
<td>5</td>
<td>-2.949</td>
<td>-0.034</td>
<td>1.59</td>
<td>0.798</td>
</tr>
<tr>
<td>E_28 (hydrocephalus)</td>
<td>5</td>
<td>-2.4</td>
<td>0.711</td>
<td>-0.075</td>
<td>-0.444</td>
</tr>
<tr>
<td>E_29 (hydrocephalus)</td>
<td>4</td>
<td>-2.254</td>
<td>-0.407</td>
<td>-0.12</td>
<td>-0.659</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>stage</th>
<th>AvEust2</th>
<th>Euang</th>
<th>ESupang</th>
<th>EuFlrang</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_31 (cleft palate)</td>
<td>5</td>
<td>-0.717</td>
<td>-0.694</td>
<td>-0.694</td>
<td>-0.694</td>
</tr>
<tr>
<td>E_32 (cleft palate)</td>
<td>5</td>
<td>-2.026</td>
<td>-1.206</td>
<td>-1.206</td>
<td>-1.206</td>
</tr>
<tr>
<td>E_30 (microencephaly)</td>
<td>5</td>
<td>-0.533</td>
<td>-1.248</td>
<td>-1.248</td>
<td>-1.248</td>
</tr>
<tr>
<td>E_28 (hydrocephalus)</td>
<td>5</td>
<td>-0.8178</td>
<td>-0.394</td>
<td>-0.394</td>
<td>-0.394</td>
</tr>
<tr>
<td>E_29 (hydrocephalus)</td>
<td>4</td>
<td>-0.557</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>stage</th>
<th>CBAang</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_31 (cleft palate)</td>
<td>5</td>
<td>-0.695</td>
</tr>
<tr>
<td>E_32 (cleft palate)</td>
<td>5</td>
<td>-0.009</td>
</tr>
<tr>
<td>E_30 (microencephaly)</td>
<td>5</td>
<td>0.292</td>
</tr>
<tr>
<td>E_28 (hydrocephalus)</td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td>E_29 (hydrocephalus)</td>
<td>4</td>
<td>-0.542</td>
</tr>
</tbody>
</table>
Figure 3.23 Note that the individual with cleft palate appears to have relatively wider choanae and laterally rotated medial pterygoid plates (likely related to the preponderance of cases in which the muscular sling of the tensor veli palatini muscle fails to contact the hamulus).
Figure 3.24 Specimens with hydrocephalus did not exhibit any significant differences ($P>0.05$) from normal crania in the relative dimensions of the choanae or overall nasopharynx.
Figure 3.25 Individuals with microencephaly exhibited no observable differences in choanal or upper respiratory tract proportions from normal humans.
Figure 3.26 Even edentulous specimens with complete alveolar resorption exhibit no observable difference from normal humans in relative choanal or upper respiratory tract dimensions. This suggests that the functional demands of respiration maintain the proportions of the nasal cavity and nasopharynx throughout postnatal life.
3.5.9.2 Population differences in nasopharyngeal dimensions

*P*-values for multiple t-tests were set at \( P=0.00076 \) as per Bonferroni adjustment of 66 orthogonal comparisons among 11 groups. The results indicated that variation in nasopharyngeal morphology did not occur along strict climatic gradients, there being much overlap in many of these relative dimensions. For example, Alaskan Inuits exhibited significantly greater choanal width than Aleutian Islanders and Australian Aborigines but did not show any significant differences from any other group. Alaskans and Australians also exhibited the lowest relative choanal height, each being significantly smaller than those of Chinese and Northern European crania. The greatest relative choanal height was exhibited by the Chinese sample, which had significantly larger values than East and West Africans, Australians, and Alaskans.

In overall choanal shape, Alaskans had a significantly higher choanal index than all groups (including Aleutian Islanders) except for Southern Europeans, North Africans, East Africans, and West Africans. Their relative midline choanal height and medial pterygoid heights were the lowest of all the populations. While the Chinese sample exhibited the greatest relative choanal area (significantly greater than half of all the groups sampled), Australians exhibited the smallest relative choanal area (significantly smaller than all populations except East Africans, but were nested among the other populations in medial pterygoid plate height), relative nasopharyngeal volume (significantly smaller than all groups except West Africans, East Africans, Alaskans, and Aleutian Islanders), and bi-ovale width (significantly smaller than all groups except Aleutian Islanders and Indians). They present with the overall smallest nasopharyngeal proportions of any group, which may bear functional implications on respiratory physiology and energy dynamics.
Figure 3.27 Box-whisker plots of significant ($P=0.00076$ as per the Bonferroni formula) differences among human populations. For each plot, abbreviations along the X axes indicate populations (Aust=Australian; N Euro=Northern European; S Euro=Southern European; N Afr=North Africa; E Afr=East Africa; W Afr=West Africa; SE Asia=South East Asia). The Y axes represent units of Procrustes-corrected length, area, and volume. Overall, the Australian sample exhibited the smallest choanal dimensions. Their nasopharyngeal dimensions appeared distinct from other human groups. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. These plots were generated using the program SAS.
Most nasopharyngeal dimensions were extremely conservative (coefficient of variation of each measure at approximately 10%; see Table 3.13) with the exception of relative nasopharyngeal roof depth (Coefficient of Variation= 29.03%). In this dimension, Alaskan Inuit and Australians (but not Aleutian Islanders) had the highest values but were not significantly different from any other population except the Chinese, which had the lowest mean value. Relative nasopharyngeal roof depth was not significantly different between Southern Chinese crania and any other population but was significantly greater in the Northern Chinese sample than Australians, Aleutian Islanders, Alaskans, and East and West Africans. This measure did not share a strong correlation with any other nasopharyngeal dimension. It thus appears likely that observed inter-population differences in relative nasopharyngeal dimensions do not correspond to any respiratory adaptation to climate and that these structures are mostly under strong functional constraint.

Table 3.13 Coefficients of variation for facial and nasopharyngeal measures among human adults. Note that most nasopharyngeal dimensions exhibit a coefficient of variation under 10% except for nasopharyngeal roof depth. Angles were converted into radians as per Schmall and Finkel (1909).

<table>
<thead>
<tr>
<th>Univariate Measures</th>
<th>Coefficient of Variation in Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi-Carotid Width</td>
<td>5.996</td>
</tr>
<tr>
<td>External Basicranial Flexion in radians (hormion-sphenobasion-basion)</td>
<td>6.094</td>
</tr>
<tr>
<td>Bi-Ovale Width</td>
<td>6.182</td>
</tr>
<tr>
<td>Facial Kyphosis in radians (prosthion-hormion-sphenobasion)</td>
<td>7.526</td>
</tr>
<tr>
<td>Palate Length</td>
<td>8.369</td>
</tr>
<tr>
<td>Choanal Orientation in radians (staphylion-hormion-sphenobasion)</td>
<td>8.785</td>
</tr>
<tr>
<td>Chonal Height</td>
<td>9.474</td>
</tr>
<tr>
<td>Nasal Index</td>
<td>17.093</td>
</tr>
<tr>
<td>Nasopharyngeal Roof Depth</td>
<td>29.030</td>
</tr>
</tbody>
</table>
When divided into cold climate (Alaskan Inuit, Aleutian Islander, Northern European, Northern Chinese) and tropical (East African, West African, South East Asian) groups, relative choanal dimensions were not significantly different ($P>0.05$; see Table 3.14). Yet, the cold climate groups exhibited significantly taller medial pterygoid plates (measured at the posterior edge; $P=0.002$) and more acutely angled choanae ($P<0.0001$). Tropical groups exhibited significantly greater palate length ($P<0.0001$) but did not have significantly longer rhinion-hormion distance ($P>0.05$), suggesting that the nasal cavity is not relatively elongated in this group.

Table 3.14 A series of Student's t-tests comparing the relative nasopharyngeal dimensions of human groups pooled into "cold" (Northern Europe, Northern China, Alaskan, Aleutian) and "tropical" (East Africa, West Africa, South East Asia) climates.

<table>
<thead>
<tr>
<th></th>
<th>P-Value</th>
<th>Larger mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choanal width</td>
<td>0.054</td>
<td>Cold Climate Group</td>
</tr>
<tr>
<td>Choanal Height</td>
<td>0.575</td>
<td>Cold Climate Group</td>
</tr>
<tr>
<td>Bi-ovale width</td>
<td>0.17</td>
<td>Tropical Climate Group</td>
</tr>
<tr>
<td>Staphylion-Sphenobasion length</td>
<td><strong>0.046</strong></td>
<td>Tropical Climate Group</td>
</tr>
<tr>
<td>Palate length</td>
<td><strong>1.3E-09</strong></td>
<td>Tropical Climate Group</td>
</tr>
<tr>
<td>Nasopharyngeal roof depth</td>
<td>0.332</td>
<td>Tropical Climate Group</td>
</tr>
<tr>
<td>Nasopharyngeal volume</td>
<td>0.893</td>
<td>Tropical Climate Group</td>
</tr>
<tr>
<td>Area of the choanae</td>
<td>0.063</td>
<td>Cold Climate Group</td>
</tr>
<tr>
<td>Angle of choanal orientation(staphylion-hormion-sphenobasion angle)</td>
<td><strong>4.479E-06</strong></td>
<td>Tropical Climate Group</td>
</tr>
<tr>
<td>Average medial pterygoid plate height</td>
<td><strong>0.002</strong></td>
<td>Cold Climate Group</td>
</tr>
<tr>
<td>Minimum nasopharyngeal height (perpendicular line intersecting Hormion and the nasopharyngeal floor at the level of staphylion)</td>
<td>0.303</td>
<td>Cold Climate Group</td>
</tr>
</tbody>
</table>
Regarding sexual dimorphism in relative choanal dimensions, none was found when the human populations were pooled. A lack of significant \( P<0.05 \) differences between males and females was also observed among four human populations for which large enough samples of known sex were available. However, among West African crania, males exhibited significantly \( P<0.024 \) taller choanae than females. This was not accompanied by any significant \( P<0.05 \) difference in choanal width, which may be more functionally constrained.

**Table 3.15** Human sexual dimorphism in relative choanal width and height.

<table>
<thead>
<tr>
<th>Population</th>
<th>Male n/Female n</th>
<th>Relative choanal width (p)</th>
<th>Relative choanal height (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inuit</td>
<td>19/13</td>
<td>0.07</td>
<td>0.517</td>
</tr>
<tr>
<td>Northern European</td>
<td>25/6</td>
<td>0.115</td>
<td>0.254</td>
</tr>
<tr>
<td>West African</td>
<td>19/11</td>
<td>0.683 *0.024</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>37/8</td>
<td>0.735</td>
<td>0.827</td>
</tr>
<tr>
<td>Indian</td>
<td>7/8</td>
<td>0.395</td>
<td>0.15</td>
</tr>
<tr>
<td>Pooled human adults</td>
<td>168/66</td>
<td>0.999</td>
<td>0.223</td>
</tr>
</tbody>
</table>

Finally, relative cartilaginous Eustachian tube (CET) floor length (distance between the Eustachian point and the point just superior to the pterygoid hamulus) showed no significant \( P<0.05 \) differences between any two populations. However, Chinese and West African crania were distinguished in possessing some of the lowest medial pterygoid heights (between its superior-most point and the superior hamulus border) and significantly \( P<0.05 \) greatest relative distances between the Eustachian point and the medial pterygoid plate posterior edge at its superior-most point among all groups. Australians had the shortest relative distance between the
Eustachian point and the superior-most point on the medial pterygoid posterior margin, which was significantly ($P<0.05$) smaller than West Africans, Chinese, and Alaskans. They also exhibited significantly ($P<0.05$) lower angles between the axis of the CET and the vertical axis of the medial pterygoid plate posterior edge than any other population with no other significant differences among the other groups. The angle made between this axis and the medial pterygoid plate posterior edge was significantly ($P<0.05$) smaller in Australians than among all other groups, which exhibited no significant ($P<0.05$) differences between each other. This evidence suggests that Australian Aborigines possess osseous Eustachian tube orifices that are vertically more vertically oriented than among all other human groups sampled. Further study is needed to confirm whether infants and young children from these populations are also characterized by such distinct nasopharyngeal morphology and if it contributes to their high rates of otitis media.

3.6 Discussion

3.6.1 Assessment of Hypothesis #1: Humans over all periods of postnatal ontogeny possess greater relative external basicranial (nasopharyngeal) width compared to other anthropoid species because of facial orthognathy and kyphosis.

The hypothesis is partially supported as both Stage 1 and Stage 5 humans are among the most orthognathic groups but did not exhibit the most facial kyphosis. The facial kyphosis angle does not distinguish Stage 1 humans from non-human hominoids nor did it separate Stage 5 humans from papionins or Stage 2 apes. It was found that papionins possessed the greatest amounts of facial kyphosis, likely an adaptation for the hafting of a prognathic face and
Anthony S. Pagano

elongated hard palate. Stage 5 humans were found to be more like *Papio* and *Macaca* in facial kyphosis than Stage 1 humans and Stages 2-5 of the other hominoids.

Among most mammals, an aiorrhynch facial morphology appears related to increased facial prognathism and decreased basicranial flexion (e.g. Hofer, 1952; Starck, 1953). However, among the anthropoid primate genera sampled here, the angle of facial kyphosis and palate length were only weakly correlated (r= 0.352) while a strong negative correlation (r=-0.67) was found between facial kyphosis and the external basicranial angle (the hormion-sphenobasion-basion angle). The angle of facial kyphosis had a strong negative correlation with the hormion-basion distance (r=-0.635), an additional measure of external basicranial flexion. These data suggest that the upper respiratory tracts of anthropoid primates may be subject to a differing set of functional demands than other mammalian groups and likely strepsirhines. This was suggested by Ross and Ravosa (1993) who found that facial kyphosis shared different morphological relationships among strepsirhine, haplorhine, and anthropoid samples.

Unexpectedly, the papionins exhibited greater facial kyphosis than hominoids with *Papio* being the most kyphosed genus during infancy and adulthood. Humans were less kyphosed than *Papio, Macaca, and Cercocebus* but more kyphosed than the non-human hominoids. The downward tilt of the papionin face is likely produced by the mechanical stresses of hafting a long, narrow rostrum. With no olfactory recess or well expressed transverse ethmoidal lamina as in macrosomatic strepsirhines or most non-primate mammals, inferior tilting of the nasal cavity and palate may not necessarily impinge on olfactory function. The papionins typically possess only one ethmoturbinal rather than the two present among most hominoids (Maier, 2000) or the four among macrosomatics such as *Microcebus* (see Smith and Rossie, 2008). Humans, on the other hand, likely achieve their advanced facial kyphosis through a combination of extreme
orthognathy, increased basicranial width, and external basicranial flexion, evolving it homoplasitcally relative to *Papio* under a separate set of functional demands.

The correlation analysis revealed that, despite strong negative correlations among hominoids between the angle of facial kyphosis and measures of nasopharyngeal width, these were not strong or significant in the pooled sample of hominoids and papionins. Thus Hypothesis 1 would be supported by the data if only the hominoid sample was used. When considered alongside the results of the t-tests of the facial kyphosis angle, these differing correlations further suggest that the papionin upper respiratory tract is under a set of functional demands separate from those acting upon the hominoids. As a model of extreme prognathism, *Papio* has thus revealed some unexpected morphologic relationships among the facial skeleton, basicranium, and nasopharyngeal boundaries.

Humans underwent substantial early growth in relative basicranial width relative to all other sampled anthropoid genera. Yet, even among intergeneric comparisons of Stage 2 crania, humans still exhibit the relatively widest basicrania between the left and right foramina ovale and carotid canals. They also exhibited the greatest relative choanal width despite the possession of significantly greater choanal index among Stage 2 *Hylobates*. Overall, Stage 2 humans appear to be distinguished from Stage 2 of all other primate genera by the same measures that distinguish adults. The rarity of crania representing newborn and young infant non-human primates hampers comparative analysis of the earliest developmental differences among the sampled genera. Future studies should focus on this stage of development as it may reflect morphological patterns established over fetal development before exposure to the functional demands of respiratory air conditioning and mastication.
The increase of bi-carotid width and decrease of bi-ovale width over human development is worth noting. These growth trends may imply that different portions of the basicranium are under separate functional constraints. The anterior portion of the basicranium corresponding to bi-ovale width is likely influenced by the demands of the upper respiratory tract while the bi-carotid width is located closer to the foramen magnum and the basiocciput, to which prevertebral muscles of head positioning attach (i.e., longus capitis and rectus capitis anterior). The intervening basioccipital element decreases significantly in relative length (sphenobasion-basion distance) between Stages 1-2 in humans and does not undergo any further growth change later in development. Indeed, this bony element broadens with the growth of the prevertebral musculature. This process is not completed within the first four postnatal months representing Stage 1, thus newborns are unable to position their heads vertically against gravity and their crania lack the rugosity associated with insertions of well-developed rectus capitis anterior and longus capitis muscles on the basiocciput. The basioccipital element may thus be excluded in estimation of the posterior nasopharyngeal boundaries as it does not appear to be directly related to the dimensions of the respiratory space. A distinction is likely warranted between the anterior half of the basicranial axis superiorly bounding the nasopharynx and the posterior half, which corresponds to a space occupied by prevertebral muscles and does not directly determine the dimensions of the upper airway.

The relative choanal area of *Hylobates* was significantly smaller than any other genus during both infancy and adulthood but they were not distinguished in nasopharyngeal depth or volume. This feature may functionally equate to a smaller amount of inspiratory air capable of entering the nasopharynx, perhaps related to a lower metabolic demand associated with brachiation relative to other locomotor modes more common among other anthropoids. Yet,
when considered alongside vertically shortened choanae that are most similar in proportion to those of humans, one may also postulate a relationship with laryngeal anatomy. *Hylobates* is known for its calling behavior and use of an enlarged laryngeal (vestibular) sac in vocal behavior (e.g. Hewitt et al., 2002). Choanal shortening may, in part, be related to this anatomical feature in some as yet unexplained morphological relationship.

Among both Stage 2 and Stage 5 male adults, *Papio* did not differ significantly from *Gorilla* in choanal index, relative palate length, or relative choanal area, in which they were distinct from all other genera. Both exhibited substantial growth changes to these dimensions between Stages 4-5. They also exhibit similar relative bi-ovale width as adults but the growth trajectory of this measure in *Gorilla* is much longer whereas it occurs solely between Stages 4-5 in *Papio*. These two taxa appear to have convergently evolved longer relative palate length and choanae that are tall and narrow in shape. Relative palate length and relative choanal width were strongly correlated ($r = -0.76$), suggesting that the possession of relatively narrow choanae may be a mechanical consequence of possessing a long, narrow rostrum. The selective pressure for this trait may have been strong as its morphologic change involves related changes to the upper respiratory tract and, by extension, the physiology of respiratory air conditioning.

3.6.2 Assessment of Hypothesis #2: The nasopharynges of basicranially flexed modern human adults are antero-posteriorly short and vertically high, growing from a long, low shape in infants.

Hypothesis 2 was supported by the results as newborns exhibited longer, less flexed basicrania (i.e., longer hormion-basion distance), greater nasopharyngeal roof depth, and
choanae that are obtusely oriented relative to adults. Humans exhibited no marked growth change in relative floor depth (the only significant difference being between Stages 2-5 but not Stages 1-5) but the depth of the posterior vomeral edge and maximum relative depth (the staphylion-sphenobasion distance) both increased between Stages 1-2, remaining unchanged between Stages 2-5. Nasopharyngeal roof depth decreased significantly between Stages 1-2 and continued shortening up until Stage 5. It is likely that simultaneous increase of relative posterior vomer depth and decrease of relative external basisphenoid length may lead to the stabilization of total nasopharyngeal floor depth throughout development.

There appear to be a large number of significant growth changes among humans relative to other hominoids, suggesting a distinct growth trajectory in this genus. Indeed, human nasopharyngeal growth is characterized by a major restructuring of the upper airways in which the tongue and hyolaryngeal complex descend inferiorly so that a permanent oropharynx exists, effectuating an intersection of alimentary and respiratory spaces (e.g., Laitman et al., 1978; Davidson et al., 2005; Vorperian et al., 2005). As such, adult humans exhibit upper respiratory tract morphology that is distinct from both human infants and all other non-human hominoids. Results of the analysis indicate that human adults exhibit significantly shorter relative hormion-basion length and wider relative bi-carotid width than any other group. The former measure appears intimately related to external basicranial flexion which, despite lack of significant difference of adult humans from Stage 1 humans and Stage 2 apes in the hormion-sphenobasion-basion angle, was still manifested in reorientation of the basicranial axis and shortening of the roof of the nasopharynx between hormion and sphenobasion. The above measures may thus be related to laryngeal descent over human development and evolution, which contribute to the formation of optimal supralaryngeal vocal tract proportions for the articulation of speech sounds.
While many growth changes distinguished Stage 1 of human development, further large scale growth trends are also occurring within Stage 2. The latter encompasses a broad period of life history, roughly approximating the fourth postnatal month to the sixth year. The entire first year of life is of vital importance as the aerodigestive tract shifts from a two-tubed system (with separation of airway and aerodigestive tract) to a single-tubed system (with crossing of the airway and aerodigestive tract). Within the first four postnatal months alone, developmental milestones in autonomic regulation, vision, hearing, laryngeal chemoreflexes, and vocalization activity are reached (Micham and Laitman, 2012). This developmental shift warrants further study so that growth changes within Stages 1 and 2 may be better understood and constrained to smaller units of time.

3.6.3 Hypothesis #3: The estimated dimensions of the cartilaginous Eustachian tube undergo growth change from infancy to adulthood, becoming longer and more vertically oriented.

Hypothesis #3 is supported by the results as increases in angulation and relative Eustachian tube floor length occur exclusively at the junction of Stages 1-2. Overall, it appears that the CET grows more acutely angled from infancy to adulthood. The subtle growth change in relative distance between the Eustachian point and medial pterygoid plate midpoint suggests that this may be a functionally constrained dimension related to the hafting of the CET to the medial pterygoid plate. The increase in relative CET floor length (between the Eustachian point and pterygoid hamulus) suggest that the functional length of the floor does indeed undergo significant and substantial growth change, which likely influences otitis media etiology. Growth
change in the relative distance from the Eustachian point to the superior-most point on the posterior medial pterygoid plate boundary suggests that relative height of the osseous Eustachian tube orifice on the temporal bone also becomes significantly taller between Stages 1-2. Relative CET length may thus be influenced by both increases in medial pterygoid plate height and increases in osseous Eustachian tube orifice height.

The relative cartilaginous Eustachian tube (CET) floor length grew significantly only between Stages 1-2 as did the angle it made with the posterior medial pterygoid plate edge. This feature was measured alongside increased relative medial pterygoid plate height between the hamulus and and posterosuperior-most point between Stages 1-2. Expansion in this dimension allows for more vertical space, which may be occupied by a larger relative CET pharyngeal orifice, expanding with more hypertrophied and mature levator veli palatini and tensor veli palatini musculature. Decreased angulation between the Eustachian point and superior-most point on the medial pterygoid plate also suggests a vertically taller orifice of the osseous Eustachian tube as well as greater robusticity at the temporal bone origin of the levator veli palatini, which is located between the Eustachian point and carotid canal (Huang et al., 1997). Its growth continued to a lesser magnitude up until the eruption of the first permanent maxillary molars, as did the midline orientation of the choanae (which grew more acute in adults). Overall, the CET appears to grow vertically taller, longer, and more acutely angled with more efficient musculature control patency. The timing of these growth changes may indicate a potential relationship with the frequent development of otitis media among infants. Its persistence into early childhood may also be related to later growth changes seen in choanal plane angulation and osseous Eustachian tube orifice height.
3.6.4 Variation among humans

The influence of cranial developmental deformities on nasopharyngeal shape

The pathologic growth of the neurocranium in the hydrocephalic individuals did not appear to influence heavily the dimensions of the osseous nasopharyngeal boundaries, which appeared normal in relative proportions (except for a relative choanal height at the lower range of normal adults in one of the crania). However, individuals with cleft palate exhibited abnormally wide choanae, which may have cascading effects on function of the Eustachian tube and velar musculature. Indeed, cases have been reported in which the tensor veli palatini muscle of cleft palate patients does not contact the medial pterygoid hamulus, causing insufficient middle ear aeration and substandard control of the soft palate (Perry and Keuhn, 2007). Patients with such a condition are at heightened risk for developing otitis media and its associated complications (Bluestone, 2005). With information on the normal growth of the nasopharyngeal boundaries presented in this study and elsewhere, the comparative rate and trajectory of upper airway development in infants and children after cleft palate corrective surgery should be investigated.

Sexual dimorphism

When grouped by sex, human males did not significantly ($P>0.05$) differ from females in choanal height, *contra* Bastir et al. (2011). However, male West Africans did possess relatively
(\(P<0.05\)) taller choanae than females, as was the case within the "Ibo-tribe" sample of Bastir et al. (2011). There was no other dimorphism found within any other population, likely a result of low sample size for each sex within each human population (and a high male bias) though a greater number of populations are represented. The sex difference in choanal height among West Africans is still smaller than the smallest significant \(P<0.05\) population difference measured, which was between pooled sex samples of West Africans and Northern Europeans. The results indicate that choanal shape differences among populations are greater than those between sexes.

**Climate and population differences**

Among all of the traits observed, there were three that differed significantly \(P<0.05\) among pooled populations from "cold climates" (Alaskan Inuits, Aleutian Islanders, Northern Europeans and Northern Chinese) and those from tropical climates (East and West Africans, South East Asia). The former group exhibited significantly \(P<0.0001\) smaller choanal plane angles (more vertically oriented choanae), smaller maximum relative nasopharyngeal depth (between staphylion and sphenobasion), and greater relative medial pterygoid plate height. The tropical group had more anteriorly rotated choanae and a relatively larger maximum nasopharyngeal depth with lower medial pterygoid plate height. The physiological importance of these shape differences is not yet understood. Few studies have considered the potential impact of choanal orientation on postnasal air conditioning whereas medial pterygoid plate height likely impacts the vertical size of both the postnasal airway and the pharyngeal orifice of the cartilaginous Eustachian tube. The above data warrant further investigation into these dimensions.
None of the above measures significantly distinguish all cold climate and tropical populations in pairwise comparisons. All of these groups tend to nest among each other in most measures with Australian Aborigines exhibiting the most distinctive nasopharyngeal morphology. They possessed the significantly smallest choanae and overall nasopharyngeal dimensions. Mlynski et al. (2001) and Bastir et al (2011) suggested that choanal dimensions could be related to metabolic demands by allowing in a greater volume of inspiratory air. Future studies should further investigate the respective interactions of climate, body mass, stature, basal metabolic rate, and sex with choanal dimensions to better understand their basic biology.

As suggested in this study by low coefficients of variation, most aspects of osseous nasopharyngeal boundary morphology are likely functionally constrained. They also tend to lack strong ($r>0.6$) correlations with facial and piriform aperture dimensions, which have been known to vary greatly among human groups (e.g., Davies, 1932; Weiner, 1954; Wolpoff, 1968). This result corroborated Bastir and Rosas (2013) who also found little correlation between piriform aperture and choanal morphology. Climatic variables likely influence nasopharyngeal morphology in a subtle, more global context. Three-dimensional geometric morphometrics techniques such as those using principal components analysis and partial least square analysis allow visualization of such changes across multi-dimensional shape vectors (e.g., Noback et al., 2011). However, when comparing individual populations as done in this study, such climatic differences are not visible. They are instead observable in studies of the external nose and paranasal sinuses (e.g., Marquez et al., in press) which distinguish climatic patterns from pairwise comparisons of populations. Overall, the degree of nasopharyngeal morphologic diversity in this human sample is minute and likely reflects a small degree of environmental adaptation along with microevolutionary differences accrued over separate population histories.
These differences are smaller in scale relative to the variation present among anthropoid primate genera, which exhibit a wide array of morphologic configurations.

**Predisposition to upper respiratory tract disease**

The results indicated that Australian aborigines exhibit nasopharyngeal anatomy distinct from all other sampled human populations with the lowest relative choanal area and volume. Unexpectedly, the estimated CET floor of Australians was found to be significantly more vertical in orientation than any other sampled population. It remains to be seen when these features emerge over development and if they are preceded by equally distinctive nasopharyngeal boundary morphology among infants and young children. Currently, no such studies have been published on population differences in nasopharyngeal morphology of this population. Australian Aborigines suffer from extremely high rates of otitis media with greater than half of all children experiencing related hearing loss in some communities (Stuart et al., 1972; Lewis et al., 1977; Moran et al., 1979; McCafferty et al., 1977, 1985; Dugdale et al., 1982; Hudson and Rockette, 1984; Foreman, 1987; Leach et al., 1994; Boswell and Nienhuys, 1995; Leach, 1999; Bluestone, 2005a; Morris et al., 2007). Judging from the relatively small choanal area of Stage 5 Australian crania, aboriginal infants and young children may possess relatively small nasopharyngeal dimensions that facilitate the passage of infectious agents from the nasal cavity and paranasal sinuses to the CET pharyngeal orifice.

The Alaskan and Aleutian Island Inuit samples, like Australian Aborigines, represent a population considered to be at “high risk” for otitis media (see review by Bluestone, 2005a). This sample did not show significant differences from any other population in measures of estimated...
cartilaginous Eustachian tube length or angulation. These results contrast Doyle (1977) who found that adult Inuit crania were distinguished from Native American, European American, and African American crania by estimated CET dimensions that were relatively longer and more horizontally oriented. However, both Alaskans and Australians in this study tended to exhibit greater relative anteroposterior nasopharyngeal roof depth between hormion and sphenobasion. The results warrant investigation of comparative growth series to test for such differences among infants and young children from these groups.

Another population of clinical interest was the Chinese, who exhibit some of the highest global rates of nasopharyngeal carcinomas. Typically, nasopharyngeal carcinoma is highest among Cantonese populations, especially those from Guangdong Province (See Section 2.5.3). It was found that the distance from the Eustachian point to the superior-most point on the medial pterygoid plate was significantly higher among Chinese crania (specifically, South Chinese) than any other population except for West Africans. This dimension may be related to a deeper lateral pharyngeal recess. The Chinese crania were also distinguished from the West Africans, East Africans, Australians, Aleuts, and Alaskans by having significantly larger choanal area and height. Future research should focus on the relationship between the osseous measures used here and the actual soft tissue dimensions of the nasopharynx and lateral pharyngeal recess. It should also be tested if the latter space is indeed deeper among individuals suffering from nasopharyngeal carcinomas.
3.7 Conclusion

The above evidence suggests that the nasopharyngeal boundaries share a morphologic relationship with the facial and basicranial skeleton. Hominoid and papionin genera with relatively great palate lengths (i.e. *Papio* and *Gorilla*) tend to exhibit narrow choanae while those exhibiting greater mediolateral facial widths (i.e. *Homo*) tend to exhibit broader choanae and nasopharynges. Greater amounts of external basicranial flexion appear to result in nasopharynges of reduced anteroposterior depth. These bear implications for the reconstruction of upper airway proportions among Plio-Pleistocene hominin fossils, which may exhibit both human-like and ape-like features of the face and basicranium.

In addition to potential usefulness in soft tissue reconstructions on fossils, analyses of nasopharyngeal boundary morphology may also bear clinical importance. These structures have been implicated in disease processes such as otitis media, arguably the most common pediatric clinical condition. Analysis of population differences in aspects of nasopharyngeal boundaries may thus contribute to understanding of middle ear disease etiology and susceptibility. Future work will investigate developmental differences among populations exhibiting high and low frequencies of otitis media.

Given its central location, aspects of the nasopharyngeal boundaries are intimately associated with functions of respiration, middle ear ventilation, olfaction, and speech. Morphological change to these structures over the respective courses of development and evolution bears profoundly on an array of physiologic functions. Nonetheless few evolutionary studies have focused exclusively on nasopharyngeal boundary morphology. Its components remain some of the least understood, yet crucial upper airway structures.
4. Histology of the primate nasopharyngeal boundaries: Testing for a relationship between air conditioning capacity and surface contractility

4.1 Aim of the chapter

This chapter will survey a range of primates to analyze distribution of the components necessary for nasal air conditioning (mucous cells, serous cells, and submucosal blood vessels; see below). Whether the patterns of distribution of these structures can be predicted from osseous nasopharyngeal boundaries will be tested. If so, this would have implications for reconstruction of soft tissue morphology among fossils.

4.2 Background

Many studies discuss histological parameters of the nasopharynx for the purposes of inferring etiology and staging of carcinomas (e.g. Yeh, 1962; Pathmanathan et al., 1995; Robinson et al., 2013) or evolutionary and developmental origins of the anterior and posterior halves (e.g., Leela et al., 1974; Tobias, 1991a). However, few have examined relationships between histology and the grossly observable osteological features that can be utilized in soft tissue reconstruction in fossils.

The distribution patterns of ciliated, pseudostratified and columnar (respiratory) epithelium, goblet cells, mucous and serous glands, and submucosal blood vessels and glands may yield useful data from which can be inferred the ability of a particular surface to aid in conditioning of postnasal nasal air. This study analyzes the mucosal and submucosal histology of the nasopharyngeal boundaries with the purpose of deducing function and structure of the
nasopharyngeal lateral walls (i.e. areas overlying the medial pterygoid plate, the torus tubarius, and the lateral pharyngeal recess), the floor (the nasopharyngeal surface of the soft palate) and the roof (the area overlying the basisphenoid bone). Specifically, two histological parameters will be described and related to gross anatomical landmarks: 1) the distribution of respiratory epithelium with goblet cells; 2) the distribution of serous and mucus-producing cells in the connective tissue layer of the nasopharyngeal boundaries, especially the posterior wall, which has been described as lacking respiratory epithelium and goblet cells (see below).

4.2.1 The respective roles of mucus-producing cells, serous cells and blood vessels in conditioning air.

The chief functions of upper respiratory tract soft tissues are the warming and humidification of inspiratory air. Warming is primarily achieved via submucosal blood vessels in the nose (Widdicombe, 1997), although heat exchange also occurs in the nasopharynx and trachea (Naclerio et al., 2007). The ability of the nose to heat inspiratory air is reduced in cold ambient conditions (Proctor et al., 1977; Naclerio et al., 2007). Thus the contributions of postnasal spaces may have taken on increased importance in conditions of extreme cold, e.g., among later Pleistocene Eurasian hominins such as Neanderthals.

Humidification of airflow occurs through the action of both mucus and serous fluid-producing cells. Mucosal cells can occur either among ciliated, pseudostratified, columnar (respiratory) epithelium or within the lamina propria, transmitting mucus to the airway surface via ducts. Serous fluid is produced solely within the lamina propria by specialized cells that may aggregate to form glands. Serous glands are present within the oral cavity (e.g., the palatine
gland on the ventral soft palate) but occur as mucouserous glands in the lining of the nasal cavity where both cell types aggregate within the lamina propria. Along respiratory surfaces, mucus is required to protect the epithelial cells from dessication by airflow (see below).

Airflow humidification occurs via a double-layered mucus covering the epithelium, which also functions in mucociliary transport [e.g., Button and Bouchard (2008), Randell and Boucher (2006), and Boucher (2003)]. This covering has a periciliary layer (the deeper layer directly overlying the epithelia), which contains serous fluid, tethered mucins (produced by goblet cells and submucosal mucous glands) and glycolipids that likely trap molecules and restrict access to underlying epithelial cells (Randell and Boucher 2006). The surface layer is comprised of mucins of higher molecular weight than those in the periciliary layer (0.5-20 µm in length) which interact with globular proteins (Thornton et al., 2004). This surface layer is also thought to be a serous fluid reservoir which can humidify inspiratory air and receive moisture from expiratory air without having the underlying epithelial cells come into direct contact with airflow. The surface layer also absorbs overflow of liquid from submucosal serous glands into the periciliary layer as these glands produce liquid at a faster rate than can be absorbed by epithelial cells (Jiang et al., 1997). The excess moisture in the periciliary layer is thus stored in the surface layer for the function of conditioning of air.

4.2.2 Previous studies of nasopharyngeal histology

Three discrete epithelial types were described lining the nasopharyngeal walls as early as 1902, when von Ebner identified a transitional type of epithelium present between respiratory epithelium and non-ciliated, stratified, squamous epithelium. The transitional epithelium
resembled the non-ciliated transitional epithelium of the urinary tract in appearance. Some suggest that the term “transitional epithelium” is not applicable to nasopharyngeal epithelia (e.g. Nakano et al., 1986) as it is already used to describe cells in other regions of the body. However, the term transitional epithelium has been retained in this thesis for convenience, in the absence of any widely accepted alternative. The term is used to describe cells intermediate in morphology between the respiratory and stratified, squamous epithelia (e.g., Harkema, 1991).

The earliest description of transitional nasopharyngeal epithelium identified the distribution as on the faucial isthmus, bordering the oropharynx (von Ebner, 1902). Bryant (1916) later described its distribution among rabbits, guinea pigs, cats, Cebus, Macaca and a human developmental series. He found that a distinct “intermediate zone” of transition was visible and that it was located at different sections of the lateral nasopharyngeal wall among the different animal specimens. Rabbits exhibited an intermediate zone anterior to and bordering the Eustachian tube orifice, while the guinea pig had an intermediate zone encompassing areas both anterior and posterior to the Eustachian tube orifice. The domesticated cat, non-human primates and the human growth series all exhibited an intermediate zone with the anterior limit overlapping the Eustachian tube orifice and the posterior boundary immediately posterior to the this orifice.

The definition of transitional epithelium was refined by Ali (1965) who analyzed the nasopharyngeal epithelial distribution in a sample of 100 human autopsy specimens whose age spanned the first to the eighth decade. He described nasopharyngeal transitional epithelium to be stratified, with no goblet cells (similar to squamous cells) but with vertically oriented nuclei and a columnar appearance like respiratory epithelium. Ali (1965) observed that roughly 65% of the nasopharyngeal surface was covered in squamous epithelium with only those portions adjacent to
the choanae covered in respiratory epithelium. He also noted the presence of transitional epithelium in patches at the salpingopharyngeal fold, lateral pharyngeal recess and on portions of the posterior nasopharyngeal wall. The presence of mast cells suggested that these islets occurred in association with irritation and/or inflammation. A wavy zone of thickened concentration was noted at the inferior aspect of the posterior nasopharyngeal wall near the faucial isthmus.

Further investigations again refined the definition of nasopharyngeal transitional epithelium. Khoo and Kanagasuntheram (1967) studied the histology of nasopharynges extracted from 99 autopsies of individuals ranging in age from the first to ninth decade. They focused on the lateral pharyngeal recess and found that respiratory, transitional and squamous epithelium were all present. Even the youngest individuals in their sample exhibited this composition. It was concluded that the native cell population was respiratory but that it underwent irritation and denuding, leading to the presence of squamous and transitional cell patches as part of a phase of regeneration and even metaplastic change in some individuals (e.g., Kanagasuntherum and Ramsbotham, 1968). This view was shared by Takahashi (1973).

Study of the nasopharyngeal histology of non-human primates (Leela and Kanagasuntheram, 1973) contradicted the hypothesis of a discrete zone of transitional epithelium on the nasopharyngeal wall. Leela and Kanagasuntheram (1973) analyzed nasopharyngeal histology on a sample of Tupaia (n=2), Nycticebus (n=2), Macaca fascicularis (n=2), and Hylobates (n=1). Like Khoo and Kanagasuntheram (1967), they also observed patchy distribution of all three epithelial types at or posterior to the Eustachian tube orifice, which they considered a heterogeneous zone of transition (contra the description of Bryant, 1916). Indeed, they noted a wide range of epithelial distribution patterns with most specimens exhibiting only squamous epithelium covering the posterior 1/4 or 1/5 of the nasopharyngeal boundaries. They
described a wide range of composition of transitional epithelia with some even remaining ciliated. Among all of the specimens used, Hylobates was the only one on which no transitional epithelium was found. Additionally, the lamina propria in all of the nasopharyngeal surfaces were found to contain extensive networks of blood vessels and mucoserous glands, which communicated with the epithelia either by distinct ducts or via submucosal crypts.

Later work by Nakano et al. (1986) directly challenged the conclusion of Bryant (1916) and Ali (1965) that there existed a zone of transitional epithelium with only one cell type. They corroborated the results of previous studies on humans (Khoo and Kanagasuntheram, 1967) and non-human primates (Leela and Kanagasuntheram, 1973) that there exist all three varieties of epithelium in the lateral pharyngeal recess and at the inferior portion of the posterior nasopharyngeal wall. They analyzed epithelia distribution among mice and found few differences from patterns previously described in humans. Nagai et al. (1997), who conducted the most recent study of normal epithelial distribution in humans, also corroborated the conclusions of Nakano et al (1986), Khoo and Kanagasuntheram (1967) and Leela and Kanagasuntheram (1973), stating that the transition from respiratory to squamous epithelium is mosaic and highly variable.

4.2.3 Contractility and the distribution of nasopharyngeal epithelia

Being a heterogenous structure, the boundaries of the nasopharynx are composed of both bony and muscular elements. Thus, some surfaces are of great contractility while others are of reduced contractility. Among the latter are the anterior nasopharyngeal wall (covering the medial pterygoid plates) and the nasopharyngeal roof (which overlies the basisphenoid bone). Leela et
al. (1974) suggested that such differences in surface contractility may also indicate the type of epithelium covering a surface. They argued that respiratory epithelium is found in the less contractile anterior half of the nasopharyngeal wall, which is innervated by the maxillary nerve (CN V2) and has goblet cells. The posterior half overlying pharyngeal constrictor muscle was argued to be covered in stratified squamous epithelium and innervated by the glossopharyngeal nerve via the pharyngeal plexus. The torus tubarius represents a zone of transition as it is also of intermediate contractility and was considered part of the anterior half of the nasopharyngeal wall [but see Tobias (1991) who uses embryologic evidence to suggest that the Eustachian tube cartilage should actually be aligned with the posterior nasopharyngeal moiety]. They supported their hypothesis using cinefluororadiography (to determine the surface contractility in several human subjects) and histological sections from humans, non-human primates, and *Tupaia*. However, no study has since independently tested for a relationship between nasopharyngeal surface contractility and epithelial distribution. Such a relationship, if confirmed, would be applicable to the estimation of nasopharyngeal histological distribution in fossils, which preserve only the contractile, bony surfaces.

4.3 Hypothesis

This study will test for functional morphologic limitations on the air conditioning ability of the nasopharynx via distribution of respiratory epithelium, goblet cells, and mucoserous glands solely over non-contractile portions of the nasopharynx overlying osseous surfaces. Given the conflicting accounts of nasopharyngeal epithelial distribution among primates and some non-primate mammals (see above), any new data on nasopharyngeal histology is of value to
understanding of the potential air conditioning capacity of this space, which has rarely been investigated. Among mammalian populations inhabiting cold climates, the possession of larger non-contractile nasopharyngeal surface areas lined with respiratory epithelium may assist the nasal cavity in more efficient warming and humidification of ambient air. If the hypothesis is supported by the evidence, direct measurements of medial pterygoid plate and external basisphenoid bone dimensions on Neanderthals could yield information on postnasal air conditioning capacity.

4.4 Materials and Methods

4.4.1 Materials

This study used a histological sample of six adult specimens representing *Homo sapiens*, *Hylobates sp.*, *Macaca fascicularis*, *Ateles sp.*, *Tarsius bancanus*, and *Otolemur crassicaudatus*. Three of the specimens (*Hylobates, Ateles, Tarsius*) were from the Cleveland Metroparks Zoo while the *Otolemur* was from the Duke University Primate Center and the *Macaca* was from the NYU Department of Anthropology. The human specimen was a cadaver from the SUNY Downstate College of Medicine.

4.4.2 Methods

Specimen preparation was performed by the author both at the Icahn School of Medicine at Mount Sinai (New York, New York) and at the laboratory of Timothy D. Smith at the School
of Physical Therapy, Slippery Rock University (Slippery Rock, Pennsylvania). Each specimen was fixed in formalin for at least two weeks before dissection. The nasopharyngeal boundaries of all of the fixed specimens were dissected from parasagittally sectioned heads with the larger hemi-nasopharynx selected for sectioning. Once extracted, each hemi-nasopharynx was perfused in a formic acid-sodium citrate solution, which is a slow acting decalcifying agent (e.g., Smith et al., 2007; DeLeon and Smith, in press). This was selected over other, faster acting agents such as Cal-Ex II which, paradoxically, damaged and discolored the mucosa but did not appear to affect the bony segments on a trial specimen (a cadaveric human hemi-nasopharynx not used in the analysis).

After decalcification, each of the specimens was dehydrated in a graded series of ethanols and embedded in paraffin. These were subsequently sectioned on a microtome with varying slice thicknesses. For each specimen, slice thickness was set at the smallest value that would not obscure the anatomy. These values were chosen for each specimen after several trials at varying slice thickness to prevent crumbling or shredding of the slices, which is more likely to occur when sections include dense portions of basisphenoid bone (the nasopharyngeal roof). The greater density of the osseous nasopharyngeal surfaces caused the microtome blade to vibrate and tear through the slices at thinner slice thickness, especially on larger blocks. The smaller non-human primates (i.e. Otolemur and Tarsius), were sectioned at 6 micrometers (µm) while the larger ones (i.e. Ateles, Macaca, Hylobates) were sectioned at 10 µm. The human specimen was the largest block and required sections of at least 12-14 µm in thickness to avoid crumbling or tearing. Every tenth section was mounted and stained with hematoxylin and eosin. The resulting slides were visualized via a Nikon Eclipse 50i light microscope with a digital sight from which images were recorded.
The nasopharyngeal boundaries were divided into six separate zones to better test for differences in epithelial distribution and submucosal histology among areas overlying bone (i.e. less contractile surfaces) and those overlying soft tissue only (i.e. surfaces of greater contractility; see Figure 4.1). Each zone was chosen to represent an area definable by gross anatomical structures, which determine surface contractility. The nasopharyngeal wall was described in terms of an anterior, paratubal, and post-tubal portion, which exhibits a lateral pharyngeal recess among humans. The anterior portion of the nasopharyngeal wall is its only non-contractile surface as it overlies the medial pterygoid plate posterior to the maxilloturbinal but anterior to the Eustachian tube cartilage. The paratubal portion overlies the Eustachian tube cartilage and salpingopharyngeal muscle, which are all contractile structures. Posterior to the torus tubarius, the post-tubal portion of the nasopharyngeal wall completely overlies contractile muscle anterior to the coronally oriented posterior nasopharyngeal wall. Overlying the basisphenoid bone, the nasopharyngeal roof is a completely non-contractile surface whereas the posterior nasopharyngeal wall and floor overly the superior pharyngeal constrictor muscle and soft palate respectively. Closer to the hard palate, the nasopharyngeal floor superficial to the central tendon of the soft palate is expected to be less contractile than more distal surfaces closer to the levels of major muscle insertions (i.e., musculus uvulae, levator veli palatini).
Figure 4.1 Illustration of a midsagittally section human cranium with nasopharyngeal soft tissues. Six separate zones have been color coded to demonstrate the anatomical relationships used to delineate contractile and non-contractile regions. The anterior portion of the nasopharyngeal wall and nasopharyngeal roof are the only exclusively contractile nasopharyngeal surfaces as each overlies bone.
4.5 Results

4.5.1 *Homo sapiens*

Some previous observations on epithelial distribution (e.g., Ali 1965) were confirmed on the human specimen. The greatest frequency of ciliated, pseudostratified, columnar (respiratory) epithelium occurs on areas overlying medial pterygoid plates, Eustachian tube cartilage, roof (overlying basisphenoid bone), and floor (soft palate). Despite being a contractile surface, respiratory epithelia still occurs over much of the dorsal soft palate as it does within the Eustachian tube. Goblet cells are not visible around the Eustachian tube lumen. Rather, specialized glands within the connective tissue layer appear to supply this surface.

Respiratory epithelium occurs with less frequency in the salpingopharyngeal fold, lateral pharyngeal recess, and the posterior nasopharyngeal wall where it grades into or is replaced by stratified, columnar epithelia. When it does occur on the posterior wall and lateral pharyngeal recess, respiratory epithelium is typically at a more superior position closer to the nasopharyngeal roof (basisphenoid bone). There are also small patches of respiratory epithelium to be found among the stratified, squamous epithelium. Yet, posterior to the torus tubarius, these small patches rarely contain goblet cells within the surface layer.

The epithelium lining clefts within the salpingopharyngeal fold and lateral pharyngeal recess exhibits hyperplasia. That is, rather than undergoing mitosis at the basal level, abnormally rapid rates of mitosis are occurring close to the surface. The resulting morphology obfuscates the organization of pseudostratified, columnar epithelia, which are instead replaced by extremely dense layers of irregularly shaped cells with mitotic nuclei stained nearly black. Such a condition
is probably the result of advanced age of the sampled individual as abnormal cell growth may be occurring in response to insult from years of high velocity air flow and muscular movement.

Figure 4.2 A slice through the nasopharyngeal floor (dorsal aspect of the soft palate). Ciliated, pseudostratified columnar epithelium covers this surface, which overlies the connective tissue layer atop the palatal musculature. (Magnification 60x; Slice #1070; Homo sapiens).
Figure 4.3 A section through the nasopharyngeal roof. At the surface lies pseudostratified, columnar epithelium while, at the connective tissue layer are mucous and serous glands in addition to a network of blood vessels that may heat inspiratory air. (Magnification 10x; Slice #1540; Homo sapiens)
Figure 4.4 Pseudostratified columnar epithelium lining the nasopharyngeal roof (Top), floor (Bottom), and lateral wall with torus tubarius (right). Large glands containing both mucus and serous cells are also visible in the connective tissue layer of the nasopharyngeal roof, suggesting that they participate in postnasal air conditioning. Note that the epithelium on some of the surfaces was damaged during sectioning. (Magnification 4x; Slice #1477; *Homo sapiens*)
Figure 4.5 Mitotic cells lining the surface of the lateral pharyngeal recess. The dark spots lining the nasopharyngeal surface are abnormally growing mitotic cells. Their thickness and location superficial to the basal layer may indicate a precancerous condition. (Magnification 40x; Slice #769; *Homo sapiens*)

Glands containing mucous cells and serous cells are present at all locations around the nasopharynx. They are located respectively within and under the lamina propria and span virtually all aspects of the nasopharyngeal boundaries. Mucous cells produce mucins that protect epithelium from dessication while serous cells aid in the humidification of inspiratory air with serous fluid. Large aggregations of these tissues were present close to the nasopharyngeal surface and to muscles on the soft palate (levator veli palatini; as part of the palatine mucous gland),
around the Eustachian tube cartilage (tensor veli palatini), and in the posterior nasopharyngeal wall (in the connective tissue layer overlying the superior pharyngeal constrictor muscle).

Figure 4.6 Section through the nasopharyngeal floor. There are mucous and serous glands alongside blood vessels in the connective tissue layer. These likely contribute to postnasal air conditioning. (Magnification 40x; Slice #1040; Homo sapiens)

Also present in the submucosal spaces of nearly all the nasopharyngeal surfaces were blood vessels. These likely transmit venous and arterial blood that aid in warming of inspiratory air. Despite lacking the "corpera cavernosa" present atop nasal conchae, there still appears to be a large volume of blood in the connective tissue layer. Considering this feature alongside the
distribution of goblet and serous glands within all of the nasopharyngeal boundaries, each of the surfaces may aid the nasal cavity in air conditioning, as would be expected for a space that receives high velocity air flow. Indeed air passing through the nasopharynx must negotiate a nearly perpendicular inferior bend before reaching the trachea (Schreider and Raab, 1981), which may increase airflow turbidity.
Figure 4.7 A section through the posterior nasopharyngeal wall. Despite a lack of pseudostratified columnar epithelia on the nasopharyngeal surface (right side), there is a rich network of mucous and serous cells and blood vessels in the connective tissue layer overlying the pharyngeal constrictor muscle. This suggests that even the contractile portions of the nasopharynx that lack pseudostratified columnar epithelium with interspersed goblet cells still play a role in postnasal airconditioning. The posterior nasopharyngeal wall may be an especially vital portion, serving as the last mucosal surface to directly contact, condition, and redirect inspiratory air before it travels toward the lungs. (Magnification 10x; Slice #500; Homo sapiens)
4.5.2 Hylobates sp.

Respiratory epithelium with thick concentrations of goblet cells covered the roof, floor, and lateral wall of the nasopharynx up to the level of the posterior torus tubarius and the anterior portion of the post-tubal nasopharyngeal wall. It was on this surface that transitional and stratified squamous epithelia became more prevalent, the latter aggregating at the tips of projections and in the furrows formed within the surface layer. Stratified squamous epithelium continued onto the posterior nasopharyngeal wall where it covered nearly the entire surface. Yet, despite epithelial type, mucous and serous glandular cells lined the posterior wall deep to the epithelium and basement membrane in the connective tissue layer. It appears that this pattern continues from the anterior half of the nasopharynx where mucous and serous glands invest the relatively shallow connective tissue layer underlying the epithelium of the nasopharyngeal roof.

The nasopharyngeal septum ended anterior to the level of the torus tubarius. It lacked muscular tissue, instead being composed of connective tissue heavily invested with blood vessels, mucous glands, and serous glands underlying respiratory epithelium with large concentrations of goblet cells at the surface. These glands likely operate independently from the enlarged palatine gland, which appears to comprise a marked proportion of velar area in cross section. The palatine gland is solely a mucous gland, which supplies the ventral soft palate surface. The septum appeared reinforced by cartilage at its inferior boundary with the soft palate. This cartilage anchors a layer of connective tissue that appears to separate the septal and palatine glands in cross section (see Figure 4.13).
Figure 4.8 A section through the lateral nasopharyngeal wall, anterior to the torus tubarius.

Ciliated, pseudostratified, columnar epithelium overlying the connective tissue atop the medial pterygoid plates. Large goblet cells are visible among the epithelial cells. (Magnification 40x; Slice # 304; Hylobates sp.).
Figure 4.9 A section through the anterior portion of the torus tubarius. Above is ciliated, pseudostratified, columnar epithelium with large goblet cells interspersed. (Magnification 60x; Slice #1455; *Hylobates* sp.)
Figure 4.10 A section through the posterior edge of the torus tubarius. The pharyngeal edge of the Eustachian tube cartilage is visible deep to the dense connective tissue layer covering it. At the nasopharyngeal surface, ciliated, pseudostratified, columnar epithelium is distributed. Interspersed among the epithelial cells are extremely large goblet cells that aggregate into small mucosal glands in some locations. (Magnification 10x; Slice #1785; Hylobates sp.)
Figure 4.11 Section through the posterior naspharyngeal wall. This surface is mostly covered by squamous epithelium, which is interspersed with patches of ciliated, pseudostratified, columnar epithelium. Large mucous glands are visible in the lamina propria. (Magnification 40x; Slice #2166; *Hylobates* sp.)
Figure 4.12 Section through the nasopharyngeal septum. This septum is lined with ciliated, pseudostratified, columnar epithelium and has a dense network of mucous glands in its connective tissue layer. Note that the dark coloration is an artifact of the cover slipping process (Magnification 4x; Slice #325; *Hylobates* sp.)
Figure 4.13 A section through the base of the nasopharyngeal septum where it meets the soft palate (bottom of the image). "Bubble artifacts" were created during the coverslipping process. The mucous glands within the septum appear separated from the palatine glands inferiorly, which can be seen occurring in lobes. This latter gland supplies the oral surface of the soft palate and contains no serous glands. A cartilaginous structure (darker purple) can be seen at the base of the nasopharyngeal septum, anchoring a thick connective tissue bundle. (Magnification 4x; Slice #335; *Hylobates* sp.)
4.5.3 *Macaca fascicularis*

Respiratory epithelium with goblet cells was present throughout the anterior reaches of the nasopharyngeal boundaries, including the floor (which did not exhibit numerous submucosal glands) and over the torus tubarius. The transitional type leading to squamous epithelia were only visible on the post-tubal portion of the lateral wall. Squamous epithelium tended to aggregate within furrows and clefts on this surface and was punctuated by patches of columnar cells. However, at the inferior-most portions of the post-tubal wall (at the level of the soft palate), squamous epithelium was present exclusively, there being a short zone of transition to this type from columnar cells more superiorly. The squamous epithelium continued onto the posterior nasopharyngeal wall, which it covered extensively.
Figure 4.14 A section through the nasopharyngeal floor. Ciliated, pseudostratified, columnar epithelium with goblet cells are present at this layer. Note the absence of large accumulations of submucosal glands. (Magnification 40x; Slice #1511; *Macaca fascicularis*)
Figure 4.15 A section through the lateral wall overlying the torus tubarius. At the surface lies ciliated, pseudostratified, columnar epithelium. It is supported by mucous and serous glands in the connective tissue layer. Note the presence of tonsilar tissue deep to the mucous glands. (Magnification 20x; Slice #1852; *Macaca fascicularis*)
Figure 4.16 The above is a slice through the torus tubarius in the lateral pharyngeal wall at the level of the Eustachian tube orifice. The nasopharyngeal surface superficial and inferior to the Eustachian tube cartilage is lined by ciliated, pseudostratified, columnar epithelium which is reinforced by mucous and serous glands. (Magnification 4x; Slice #1852; *Macaca fascicularis*)
Figure 4.17 The above image is a section through the superior portion of the post-tubal portion of the lateral nasopharyngeal wall. Note the transition from pseudostratified, columnar epithelium above to squamous epithelium below. (Magnification 40x; Slice #1144; *Macaca fascicularis*)
As described by Leela et al. (1974) and Doyle and Rood (1979), a nasopharyngeal septum was present and continued well posterior to the level of the torus tubarius. It was lined with respiratory epithelium atop a connective tissue layer. None of the muscle fibers from the soft palate communicated with the connective tissue within the septum. This arrangement suggests that the septum does not directly play a role in the elevation of the soft palate.
Figure 4.19 The above is a section through the epithelial surface of the nasopharyngeal septum. Note the ciliated, pseudostratified, columnar epithelia at the surface are extremely tall and densely packed with goblet cells. They lie atop a thick layer of connective tissue. (Magnification 40x; Slice #1535; Macaca fascicularis)
Figure 4.20 The above is a section through the nasopharyngeal septum, which appears to be comprised primarily of connective tissue and is covered in respiratory epithelium. Muscle fibers from the soft palate inferiorly do not continue up the septum. (Magnification 4x; Slice #1543; *Macaca fascicularis*)
Figure 4.21 Sagittal view of a *Macaca fascicularis* specimen. In the bottom image, the boundaries of the vomer (green), basisphenoid (red), and soft palate (black) are outlined to better define the nasopharyngeal septum,
No mucus or serous glands were present within the connective tissue layer of the nasopharyngeal septum but there were goblet cells among the respiratory epithelia covering it. Glandular tissue appeared concentrated around the nasopharyngeal floor (potentially as part of the palatine gland) and superior half of the lateral wall. At the level of the torus tubarius, these glands continued along the lateral wall up to the superolateral boundary of the septum. This pattern of distribution continues posteriorly into the lateral pharyngeal recess where collections of mucous and serous glands underlie the inferior-most aspects of the nasopharyngeal wall that are lined with squamous epithelium and contact the soft palate in life. They also occur at the superolateral edge of the lateral wall where pseudostratified, columnar epithelia are punctuated by patches of squamous epithelia. The glands continued onto the posterior nasopharyngeal wall where they were invested in the connective tissue layer with an extensive network of blood vessels.
Figure 4.22 The above is a section through the posterior nasopharyngeal wall. The surface on the right side is lined with squamous epithelium, which overlies mucosal cells in the lamina propria. These are also embedded within the pharyngeal constrictor muscle. Deep to the lamina propria, in the connective tissue layer, are aggregations of serous cells (on the left side of the figure). This nasopharyngeal surface thus receives both mucus and serous fluid. (Magnification x40; Slice #1034; Macaca fascicularis)
Figure 4.23 A magnified view of submucosal serous cells under the posterior nasopharyngeal wall. These cells allow for the humidification of airflow. (Magnification 40x; Slice #895; *Macaca fascicularis*).

4.5.4 *Ateles* sp.

Respiratory epithelium was present on almost the entire nasopharyngeal surface. There is a dense concentration of goblet cells anteriorly but these become less common in the lateral pharyngeal recess. Some epithelia with a transitional appearance (between respiratory and squamous types) were present in the furrows between surfaces of the nasopharyngeal floor and lateral wall posterior to the level of the torus tubarius. These were thicker with less columnar cell
shape and few goblet cells but were still ciliated. Mucous and serous glands were present throughout the connective tissue layers of all of the nasopharyngeal surfaces, suggesting participation in respiratory function. Unlike other primates sampled here, the submucosal glandular tissue is only sparsely distributed under the posterior wall overlying the superior pharyngeal constrictor muscle.

Figure 4.24 A section through the nasopharyngeal floor at the anteroposterior level of the medial pterygoid plates. Note the presence of ciliated, pseudostratified, columnar epithelium with goblet cells. This epithelial type covers most of the nasopharynx. (Magnification 60x; Slice #225; *Ateles* sp.)
Figure 4.25 The above is a slice through posterior nasopharyngeal wall, preserving only an irregular, circular patch of epithelial surface area. This surface appears surrounded by connective tissue and muscle fibers from the superior pharyngeal constrictor muscle. Note the sparse presence of mucous and serous glands. The arrow points to one of the few small serous glands observable in this slice. The area within the rectangle is magnified in Figure 4.26 and the structure identified by the arrow is magnified in Figure 4.27 (Magnified 4x; Slice #1345; *Ateles* sp.)
Figure 4.26 Section through the posterior nasopharyngeal wall of *Ateles*. It is almost completely covered in ciliated, pseudostratified, columnar epithelium unlike other sampled primate genera. (Magnification 40x; Slice #1345; *Ateles* sp.)
Figure 4.27 Section through the serous gland in Figure 4.25. (Magnification 40x; Slice #1345; Ateles sp.)

4.5.5 *Tarsius bancanus*

The tarsier nasopharynx is vertically short and narrow, likely as a consequence of its extreme orbital hypertrophy. Posterior to the torus tubarius, the nasopharyngeal roof is long and sloping. The surface overlying the basisphenoid is extremely narrow but the mucosa curve laterally and inferiorly for a long distance, grading into a surface intermediate in position between the nasopharyngeal roof and lateral wall. This surface eventually meets an extremely
short lateral wall with little space remaining between the lateral edge of the intermediate surface and dorsal velar surface with the lateral pharyngeal recesses appearing diminutive (Figure 4.27).

Overall nasopharyngeal shape is largely defined by small, anteroposteriorly abbreviated medial pterygoid plates, which are far smaller than the lateral pterygoid plates. The latter structures extend laterally around the inflated tympanic bullae, which are so large that they encroach upon the nasopharynx at its posterior-most extent (Figures 4.27 & 4.28). This configuration results in a cartilaginous Eustachian tube that is oriented vertically relative to the other primates sampled. It also appears that a larger proportion of the epiglottis lies in an intranarial position relative to the other non-human haplorhines sampled, instead resembling the *Otolemur* specimen in gross morphology. Such a configuration is likely symplesiomorphic.

Respiratory epithelium covers the majority of the nasopharyngeal surface, including the anterior half of the floor, roof, and lateral walls including the torus tubarius. The transition to squamous epithelium may be seen distally on the velum approaching its point of contact with the epiglottis. It also occurs on the nasopharyngeal roof, which exhibits patches of squamous, respiratory, and transitional epithelium between the two types. These are reinforced with both mucous and serous glands in the connective tissue layer but accompanying submucosal blood vessels were not visible. Mucous and serous glands were also present in the anterior portion of the soft palate (i.e. nasopharyngeal floor) with ducts visibly opening to both the dorsal and ventral surfaces.
Figure 4.28 Note the constricted recess inferolaterally (left) and the close proximity of the tympanic bulla to the superolateral boundary of the nasopharynx (Magnification 4x, Slice# 1995; *Tarsius bancanus*).
Figure 4.29 Basal view of a *Tarsius* sp. dry cranium. Note that the medial pterygoid plates (MP) are anteroposteriorly short relative to the relatively large lateral pterygoid plates (LP). The tympanic bullae (TB) are extremely hypertrophied, encroaching on the posterior aspect of the nasopharynx.
Figure 4.30 The above slice demonstrates the distal portion of the cartilaginous Eustachian tube and its pharyngeal orifice, which are oriented at a nearly vertical position. The orifice is at the same horizontal level as the nasopharyngeal roof. Note the extensive mucoserous submucosal tissue supplying the Eustachian tube and nasopharyngeal floor. (Magnification x40; Slice #1685; Tarsius bancanus)
Figure 4.31 The anterior portion of the nasopharynx is visible with lateral wall against the medial pterygoid plate. Inferiorly, the soft palate is densely packed with mucous glands and a smaller number of serous glands. Ducts within these structures appear to be reaching both the oral surface inferiorly and the nasopharyngeal surface superiorly. Note the posterior tongue at the bottom of the image with a thin connective tissue layer (Magnification 4x; Slice #1675; *Tarsius bancanus*). The area encompassed within the black square is magnified in Figure 4.31
Figure 4.32 At the anterior portion of the nasopharyngeal floor, ciliated respiratory epithelium is present with thick goblet cells providing mucins to protect this surface from dessication.

(Magnification 20x; Slice 1675; *Tarsius bancanus*)
Figure 4.33 Anterior portion of the post-tubal nasopharyngeal wall lined with respiratory epithelium with goblet cells. Also note the dense concentration of mucous and serous glands in the lamina propria. (Magnification 40x Slice #1825; *Tarsius bancanus*)
Figure 4.34 The area within the black square of the top image is magnified within the bottom image. This slice is located in the posterior half of the post-tubal nasopharyngeal wall. Note the respiratory epithelium transitioning into stratified squamous epithelium on the dorsal velum (Bottom Image). The ribbon of bone in the top image is an artifact but does not interfere with visualization of the nasopharyngeal boundaries. (Top Magnification 4x, Bottom Magnification 40x; Slice #1965; *Tarsius bancanus*)
4.5.6 *Otolemur* sp.

No vertically oriented posterior nasopharyngeal wall was present, rather the nasopharyngeal space communicates caudally with the larynx. Thus the roof overlying the basisphenoid and the posterior wall overlying the pharyngeal constrictor muscles were continuous craniocaudally. All of the nasopharyngeal surfaces are covered in respiratory epithelium with additional mucous and serous cells within the connective tissue layer of the nasopharyngeal roof. The epiglottis was visible in the nasoharynx. It was lined by ciliated, pseudostratified columnar epithelium but did not exhibit any goblet cells. The nasopharyngeal septum ended far anterior to the torus tubarius, which appeared anteroposteriorly long, encompassing a large portion of nasopharyngeal depth.
Figure 4.35 The above is a section through the torus tubarius. Ciliated pseudostratified columnar epithelial cells cover the connective tissue layer overlying the Eustachian tube cartilage.

(Magnification 40x; Slice #1295; *Otolemur sp.*)
Figure 4.36 The above is a section through the nasopharynx at the craniocaudal level of the Eustachian tube pharyngeal orifice. Note that mucouserous glands underly all of the nasopharyngeal boundaries in the poorly defined junction between lamina propria and submucosal layer. The tensor veli palatini muscle is visible at the lower right side of the image inserting into the Eustachian tube cartilage. (Magnification 10x; Slice #1433; Otolemur sp.)
Figure 4.37 A section through the posterior pharyngeal wall. Note that it is covered in respiratory epithelium. (Magnification 40x; Slice #1665; Otolemur sp).
Figure 4.38 Section through the distal-most (caudal) nasopharynx. Note the epiglottis (inferiorly bounding the nasopharyngeal space). The entire surface is covered in ciliated, pseudostratified, columnar epithelium with mucoserous glands just lateral to the epiglottic cartilage on the nasopharyngeal floor and within the lateral and posterior walls. (Magnification x10; Slice #1655; Otolemur sp.)
4.6 Discussion

4.6.1 The nasopharyngeal septum

The function of the nasopharyngeal septum of some non-human primates remains unknown but it may promote laminar postnasal airflow. Among both *Hylobates* and *Macaca*, it does not appear continuous with velar muscles and does not likely participate in movement of the soft palate. The septum is instead filled with connective and glandular tissue. There also appears to be a C-shaped cartilage at the base of the septum in *Hylobates*, embedded within the soft palate as noted by Leela and Kanagasuntheram (1973). Among both genera, the septum is lined with respiratory epithelium and likely continues the air conditioning function of the mucosa and submucosal glands covering the vomer part of the nasal septum anteriorly. Future studies should investigate a greater number of primate taxa to determine the phylogenetic polarity of the nasopharyngeal septum. Additionally, its role in postnasal airflow dynamics should also be analyzed.

4.6.2 Epithelial distribution and submucosal glands

Despite minor idiosyncrasies among genera, the specimens tended to exhibit similarities in epithelial distribution and submucosal histology. Ciliated, pseudostratified, columnar (respiratory) epithelium appeared to cover most of the nasopharyngeal surface. The roof, lateral walls overlying the medial pterygoid plates, and floor were all covered in this epithelial type as were the torus tubarius and anterior aspects of the post-tubal nasopharyngeal wall. *Contra* Bryant
transitional epithelium was not found here as a discreet type with a distinct morphology. Rather, pseudostratified, columnar epithelia graded into this type before transitioning into stratified squamous epithelium further posteriorly in the human, *Hylobates*, and *Macaca* specimens. This pattern was observed by Leela and Kanagasuntheram (1973) and Klein et al. (1979) in non-human primates and by Nakano et al. (1986) in mice. Thickenings of respiratory epithelium were observed in the ridges and furrows created by the superior pharyngeal constrictor muscle in posterior-most extent of the post-tubal nasopharyngeal walls of most specimens. These appeared slightly less columnar with few to no goblet cells. Squamous epithelium was found covering most of the posterior nasopharyngeal wall, except at its junction with the nasopharyngeal roof superiorly. At such areas, covered by squamous epithelium, there were no goblet cells but mucous glands were present in the lamina propria. Among *Macaca* were serous glands, which were located deep to the pharyngeal constrictor muscle and likely communicated with the surface via ducts. Such tissue depth was not visible in *Hylobates* but it also appears likely that this genus possesses similarly deep serous glands underlying the posterior nasopharyngeal wall.

The presence of respiratory epithelium on the posterior nasopharyngeal wall of *Ateles* was unexpected as it is a contractile surface and may serve as evidence against a strict relationship between epithelial type and contractility of the substrate. This is further reinforced by the near universal presence of respiratory epithelium on most of the soft palate and salpingopharyngeal fold, two extremely contractile surfaces overlying several large muscles. Indeed several studies (Kuehn and Kahane, 1990; Nagai et al., 1997; Kuehn and Moon, 2005) also observed respiratory epithelium on the dorsum of the soft palate of human cadavers and *Macaca* (Klein et al., 1979), noting a transition to squamous epithelium on the posterior portion,
which makes direct contact with the posterior nasopharyngeal wall in humans and the epiglottis among nonhuman primates. It thus appears more likely that the distribution of squamous epithelium on the posterior nasopharyngeal wall is the combined result of substrate contractility, sustained contact with high velocity inspiratory air, and (among humans) contact with the velum. The conditions of *Ateles* and *Otolemur* as the only primates in this sample to completely lack squamous epithelium on the posterior nasopharyngeal wall suggest that it did not experience denuding or irritation of this surface in the way other primates had. This may be due to as yet unidentified properties of nasal airflow dynamics or to life in a climate-controlled enclosure over the course of the individuals’ lives. Future studies will include a greater sample size of *Ateles* and *Otolemur* to analyze intraspecific variation in epithelial distribution patterns.

Despite previous descriptions of the torus tubarius as a zone of transition between respiratory and squamous epithelia, it was found to be covered by ciliated, pseudostratified, columnar epithelium among all of the specimens. The torus tubarius is also the site of some of the thickest concentrations of mucous and serous glands within the nasopharynx. They likely contribute to air conditioning functions as does the posterior nasopharyngeal wall.

The posterior nasopharyngeal wall in all the examined specimens has mucous glands within the lamina propria and an extensive network of blood vessels within the connective tissue layer. Each of these components is vital for performing the air conditioning function of the upper respiratory system. Thus one would expect these features to be present at an area regularly subjected to high velocity airflow and air turbidity that may rival the conditions within the nasal valve. Indeed inspiratory air must negotiate a nearly 90 degree inferior turn to exit the nasopharynx of humans and other anthropoids (e.g., Schreider and Raab, 1981), eventually
reaching the larynx and lower respiratory system. The posterior nasopharyngeal wall may thus be an underappreciated but important component of the upper airway.

4.6.3 Relevance to reconstruction of the Neanderthal nasopharynx

The nasopharynx has been measured warming inspiratory air by 2° Celsius (see classic study by Perwitzschky, 1927) while it has also been argued to aid in humidification (Negus, 1956). This may not appear to be a marked contribution but, under conditions of cold stress, the temperature and humidity of air reaching the nasopharynx is considerably reduced as the air conditioning capacity of the nasal cavity is relatively less than in warmer and more humid ambient conditions (e.g. MacDonald, 1889; Perwitzschky, 1927; Moe, 1942; Naclerio et al., 2007). Thus any increase in temperature within the nasopharynx may aid in preventing lethal chilling and desiccation of pulmonary tissues. This could render any differences in nasopharyngeal airway proportions of Neanderthals from modern humans directly translatable to their respiratory air conditioning capacity, especially in the cold, dry climates of later Pleistocene Western Eurasia.

Despite being comprised of soft tissue on most of its surfaces, the dimensions of the choanae and external basisphenoid bone may allow reconstruction of the nasopharyngeal dimensions among Neanderthals and other mid-Pleistocene hominins. Such reconstructions may allow for estimation of nasopharyngeal surface area. Given the nearly ubiquitous distribution of mucoserous glands and networks of blood vessels in the lamina propria and connective tissue layers of all nasopharyngeal surfaces, both contractile and non-contractile surfaces may assist in inspiratory and expiratory air conditioning. Additionally, the results of this study suggest that the
torus tubarius of primates contains some of the densest concentrations of mucoserous glands and goblet cells. As this structure projects far medially beyond the human nasopharyngeal wall relative to other primates (Doyle and Rood, 1979), so too may the Neanderthal torus tubarius have projected into the path of postnasal airflow to offer additional surface area. Improved understanding of the morphologic relationships of the nasopharyngeal boundaries and their components are needed to compliment histological data as presented here.

4.7 Conclusion

A wide sample of primate genera was analyzed histologically to test whether the distribution of mucous cells, serous cells, and submucosal blood vessels (the components necessary for postnasal air conditioning) is restricted to bony, non-concontractile surfaces. Many classic studies (e.g., Bryant, 1916) have asserted that respiratory epithelia and goblet cells may only be found overlying the medial pterygoid plates with only limited distribution over the torus tubarius and absence posterior to it. To the contrary, this study found that all three postnasal air conditioning components occur on all the nasopharyngeal surfaces. Despite variation among the genera represented in the location of mucous cells (i.e. as goblet cells at the epithelial surface or mucous cells lodged within the lamina propria), all nasopharyngeal surfaces appear capable air conditioning. These results suggest that any differences among groups in nasopharyngeal surface area could potentially impact air conditioning capacity. One such analysis may be between Neanderthals and modern humans whose respective nasal and paranasal sinus morphologies are distinct (e.g., Marquez et al., in press).
5. Testing for the epigenetic influence of local climate on development of nasopharyngeal hard and soft tissue dimensions: A CT and geometric morphometrics study of two *Macaca mulatta* colonies

5.1 Aim of the chapter

This chapter will test whether the development of the nasopharyngeal boundaries can be influenced epigenetically by ambient climate. Two outdoor colonies of *Macaca mulatta* representing cold- and warm-reared individuals will be analyzed for such morphological differences.

5.2 Background

5.2.1 Previous studies of the nasal complex and its relationship with climate

As a prerequisite to respiration among air breathing mammals, inspired air must be warmed to core body temperature and achieve 100% humidity or saturation. The upper respiratory tract (URT) is a vitally important series of spaces as it is the mechanism largely responsible for this exchange of heat and moisture. The mucosa and submucous venous networks lining its boundaries serve to moisturize and heat airflow, respectively. This region of anatomy is thus a likely site for climatic adaptations.

There has been a relatively long history of study of the interaction of climatic stresses and URT morphology among humans. As a tropical species inhabiting a wide range of habitats,
respiratory adaptations to cold, dry climates have been argued to accompany those in postcranial morphology (e.g. Allen, 1877; Schneider, 1951; Baker, 1958; Katzmarzyk and Leonard, 1998) and behavior (e.g., clothing, fire). As reviewed in detail by Marquez (2002), variation in piriform aperture dimensions has been the most extensively studied aspect of human URT variation. As early as the 18th century, data had been collected on the piriform aperture dimensions of a wide range of human populations. These were often monographs (e.g., Topinard, 1885) that offered descriptions of varied biological phenomena without offering deeper analyses of specific hypotheses or their physiologic/evolutionary implications.

It was not until Hrdlicka's (1910) study on the cranial morphology of the Inuit that a relationship was considered between piriform aperture shape and climate. Nonetheless, he did not specify the functional importance of the relatively narrow piriform apertures within this population. Later studies (e.g., Thomson and Buxton, 1923; Davies, 1932; Weiner, 1954; Franciscus and Long, 1991) concluded that differences among populations from cold, dry and warm, wet climates in the nasal index (nasal width/height *100) were related to an increased area of nasal mucosa for warming and moisturizing airflow. However, Wolpoff (1968) questioned the use of piriform aperture height as it did not correspond to internal nasal cavity height. He instead argued that external nasal width was a better indicator of climatic adaptation as it bears a closer relationship with nasal cavity width among Inuits and Aboriginal Australians as estimated by hard palate width. Carey and Steegman (1981) later proposed that nasal projection is related to humidity using data from Woo and Morant (1934).

Few studies have examined aspects of the internal nasal cavity as potential sites for climatic adaptation. Charles (1930) examined internal nasal morphology among a group of African and European American crania and found that the latter group exhibited a longer nasal
cavity but there was little difference in the height or width of the internal nasal fossa. However, Franciscus (1995) collected many of the same measures on a diverse group of Old World crania spanning from Northern Europe to Sub-Saharan Africa and concluded that nasal fossa breadth, especially at its superior-most extent, was narrower among Supra-Saharan populations of both modern human and archaic Homo. Yokley and Franciscus (2005) later combined measures from both of these studies to perform a principal components analysis. On both the first and second principal components vectors, the data indicated a separation of Supra- and Sub-Saharan groups (including African and European Americans) where the former is characterized by a taller and longer nasal cavity while the latter exhibits greater nasal cavity breadth. It was not until Yokley (2006, 2009) that the actual surface area of the internal nasal cavity was analyzed for a relationship with climate. He used a sample of European (n=40) and African American (n=9) live subjects who underwent CT imaging of the head. Measurement of cross-sectional surface area revealed that the European American sample had a greater endonasal surface area, likely an adaptation for colder, drier climate.

Considerably fewer studies have focused on the relationship between climate and paranasal sinus size. Koertvelyessy (1972) and Shea (1977) both used Inuit samples from varying latitudes and discovered that those farther north, in colder, drier conditions exhibited smaller paranasal sinuses. Rae et al. (2003) reproduced this study design on Macaca fuscata from different latitudes within the Japanese archipelago and showed that samples from colder, drier habitats exhibited smaller maxillary sinuses (the only paranasal sinus present among Macaca). However, in another study of Macaca, Marquez (2002) and Marquez and Laitman (2008) revealed a more complicated dynamic in which M. mulatta from colder climates and higher altitudes exhibited patterns of functional integration of the maxillary sinus different from
those in *M. fascicularis* from the warmer lowlands. Their results suggest that climatic stresses of cold, dry and warm, wet habitats exert different stresses on the paranasal sinuses and that they function as a part of the larger nasal complex.

5.2.2 Animal models

Most studies of craniofacial adaptations to climate have focused on human crania and not on non-human primate or other animal models. A drawback of using humans as models of climatic stress is their highly mobile nature. *Homo sapiens* has consistently exhibited the potential for large-scale migrations between separate climatic zones, as demonstrated from both genetics (e.g., Haak et al., 2012; Spurdle and Jenkins, 1996) and the distribution of language families such as Algonquian, Altaic, Athabascan, Austronesian, Indo-European, Semitic, and Sino-Tibetan across a wide range of climatic zones before the advent of modern modes of transportation. The influence of climatic stresses on upper respiratory tract anatomy may thus be less clear when considering the extensive amounts of gene flow that have characterized humans since at least the Neolithic Age.

In contrast to humans, many mammals exhibit smaller ranges and a reduced propensity for large-scale migration and population movement. Non-human mammals may thus be a better model for the interaction of the upper respiratory tract (URT) and climatic stresses. Among non-human primates, *Macaca* is among the few genera that has settled into snowy temperate zones for as long as nearly half a million years (Delson, 1980). Chu et al (2007) argue from mitochondrial DNA data that the clade composed of *M. mulatta, M. fascicularis*, and *M. fuscata* may have originally been descended from northerly populations that relocated to Southern China.
and Northern Vietnam under glacial conditions and subsequently repopulated northward at approximately 120 to 180 ka. There appeared to be some divergence in the mitochondrial signals between Western populations inhabiting Pakistan and India and Eastern populations inhabiting a north-south axis between China and South East Asia, which suggested that gene flow was maintained via male dispersal. Fooden and Albrecht (1999) noted that South East Asian populations of *M. mulatta* violate Allen’s Rule in which the limbs and tails of populations closer to the equator are expected to be longer than those of others farther from the equator. All populations of *M. mulatta* exhibit relatively short tails, which they used as evidence to argue for a northern origin of the population now ranging between tropical South East Asia and temperate Central and Northern China. The current pattern of geographic distribution of *M. mulatta* may thus have remained the same since the Middle Pleistocene. Additionally, these data attest to the lack of migratory activity in *M. mulatta* relative to *Homo sapiens*, rendering them a more suitable model for genetically coded adaptations to climatic stress.

Most studies on the interaction of climate and URT morphology has focused on genetically coded adaptations, despite the lack of explicit mention of this dynamic. The work of Steegmann and Platner (1968) and subsequently Rae et al. (2006) tested the degree to which observed patterns of URT morphology are related to natural selection versus epigenetic processes acting over postnatal development. Both of these studies utilized the same sample of Sprague-Dawly albino rats (n=44) divided into a group raised at an ambient temperature of 1°C and another control group raised at an ambient temperature of 22°C. All individuals were sacrificed at 90 days of age. Steegman and Platner (1968) collected cranial and postcranial measures using dial calipers and found that the cold group was characterized by a distinct morphologic pattern. They exhibited nasal narrowing at the pre-palatal foramen and premalars.
and an overall appearance of short, broad malars, which may be related to increased use of the muscles of mastication to consume greater amounts of food and, in turn, to maintain a higher body weight. Rae et al. (2006) subjected this cranial sample to micro-CT scanning and Generalized Procrustes Analysis of three-dimensional landmark coordinate data. Their results showed anterosuperior displacement of the nasal complex in the cold group with smaller volumes of the nasal cavity, maxillary sinus, and overall cranium when scaled over either femoral head diameter or endocranial volume. They concluded that the small volume of the nasal cavity may be counteracted by genetics among wild populations that exhibit the opposite trend.

5.2.3 Hypothesis: Climatic stresses, namely temperature and humidity, influence the growth of the nasopharyngeal boundaries via an epigenetic process.

The current study examined whether climatic stresses may share a relationship with any aspect of nasopharyngeal boundary morphology among a sample of *M. mulatta* raised in a warm (California) and cold (Oregon) outdoor environment. It was expected that this group exhibits a nasopharynx of larger surface area-to-volume ratio to promote greater air conditioning and a relatively longer cartilaginous Eustachian tube, which may confer benefits to middle ear health and function. The hypothesis was to be rejected if no differences were found in the relative proportions of the nasopharyngeal boundaries.
5.3 Materials and Methods

5.3.1 Materials

This study utilized a sample of 15 postmortem heads of adult male *Macaca mulatta* and five sub-adult males. Body weights were recorded immediately after death to ensure the greatest accuracy. Adult males were chosen as the morphological consequences of sexual dimorphism may have confounded any climatic signals in nasopharyngeal boundary morphology. Adulthood was determined by the eruption of the third maxillary molar, ensuring that craniofacial growth was mostly complete. Age was confirmed via parasagittal CT sections through the maxillary alveoli and sub-adults were defined by unerupted third molars (see Figure 5.1). These specimens were sacrificed for use in previous neuronatomical studies and most had the brain removed. One individual also had its orbits removed postmortem as part of a separate study on ophthalmic function. For the purposes of this study, each specimen was subsequently sectioned inferior to the cricoid cartilage and frozen for preservation.

Two populations of *M. mulatta* were chosen for this sample. One is from the Oregon National Primate Research Center and the other is from the California National Primate Research Center. The location of the Oregon National Primate Research Center (Beaverton, Oregon) experiences winter temperatures below 00 Celsius. Between January and March of 2010, the lowest temperature was -9.40 Celsius while the location of the California National Primate Research Center (Berkeley, California) experienced a low temperature of 4.720 Celsius (Weather Underground Incorporated, 2010). While not as stark a climatic contrast as the one among the habitats of sub-arctic and tropical *Macaca* species, a large enough difference may exist between
the two localities to exert some epigenetic influence on the individuals within the sample. Only animals raised in outdoor colonies at these two sites for the entirety of their lives were selected for this study.

Figure 5.1 Adults were identified via an erupted third molar. The location of the parasagittal plane is demonstrated in (a). From this view, the unerupted third maxillary molar is visible (b). The below CT is from a subadult specimen of the Oregon sample. Note that the slice is through the right side of the head.
5.3.1.1 Postmortem distortion

While issues of tissue shrinkage or swelling may be of concern in studies of postmortem anatomy, the nasopharyngeal boundaries are reinforced by skeletal structures in all but the posterolateral wall and floor. However, the anterior-most extent of the nasopharyngeal floor is reinforced by fibers of the central tendon of the soft palate, which likely reduce some postmortem shape change. Menache et al. (1997) reported an absence of postmortem tissue shrinkage in the nasal cavity and attributed this observation to the close relationship between nasal tissue and osseous structures. Similar soft tissues cover the medial pterygoid plate and basisphenoid bone, suggesting that, at least in the dimensions of mediolateral breadth and anteroposterior depth, the impact of shape changes is likely small.

An additional challenge to visualization of normal anatomy came from the process of freezing the specimens, which resulted in large blocks of solid ice filling much of the nasopharynx in some specimens but not others. This gave the appearance of solid tissue, as a homogeneous gray mass. However, the lateral and posterior nasopharyngeal wall boundaries took on a darker shade of gray, making them distinguishable from the lighter colored ice block. The surface of the pharyngeal constrictor muscle also tended to coincide with the line intersecting the posterior edges of the left and right lateral pterygoid plates while the lateral nasopharyngeal walls tended to project directly posteriorly from the medial pterygoid plate edges. These enabled distinction of the nasopharyngeal boundaries at the level of each axial CT slice (see Figures 5.2, 5.3).

To test for any inconsistencies produced by postmortem shape change or ice crystals, separate tests of normality (Shapiro-Wilk W test) were calculated on the pooled sample of 20
specimens using both absolute nasopharyngeal volume and absolute nasopharyngeal surface area. Both returned insignificant results ($P = 0.1009$ for absolute nasopharyngeal volume; $P = 0.1688$ for absolute nasopharyngeal surface area), indicating that these values are normally distributed. Thus, neither postmortem factor appreciably influenced measurement of the nasopharyngeal boundaries. Any observed differences between the Oregon and California groups in absolute or relative measures of surface area and volume are likely the result of biological differences.
Figure 5.2 A sagittal slice through the head of an adult *Macaca mulatta* from the California National Primate Research Center. The red line represents the respective level and the plane of images in Figure 5.3.
Figure 5.3 Above are two separate axial slices through the nasopharyngeal orifices of Macaca raised in an outdoor colony in California. The scans are taken at similar vertical levels and should thus represent homologous regions of anatomy. Note that the specimen on the left has its nasopharynx filled with ice, which mimics a soft tissue signal on CT scans. The specimen on the right does not have any ice visible in the nasopharynx. Note that Blue circles are placed over the posterior edges of the lateral pterygoid plates, which are connected by a red line. This represents the location of the posterior nasopharyngeal wall. When intersected by the perpendicular lines at the horizontal level of the scans, the nasopharyngeal boundaries are completely outlined.
5.3.2 Methods

5.3.2.1 Radiology

CT imaging was chosen for data collection on all specimens. When compared to MRI (see Figure 5.5), CT was more efficient at distinguishing hard tissue and empty space while MRI was better able to distinguish muscle groups. A kilovolt potential of 120 and a tube current of 120 milliamps was used. Scanning of the California sample was performed at the California National Primate Research Center in Berkley while the Oregon sample was scanned at the Mount Sinai Hospital, Radiology Associates in New York. For the California sample, a slice thickness of 0.6mm and a slice interval of 1 mm were used. The Oregon sample was scanned with a slice thickness of 0.625 mm and a slice interval of 1mm.

5.3.2.2 Geometric Morphometrics and Procrustes Scaling

CT scanning of each specimen produced an image stack or series of image files (each representing an individual slice), which were analyzed via the computer program ImageJ. Nasopharyngeal volume and surface area were calculated by manually tracing the nasopharyngeal boundaries on each axial slice. The area and perimeter of each traced section were collected and respectively multiplied by the sum of slice thickness and slice interval. The respective sums of these products comprised measures of nasopharyngeal volume and surface
Figure 5.4 Images from a CT scan (b) and an MRI (c) of an adult male *Macaca mulatta* at the Mount Sinai School of Medicine. The specimen was a frozen head with the brain removed. The orientation (a) of the scans is shown on a CT image of the head in gross appearance and a mid-sagittal slice. In (b) the course of the Eustachian tube (ET) is visible lateral to the choanae (CH). In (c) the ET is not clearly visible lateral to the CH. Also, the boundaries of the petrous temporal (PT) are not well defined in (c).
area. Three-dimensional coordinate data were also collected from locations around the entire upper respiratory tract. Landmark locations were visualized through stacks of axial slices. The X and Y coordinates were provided by ImageJ in millimeters while the Z coordinate was derived by multiplying slice number by the sum of slice thickness and slice interval, expressed in millimeters. These data were analyzed via Morphologika so that a Generalized Procrustes Analysis could be performed. For examples of geometric morphometrics analysis of landmark data acquired from CT imaged specimens, see Bookstein et al. (1999), Zollikofer et al. (2005), Braga and Treil (2007), Bastir et al. (2008), and Holton et al. (2013).

When the data were first entered into Morphologika during a preliminary analysis, there was an extremely large division in shape space between the Oregon and California samples on the first principal component (PC 1). It appeared that, despite being scanned at similar slice thickness and interval, the specimens from California and Oregon were systematically oriented within the CT scanners in different positions, affecting the locations at which some landmark locations could be visualized. This artifact constituted roughly 60% of all sample variance in the Principal Components Analysis of shape vectors. Nonetheless, it also served as a useful tool in visualizing which landmark locations were the most reliably located. These determined which measures should be compared among the two groups of *Macaca* to assess relative shape differences. The measures chosen were:

- Hard palate length (prosthion-posterior nasal spine chord length)
- Nasal Height (rhinion-prosthion chord length)
- Average maxilloturbingal length (mean length between the anterior- and posterior-most points of attachment of the maxilloturbinal bone to the lateral nasal wall on the left and right side)
-Cartilaginous Eustachian tube length (estimated as the mean chord length between the superior-most point on the osseous Eustachian tube orifice and the superior-most point on medial pterygoid plate edge on the left and right sides)

The centroid size of each specimen was used to scale nasopharyngeal surface area and volume for direct comparison [see Holton et al. (2013) who also used centroid size to scale human maxillary sinus volume from CT slices]. These measures are not impacted by differences in specimen orientation as the boundaries of the nasopharynx remain visible among the many slices comprising them. Centroid size also remained a reliable measure as it is based on the square root of the sum of squared distances from each landmark to the centroid (average coordinate value). Thus, as a composite of length measurements, it is not noticeably influenced by small amounts of variation in landmark location. Relative nasopharyngeal volume was calculated using the formula:

\[ \frac{1/3 \text{ (Absolute Volume)}}{\text{Centroid Size}} \]

Relative nasopharyngeal surface area was calculated using the formula:

\[ \frac{1/2 \text{ (Absolute Area)}}{\text{Centroid Size}} \]
Table 5.1. Three-dimensional landmarks used in the Generalized Procrustes Analysis.

<table>
<thead>
<tr>
<th>Order</th>
<th>Landmark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>posterior nasal spine</td>
</tr>
<tr>
<td>2.</td>
<td>Hormion</td>
</tr>
<tr>
<td>3.</td>
<td>Basion</td>
</tr>
<tr>
<td>4.</td>
<td>Left petrous apex</td>
</tr>
<tr>
<td>5.</td>
<td>Right petrous apex</td>
</tr>
<tr>
<td>6.</td>
<td>Left carotid canal</td>
</tr>
<tr>
<td>7.</td>
<td>Right carotid canal</td>
</tr>
<tr>
<td>8.</td>
<td>Left lateral-most point on piriform aperture rim</td>
</tr>
<tr>
<td>9.</td>
<td>Right lateral-most point on piriform aperture rim</td>
</tr>
<tr>
<td>10.</td>
<td>Left superior-most point on medial pterygoid</td>
</tr>
<tr>
<td>11.</td>
<td>Right superior-most point on medial pterygoid</td>
</tr>
<tr>
<td>12.</td>
<td>Left superior-most point on medial pterygoid edge</td>
</tr>
<tr>
<td>13.</td>
<td>Right inferior-most point on medial pterygoid edge</td>
</tr>
<tr>
<td>14.</td>
<td>Left midpoint on medial pterygoid edge</td>
</tr>
<tr>
<td>15.</td>
<td>Right midpoint on medial pterygoid edge</td>
</tr>
<tr>
<td>16.</td>
<td>Left anterior-most point on maxilloturbinal</td>
</tr>
<tr>
<td>17.</td>
<td>Right anterior-most point on maxilloturbinal</td>
</tr>
<tr>
<td>18.</td>
<td>Left posterior-most point on maxilloturbinal</td>
</tr>
<tr>
<td>19.</td>
<td>Right posterior-most point on maxilloturbinal</td>
</tr>
<tr>
<td>20.</td>
<td>Left anterior-most point on ethmoturbinal</td>
</tr>
<tr>
<td>21.</td>
<td>Right anterior-most point on ethmoturbinal</td>
</tr>
<tr>
<td>22.</td>
<td>Superior-most midline point on C1 vertebra</td>
</tr>
<tr>
<td>23.</td>
<td>Inferior-most point on the Left bony Eustachian tube</td>
</tr>
<tr>
<td>24.</td>
<td>Inferior-most point on the Right bony Eustachian tube</td>
</tr>
<tr>
<td>25.</td>
<td>Rhinion</td>
</tr>
<tr>
<td>26.</td>
<td>Prosthion</td>
</tr>
<tr>
<td>27.</td>
<td>Left Eustachian process tip</td>
</tr>
<tr>
<td>28.</td>
<td>Right Eustachian process tip</td>
</tr>
</tbody>
</table>

5.4 Results

5.4.1 Generalized Procrustes Analysis

Three-dimensional landmark coordinate data collected from stacks of axial slices were subjected to a Generalized Procrustes Analysis. As mentioned above, a consistent distortion was
present among the data. This was likely caused by differences in the position of the specimens inside of the CT scanner, which partially impeded the discernment of three-dimensional landmark locations in fine detail as would be possible on dry crania. For example, some landmark locations are situated between slices or are only available at an angle that obfuscates visualization of local anatomic features otherwise necessary for estimating landmark positions. The distortion accounted for 64.5% of shape variance when only unilateral and left-side landmarks were used (so as not to assign double weighting to bilateral landmark coordinates). Interestingly, one adult individual from the California sample clustered among the Oregon specimens, potentially as a result of similar positional orientation during CT scanning. Most of this distortion appeared constrained within PC 1.

The second and third principal components accounted for 10.4% and 7.38% of shape variance, respectively. Judging from the resulting wireframe models and plotting of specimens along their axes, they did not include the vector of shape distortion and thus probably represent a more accurate quantification of shape diversity among the California and Oregon samples. Extensive overlap is visible on both PC 2 and PC 3 with the California sample occupying both extremes of the latter axis. Higher values along PC 2 are associated with vertical increases in upper respiratory tract (URT) dimensions. That is, the height of the piriform aperture and medial
Figure 5.5 The wireframe model represents a midsagittal view of the nasal cavity (green), left choana (red), cartilaginous Eustachian tube (blue), and hormion-basion chord (black).

pterygoid plates increase while the choanae become more vertically oriented. Additionally, the tract of the cartilaginous Eustachian tube elongates but also becomes more horizontally oriented. Higher values along PC 3 are associated with increased facial aerorhynchie, basicranial retroflexion (along the hormion-basion chord), decreasing piriform aperture height, and vertical orientation of the cartilaginous Eustachian tube.

With respect to growth trends, two out of the three subadults in the California sample cluster among the adults while both of the subadults from the Oregon sample are clustered with one California sub-adult on the high end of PC 2. This may be interpreted as either the California sample reaching adult URT shape at a quicker rate or that adult URT shape is typically achieved at some point between the eruption of the second and third maxillary molars among both groups. However, all of the California subadults clustered among the adults in centroid size while the
Oregon sample formed a separate cluster below even the California subadult that overlapped them on PC 2. When centroid sizes were compared via a Student's t-test, the California sample
Figure 5.6

Note the distortion on PC 1, which almost completely separates the California and Oregon specimens. Individuals located higher on PC 1 take on a vertically compressed shape and laterally wide appearance whereas those on the low end of PC 2 are vertically higher but mediolaterally narrower. Measures affected by this distortion exhibit no overlap among the Oregon and California specimens.

Sample in blue: adults represented by diamonds; subadults represented by rectangles. Oregon sample in green; California sample in Green; Oregon sample in Blue; Adults represented by squares.
Adults from the Oregon and California samples exhibit a fair amount of overlap on both PC 2 and PC 3. The Oregon subadults (n=2) were located at the high end of PC 2 and distinguished by more vertically oriented piriform apertures and choanae. Specimens at the low end of PC 2 exhibited piriform apertures that were vertically taller, choanae that appear wider, and more vertically oriented Eustachian tubes. California samples in green; Oregon sample in blue; adults represented by diamonds; subadults represented by rectangles. Note: California sample in green; Oregon sample in blue; adults represented by diamonds; subadults represented by rectangles.

**Figure 5.7** Adults from the Oregon and California samples exhibit a fair amount of overlap on both PC 2 and PC 3. The Oregon subadults (n=2) were located at the high end of PC 2 and distinguished by more vertically oriented piriform apertures and choanae.
Adults from the Oregon sample tended to exhibit higher centroid size than those from the California sample. Nonetheless, Oregon subadults had smaller centroid size than the California subadults, who clustered at the lower end of the adult range.

Note: California sample in Green; Oregon sample in Blue; adults represented by diamonds; subadults represented by rectangles.
exhibited significantly ($P=0.025$) greater centroid size, suggesting that their rate of growth is faster despite a less distinct difference in rate of attainment of adult URT shape. Indeed body weight was also significantly greater ($P=0.0026$) among the California subadults, suggesting larger overall size.

There was an appreciable difference in centroid size between the two adult samples with the Oregon specimens exhibiting larger values. Only minor overlap was visible, suggesting that cold climate may have affected URT size more than it did shape among these groups. This difference was significant ($P=0.009$) when tested via a Student's t-test. However, the opposite trend occurred in body weight with the California specimens weighing significantly ($P=0.024$) more than the Oregon specimens. It appears that the Oregon individuals exhibit larger URT proportions despite smaller overall body size.

5.4.2 Univariate Measures

Of the linear measures that were synthesized from the Procrustes-corrected landmark coordinate data, there was no significant ($P<0.05$) difference in palate length (between prosthion and the posterior nasal spine), piriform aperture height (between rhinion and prosthion), average maxilloturbinal length, or Eustachian tube length among adults of the Oregon and California groups. There were also no significant ($P<0.05$) differences found in Procrustes-scaled measures of nasopharyngeal volume, surface area, or surface area: volume ratios. However, the Oregon sample exhibited significantly ($P=0.032$) greater absolute nasopharyngeal surface area despite significantly ($P=0.024$) smaller body weight.
Table 5.2 Descriptive statistics of body mass, centroid size (derived from three-dimensional coordinate data), and the ratio of nasopharyngeal surface area (square root) to volume (cube root) measured in millimeters.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>17.027</td>
<td>2.662</td>
<td>173.204</td>
<td>9.838</td>
<td>2.305</td>
<td>0.0799</td>
</tr>
<tr>
<td>Oregon</td>
<td>10.964</td>
<td>1.231</td>
<td>200.19</td>
<td>21.261</td>
<td>2.296</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Subadults from each sample also exhibited a lack of significant \((P<0.05)\) differences in all of the above measures. They were not significantly different \((P<0.05)\) from adults of either group or the pooled adult sample \((n=15)\) in scaled and absolute nasopharyngeal volume and surface area, even when combined into a pooled subadult groups \((n=5)\). However, subadults from the Oregon sample exhibited significantly \((P=0.035)\) greater maxilloturbinal length on the left side but not the right. This was unexpected as there were no significant \((P<0.05)\) differences in maxilloturbinal length asymmetry between the pooled Oregon and California samples or between subadults and adults.

5.5 Discussion

Previous studies have identified potential heritable differences in upper respiratory tract (URT) measurements, distinguishing separate *Macaca* species that evolved in cold, dry and warm, wet
habitat (Marquez and Laitman, 2008). This study tests for the epigenetic influences of climate on upper respiratory tract morphology. The two samples used were conspecifics of *Macaca mulatta*, a species that inhabits a wide range of habitats with differing climatic stresses. It was found that adults and subadults from both groups exhibited extensive overlap in many aspects of upper respiratory tract shape, as judged from both the principal components analysis and comparisons of linear dimensions. However, centroid size and absolute nasopharyngeal surface area were significantly ($P<0.05$) greater among the Oregon specimens despite smaller body weight. Thus a larger URT may grow to accommodate the need for greater mucosal surface area, allowing for better conditioning of inspiratory air.

The centroid sizes of the Oregon subadults was significantly ($P=0.025$) smaller than those of the California subadults as was body weight ($P<0.0026$). Yet, despite the California subadults weighing over twice as much as the Oregon subadults, there were no significant ($P<0.05$) differences in absolute nasopharyngeal volume or area. Such a result could signify that the epigenetic influence of climate on upper respiratory tract dimensions is exerted earlier in development. Additionally, larger URT centroid size among the Oregon adults could occur through longer periods of growth whereas the smaller California adults achieve their URT proportions much earlier in development. Indeed, when viewed on a chart, the California subadults appear to cluster among sympatric adults on the axis of centroid size while the Oregon subadults cluster far below both the California and Oregon adults.

Attempts at measuring precisely the mediolateral widths of the piriform aperture and choanae were hampered by warping of the coordinate data. Measurement of choanal and medial pterygoid plate height were also hindered by this pattern of distortion, but comparisons of nasopharyngeal volume and surface area scaled over centroid size yielded no significant
(P<0.05) differences. It appears from the above results that nasopharyngeal shape (but not relative size) is functionally constrained in primate species that range among diverse habitats. Not only do the nasopharyngeal boundaries lie in close proximity to the nasal cavity, oral cavity, brain case, and atlanto-occipital articulation, but they also directly influence the dimensions of the cartilaginous Eustachian tube. The lack of significant (P<0.05) difference found in relative Eustachian tube length indicated that middle ear aeration among *Macaca mulatta* is not observably influenced by aspects of local temperature.

Relevance to the study of Pleistocene *Homo*

Further research is warranted into the epigenetic influence of climate so that the scope of genetically heritable morphologic differences can be better understood. Studies of Middle and Late Pleistocene paleoanthropology are focused on groups such as *Homo heidelbergensis* (or mid-Pleistocene *Homo*) and early anatomically modern *Homo sapiens*, which all inhabited a wide range of habitats from tropical Africa to temperate Eurasia. An understanding of epigenetic shape differences related to local climate may better help in analyses of genetically heritable shape change and climatic adaptation. Even data from animal models such as *Rattus* (Steegman and Platner, 1968; Rae et al., 2006) and *Macaca* (Rae et al., 2003; this study) may reveal information about the manner in which the upper respiratory tract develops in response to local temperature and humidity stresses. Preliminary evidence presented here suggests that use of Procrustes-corrected coordinate data may be an ideal means for contrasting separately the influences of heritable shape differences and epigenetic size differences in analyses of nasopharyngeal boundary morphology.
5.6 Conclusions

This study analyzed two outdoor colonies of *Macaca mulatta* from Oregon and California, the former representing a colder climate than the latter. The frozen heads of adult males were imaged via CT and three-dimensional landmark coordinate data was collected as part of a geometric morphometric study. Few shape differences were found between the two colonies but there was a pronounced difference in centroid size of the upper respiratory tract. The Oregon individuals reared in a colder climate exhibited larger upper respiratory tracts but markedly smaller body size than conspecifics from the warmer California colony. These results suggest that upper respiratory tract growth may be more responsive to local temperature than overall body size. However, this effect on growth trajectory is manifested in size and not shape, the latter appearing more closely related to heritable patterns of cranial morphology.
6. Geometric morphometric analysis of the osseous nasopharyngeal boundaries of modern humans, Neanderthals, and mid-Pleistocene *Homo*

### 6.1 Aim of the chapter

This chapter aims to test for 1) climatic adaptation in the dimensions of the Neanderthal nasopharynx and 2) differences in the cartilaginous Eustachian tube tract of Neanderthals relative to humans and mid-Pleistocene hominins. The first prediction would be supported if Neanderthals exhibit taller and narrower nasopharynges than modern humans and the Kabwe 1 cranium, a mid-Pleistocene hominin from tropical Zambia. The second prediction would be supported if Neanderthals exhibited longer and more horizontally oriented cartilaginous Eustachian tube tracts than modern human adults.

### 6.2 Background

#### 6.2.1 Previous studies of osseous nasopharyngeal boundary anatomy in Plio-Pleistocene hominins

The nasopharyngeal boundaries of fossil hominins have received relatively little attention as they are not as readily abundant as dental, facial, and neurocranial elements. As such, the respiratory abilities of australopith-grade hominins and early *Homo* have been seldom considered despite their vital evolutionary importance. A great functional shift likely occurred in their upper respiratory tract morphology during the Pleistocene as has been reconstructed by Laitman
(1977). Facial orthognathy and increased external basicranial flexion produced horizontal (encompassing the oral cavity and nasopharynx) and vertical (between the glottis and velum) supralaryngeal vocal tract components of roughly equal proportions, as exhibited by anatomically modern human adults. Most studies of the external basicranial morphology of fossil hominins have focused on the dimensions of the midline basicranial axis in the context of vocal abilities (e.g., Laitman et al., 1979, 1982; Grosmangin, 1979; Budil, 1994) or positioning of the foramen magnum and locomotion (e.g., Dart, 1925; Tobias, 1991b; Brunet et al., 2002; Kimbel and Rak, 2010). However, relatively little attention has been given to the dimensions of the choanae, cartilaginous Eustachian tube length, or overall naopharyngeal boundary morphology. These structures may be highly integrated in the functional morphology of the upper respiratory tract and its facial and basicranial components. As this study focuses on fossil specimens dating to the Middle and Late Pleistocene, a review is given below of the study of the osseous nasopharyngeal boundaries among Plio-Pleistocene fossils antedating the samples used here, for which relatively little descriptive data existst.

6.2.1.1 Choanal morphology of fossil hominins

Descriptions of choanal dimensions among fossil hominin crania are infrequent as the medial pterygoid plates bounding the choanae are arguably among the most rarely preserved skeletal elements. Even when present, aspects of dental, facial, and basioccipital morphology receive greater attention. Thus, descriptions of choanal morphology among australopith-grade hominins and early Homo are limited. Suwa et al. (2009) argued that Ardipithecus ramidus had vertically oriented choanae and a less elongated nasopharynx relative to Pan troglodytes, instead
resembling *Pan paniscus*. However, the only osseous elements of the nasopharyngeal boundaries preserved are a fragmentary midline portion of the horizontal plates of the palatine bones, a fragmentary right pterygoid element (it is not specified whether a medial or lateral pterygoid plate is preserved, nor is it clear from the image provided), and a posterior portion of the basioccipital bone. They instead inferred the condition of this fossil from the possession of an anteroposteriorly short glenoid and temporal fossa. In these dimensions, *A. ramidus* also resembles *P. paniscus* more closely than *P. troglodytes*, which they claim is "derived" in its nasopharyngeal morphology relative to the former two.

A brief description is given by Wood (1991) of the medial pterygoid plate in KNM-ER 406, a fossil specimen attributed to *Paranthropus boisei*. He gives a measurement of its length as 32 millimeters and describes its course as straight, lacking the posterolateral "twisting" of the adjacent lateral pterygoid plate. This latter structure was measured 43 millimeters in length and 20 millimeters in bilateral width. However, neither the functional nor phylogenetic importance of these measures was discussed. Wood (1991) did not compare these measures to any other intact specimens such as Sts 5 or MLD 37/8 on which the choanal dimensions are still measurable.

Tobias (1991b) described the medial pterygoid plates of OH 24 as less robust than Sts 5 and MLD 37/38. He considered the scaphoid fossa to be relatively shallow and that the overall morphology of the medial pterygoid plates was closer to the modern human condition than those of australopiths. However, Schwartz and Tattersall (2003) described the choanal breadth as relatively narrow in appearance, which created a large infratemporal fossa. Tobias (1991b) also described the pyramidal processes as large relative to both anatomically modern humans and the great apes, an assessment he also gave for OH 5 (Tobias, 1967) despite no mention of its choanal dimensions (despite inferior width being estimable in norma basilaris).
Though at least partially preserved or estimable in some specimens attributed to *Homo erectus sensu lato* (e.g. D2700, KNM-ER 3733), surprisingly little metric data and descriptive analysis has been performed on the choanal dimensions and postnasal morphology of these fossil crania. An exception is the thorough description by Weidenreich (1951) of the superior portion of the choanae on the Javanese Solo (Ngandong) Skulls VI and XI. The superior "root" of the pterygoids is described as wide relative to modern humans. He gives a breadth of 16 mm for the former specimen and contrasts it to a range of 12 mm (Tasmanians) to 8 mm (Europeans) among modern humans. The superior choanal width of Solo Skull VI was reported as 35 mm, which is higher than any human or non-human hominoid measured. Kabwe 1 achieved the closest value, at 28.5 mm in width. While noteworthy, the functional importance of such choanal breadth remains unclear.

6.2.1.2 The nasopharyngeal roof and midline basicranial axis

*Australopithecus (Praeanthropus) afarensis* is the earliest hominin hypodigm for which aspects of the osseous nasopharyngeal roof have been studied. Kimbel et al. (2004) measured bicarotid breadth scaled over bi-ovale breadth in two specimens of *A. afarensis* (A.L. 444-2, A.L. 333-45) as well as fossil crania representing several other Plio-Pleistocene hypodigms and samples of *Homo sapiens*, *Pan*, and *Gorilla*. The fossil sample included specimens of *Australopithecus africanus* (Sts 5m MLD 37/38), *Paranthropus boisei* (OH 5, KNM-ER 406, KNM-ER 13750, KNM-ER 23000), *Paranthropus aethiopicus* (KNM-WT 17000), and early *Homo* (Sts 19, KNM-ER 1813, OH 24). Their scaled measure was used as a proxy for the respective widths of the basioccipital element at the carotid canals and spheno-occipital
synchondrosis at the foramina ovale (see Laitman, 1977). The latter component thus accounts for
the width of the nasopharyngeal roof at its posterior-most extent. It was found that the specimen
A.L. 444-2 exhibited a human-like value of 113 while A.L. 333-45 had a low value of 91, closest
to the male *Pan* average. *A. africanus* also exhibited low, apelike values as did the one specimen
of *A. aethiopicus*. However, most specimens attributed to early *Homo* and *P. boisei* were within
or close to the human range, with the exception of OH 24. These results indicate that *A. afarensis*
was likely polymorphic (if not dimorphic) with regard to basicranial breadth and that the relative
orthognathy exhibited by both early *Homo* and *P. boisei* may have served as a factor in the
broadening of the nasopharyngeal roof to human-like proportions. It is worth noting that the OH
24 specimen exhibits damage at the midline basicranial axis and vomeral articulation (Tobias,
1991b), which may have altered its value in the analysis. As noted by Laitman et al. (1982), this
specimen has undergone extensive reconstruction and did not cluster with other australopith
crania in their multivariate statistical analyses.

Kimbel and Rak (2010) later measured relative basicranial width in the fossils AL 58-22
and AL 8221 as the index of bilateral entoglenoid width to bi-articular tubercle width and, in the
latter specimen, also measured the index of bi-entoglenoid width to bi-orbital width. Both of
these specimens were found to exhibit narrow basicrania, which lay within the range of great
apes. Other specimens from Hadar representing presumed conspecifics attained human-like
proportions for scaled entoglenoid width as did other Plio-Pleistocene hominin fossils such Sts 5,
OH 5, KNM-ER 406, KNM-ER 1370, and KNM-WT 17 000. They concluded that basicranial
breadth was polymorphic among *A. afarensis* and may be tied to sexual dimorphism in which
males exhibit the derived condition of relatively greater basicranial breadth.
The midline upper respiratory tracts (URT) of Plio-Pleistocene hominins have been described as ape-like in morphology. Laitman et al. (1982) reconstructed the URT's of several fossil crania, which have been attributed to the genera *Australopithecus* (Taung 1, STS 5, MLD 37/38), *Paranthropus* (KNM-ER 406, OH 5, SK 47, SK 48, SK 83) and early *Homo* (OH 24). All exhibited non-flexed basicrania between staphylion and basion, relatively deep nasopharyngeal roofs, and relatively short basioccipitals. A discriminant functions analysis consistently placed all specimens among the great apes and newborn humans. It was thus concluded that Plio-Pleistocene hominins exhibited a high placement of the larynx and hyoid bone in life. This anatomic configuration may thus be related to possession of an anteroposteriorly deep nasopharyngeal roof and choanae with a relatively obtuse or horizontal midline orientation.

Tobias (1991b) used an index of hormion-sphenobasion length scaled over hormion-basion length. He found that early *Homo* (OH 16, OH 24, KNM-ER 1470, KNM-ER 1813) and *P. boisei* (OH 5, KNM-ER 406) exhibited relatively short basisphenoid (nasopharyngeal roof) depth as did *Homo sapiens* while those of *A. africanus* were intermediate between *Homo sapiens* and the deep nasopharyngeal roofs of the great apes. Kimbel et al. (2004) later scaled basioccipital length against bi-orbital breadth to represent the shorter overall length of the basiocciput in humans relative to the great apes. Their measures of A.L. 444-2 and A.L. 417-1 both yielded values close to the human mean, as did measures of *A. boisei* (OH 5, KNM-ER 406). However, *A. africanus* (Sts 5) and *P. aethiopicus* (KNM-WT 17000) exhibited values well within the great ape range. The combined results of Laitman et al. (1982), Tobias (1991b), and Kimbel et al. (2004) suggest that Plio-Pleistocene hominins exhibited a range of basicranial morphology intermediate between the great ape and human condition. Interestingly, crania
associated with *P. boisei* have been argued to exhibit human-like basicranial proportions. However, Dean (1982) distinguished *Paranthropus* from *Homo* by the relatively long length between the midpoints of the bi-infratemporal fossa distance and the bi-foramen ovale distance, which is close to the great ape value and signifies a relatively longer basisphenoid. Rak (1983) also distinguishes these "robust" australopiths as possessing relatively greater palatal length, which impacts the overall URT length.

Despite the great detail in which Weidenreich studied the Ngandong (Weidenreich, 1943) and Zhoukoutien (Weidenreich, 1951) *Homo erectus* crania, he provides little in the way of measures of the basisphenoid or nasopharyngeal roof dimensions. However, the morphology of the basicranial axis among the Ngandong fossils were described as less flexed than anatomically modern humans (Wiedenreich 1943). As illustrated by Maier and Nkinni (1984), the midline basicranial axis of OH 9 appears superiorly positioned and less flexed at its pharyngeal surface. The authors consider this one of several primitive basicranial traits exhibited in the *Homo erectus* hypodigm. Dean (1982), however, called attention to the similarity in basisphenoid breadth between *Homo erectus* (KNM-ER 3733, KNM-ER 3883, OH 9) and modern humans. The bi-infratemporal fossa width in these specimens exceeds the bi-foramen ovale width and the bi-carotid canal widths, though this morphology is not as well expressed as in humans.
6.2.2 Previous studies on upper respiratory tract morphology among Neanderthals and mid-Pleistocene hominins

6.2.2.1 Cold adaptation in the Neanderthal upper respiratory tract

Neanderthals may be characterized by their possession of tall, broad piriform apertures (Holton and Franciscus, 2008; Marquez et al., in press) as well as marked midfacial projection and large paranasal sinuses (Rae et al., 2011; Marquez et al., in press). Many explanations have been posited for this unique suite of features, which does not reflect the upper respiratory tract morphology of modern human populations from cold, dry climates but instead resembles those from tropical populations (Marquez et al., in press). Biomechanical models have been proposed in which the sagittally oriented zygoma and broad piriform aperture were functionally related to the stresses of anterior dental loading (e.g., Rak, 1986; Demes, 1987).

Among the earliest models of climatic adaptation in Neanderthal nasal morphology was that of Coon (1962) who argued that their increased midfacial prognathism functioned to increase the distance between the nasal apparatus and arteries supplying the brain. This would extend the nasal cavity and allow for greater air conditioning to protect the brain from cold stresses. However, the brain is more likely to undergo heat stress than hypothermia (Edholm, 1978; Dean, 1988). Dean (1988) proposed that the relatively large nasal cavities of Neanderthals may have instead provided greater surface area for nasal mucosa to offset increases in core body temperature during bouts of intense physical activity. He suggested that the relatively large Neanderthal brain would have had run an increased risk of hyperthermia, especially when combined with the effects of other potential sources of insulation such as body fat or clothing.
A suite of features has been identified among Neanderthals and the Steinheim 1 cranium that include a vertically oriented, continuous crest running along the lateral edge of the piriform aperture and a medial projection from this crest (Schwartz and Tattersall, 1996). These traits increase the amount of surface area available for the air conditioning function of nasal mucosa, serving as an adaptation to cold climate. The authors consider such a condition to be autapomorphic relative to other hominin groups and unlike any nasal morphology exhibited among other primates or non-primate mammalian taxa (Schwartz et al., 2008). Nonetheless, they acknowledged that the seldom preserved internal conchal morphology of Neanderthals would be needed to fully assess the adaptive benefit of this suite of features.

The medial projection and lateral crest of Schwartz and Tattersall (1996) was considered by Arsuaga et al. (1997) to be a superior swelling continuous with the confluence of the maxillary conchal crest and the nasal spine. The latter study described this "spino-turbinal crest" on the Monte Circeo 1 specimen and several of the Sima de los Huesos fossils, stating that it can take on a variety of appearances among modern humans. Its appearance in Atapuerca 5 is similar to that of the Neanderthals and is used as evidence that the population represented at Sima de los Huesos is ancestral to them. Additionally, Franciscus (1999) contended that the pronounced, continuous crest lining the lateral piriform aperture rim of Neanderthals is a morphologic pattern present among modern humans, which was previously described by Gower (1923) in his Stage 5 of a set of six anatomic stages/configurations observed among modern humans. This morphologic pattern includes a fusion of the crista lateralis (from the lateral piriform margin), crista spinalis (from the nasal spine), and crista turbinalis (from the maxilloturbinal). Franciscus (1999) also argued that the medial projection observed by Schwartz and Tattersall (1996) is actually a maxillary conchal crest, not an autapomorphic feature.
Marquez (2002) challenged the interpretations of Arsuaga (1997) and Franciscus (1999) on several grounds. He specified that, according to Gower's (1923) definition, the crista turbinalis does not vertically exceed the superior boundary of the maxilloturbinal [as was described in Monte Circeo 1 and specimens from Sima de los Huesos by Arsuaga et al. (1997)]. Also, both Marquez (2002) and McCown and Keith (1937) noted that the continuous crest of bone visible in the Forbe's Quarry 1 cranium creates a prenasal fossa, which Gower (1923) precludes from the Stage 5 morphology. The medial projection rooted from this crest projects far medially relative to the modern human conchal crest. Marquez (2002) noted that its presence among Neanderthals does not necessarily produce a narrower internal nasal cavity breadth as argued by Franciscus (1999). Indeed, a medially projecting crest may provide extra surface area to an otherwise broad nasal cavity.

Schwartz et al. (2008) refined the description of the lateral nasal morphology of Neanderthals and expanded their discussion to a larger sample of fossil hominins. A taxonomically and temporally broad range of fossils were cited as lacking a clearly defined anterior maxillary conchal crest or any kind of lateral nasal swelling (OH 24, KNM-ER 1470, KNM-ER 3733, SK 847, Jinniushan, Arago) whereas some modern humans and other fossil hominins (from Sima de los Huesos, Kabwe, Petralona, and Nariokotome) exhibit a horizontally oriented conchal swelling anteriorly in the nasal cavity. Neanderthals and Steinheim 1, unlike all other hominins sampled, show some form of a vertically oriented strut or swelling on the lateral nasal wall near the piriform aperture located partially or completely superior to the location of the maxilloturbinal. This condition was again interpreted as an autapomorphy.

Some (e.g., Holton and Franciscus, 2008; Rae et al., 2011) have proposed that aspects of Neanderthal facial and nasal morphology were not cold adapted. They cite their large paranasal
sinuses, marked midfacial prognathism, and tall, broad piriform aperture dimensions as a functional consequence of retaining ancestral facial morphology. The Neanderthals and their ancestors were thus a hypodigm that inhabited a glacial climate and yet retained broad piriform apertures and large paranasal sinuses similar to those of tropical modern human groups. Weaver et al. (2007) argued that genetic drift (rather than cold adaptation) was a more likely means by which the Neanderthals acquired aspects of their nasal and overall cranial morphology that may be considered derived relative other members of *Homo*.

Most recently, work by Marquez et al. (in press) confirmed that, relative to modern humans from cold climates, Neanderthals may be distinguished by their voluminous paranasal sinuses, variable nasofrontal angles (rhinion-nasion-glabella angle), greater piriform aperture area, high nasal index [(piriform aperture width/height) X 100], and expression of a medial nasal projection. In most of these measures, Neanderthals and mid-Pleistocene *Homo* consistently fell at the extreme range of variation among tropical and South African crania or was outside the modern human range. The authors suggest that a separate set of functional constraints likely acted upon the Neanderthal cranium relative to modern humans. Analyses of potential cold adaptations (or lack thereof) among Neanderthals that use contemporary humans as reference groups for “cold adapted” and “warm adapted” nasal anatomy should consider the inherent limitations due to differences in overall craniofacial structure. Modern humans exhibit extreme facial orthognathy and external basicranial flexion during adulthood while Neanderthal crania were anteroposteriorly long and vertically short in appearance with pronounced midfacial prognathism and relatively great hard palate width. Separate functional constraints may have acted upon the morphology of each nasal complex component among modern humans and...
Neanderthals, constraining the manner in which cold adaptations may have manifested in the dimensions of the external nose and paranasal sinuses.

6.2.2.2 Reconstructions of vocal tract dimensions and articulatory abilities among Neanderthals and mid-Pleistocene hominins

The supralaryngeal vocal tract (SVT) anatomy of Neanderthals has been a source of controversy with most studies attributing either human-like vocal abilities or those of human infants. Lieberman and Crelin (1971) were among the earliest to reconstruct the Neanderthal SVT. They found that the La Chapelle-aux-Saints cranium exhibited a lack of flexion of the sphenoorbital synchondrosis and a relatively long prosthion-basion length among other features. These aspects of SVT morphology were similar to the condition of human infants and, based on analogy, it was concluded that the Neanderthal tongue was mostly positioned within the oral cavity and that the position of the larynx would have been relatively high. They thus would have lacked the ability to produce the point or quantal vowels [i], [u], or [a], which require an abrupt 10:1 area discontinuity at the midpoint of the SVT. The bulk of the adult human tongue, however, lies in a relative posterior and inferior position that allows for optimal control of SVT constriction.

Laitman (1977) and Laitman et al. (1979) focused on the upper respiratory tract and basicranial axis to assess the overall SVT morphology of Neanderthals (La Chapelle-aux-Saints, La Ferrassie 1, Forbe's Quarry 1, Monte Circeo 1, Saccopastore 2, Teshik-Tash) as well as early modern humans (Afalou 5, Ain Dokhara, Cro Magnon 1, Predmosti 4), Mid-Pleistocene hominins (Kabwe 1, Steinheim 1), and an australopith (Sts 5). Two dimensional coordinate data
were collected for the locations of the landmarks prosthion, staphyion, hormion, sphenobasion, and basion. Their relative positions were assessed via discriminant functions analysis so that the fossil data could be fitted to a range of morphologic patterns among growth series of human and non-human hominoids. Their results indicated that La Ferrassie 1, Monte Circeo 1, and Saccpastore 2 were most similar to modern humans between the ages of 6 and 12 years while the La Chapelle-aux-Saints cranium was most similar to human infants (at the time of eruption of the deciduous dentition). They argued that Neanderthals likely did not exhibit the interlocking velum and epiglottis as seen in non-human hominoids but also did not achieve SVT dimensions like those of adult modern humans. Instead, they likely ranged in morphology between the condition of two year-old infants and 12 year-old children with less flexion of the basiocciput and a larger relative distance between the hard palate and foramen magnum. The Steinheim 1 cranium fell within the Neanderthal range of variation while the Kabwe 1 specimen was more like adult humans in overall morphology. The strength of this study design was that it did not rely on any single metric of flexion or basicranial length, instead utilizing a combination of features to assess the overall morphology of fossil crania. Other studies by Grosmangin (1979) and Budil (1994) confirmed the results of Laitman and colleagues (1979), using similar methods.

Several studies have concluded that the Neanderthal SVT was likely within the adult human range of variation. However, some used methods that were less than ideal. Arensburg et al. (1989) collected various measures from a Neanderthal hyoid bone recovered from Kebara cave, Israel. It was concluded that the size and shape of this bone falls within the human range and that Neanderthals must have had fully modern speech capabilities. However, the authors did not consider that it is the vertical placement of the hyoid bone and larynx that determines the 1:1 ratio between the length of the oral cavity and the vertical height of the pharynx (e.g., Lieberman
et al., 1992). Other studies using the same measurements (Laitman et al., 1990) showed that the hyoids of pigs could also fall within the human range. The value of hyoid shape in determining location was thus not established.

Lieberman and McCarthy (1999) later disputed the usefulness of external basicranial flexion in deducing laryngeal descent, which is functionally related to the vertical height of the SVT. Laterally-oriented radiographs from the Denver Growth Study were measured for vertical height of the vocal folds, several measures of internal basicranial flexion, one measure of external basicranial flexion (the hormion-sphenobasion-basion angle), and several measures of the face and lateral mandible. It was determined that, after the majority of basicranial flexion occurred between three and six years of age, the vocal folds of the larynx continued their descent thus disputing the mechanical relationship between flexion of the spheno-occipital synchondrosis and laryngeal placement. However, the internal basicranial measures used here were not necessarily related to external basicranial flexion, which was represented by only one measure. Additionally, lateral plain film radiography is not an optimal technique for visualizing soft tissue structures (such as the larynx) and the amounts of KVP (kilovolt potential, a measure of image refinement) were not specified. Furthermore, bilateral mandibular landmarks were rendered unreliable as there was no true control for parallax.

Despite the issues with the methods of Lieberman and McCarthy (1999), Lieberman (2007) considers their results cogent enough to dispute the functional relationship between (presumably external) basicranial flexion and laryngeal descent. Lieberman (2007) used this evidence in addressing the assessment of Boe et al. (2002) that Neanderthals were capable of the full repertoire of modern human speech. Boe et al. (2002) used prosthion-basion length and measures of the cervical vertebrae of fossil specimens from Le Ferrassie and Kebara to model
the Neanderthal vertical SVT as attaining similar proportions to those of modern human children (between the ages of 4 and 10). While their morphologic results agree with Laitman et al. (1979), Boe et al. (2002, 2007) further extrapolated that Neanderthals, like human children, possessed a ratio of the vertical to horizontal SVT components of .8 as compared to the average modern human mean of 1. They argued that, as human children are physically capable of producing the full range of human speech sounds, so too were the Neanderthals. However, Lieberman (2012) questions their Variable Linear Articulatory Model (VLAM), which warps the infant tongue to the standardized anatomic configuration of two women so that the production of quantal vowel sounds is made possible. This results in a tongue position that is placed high in the nasal cavity. Furthermore, Lieberman (2012) cites previous studies that argue against the ability of human infants to produce such vowel sounds (Buhr, 1980; Lieberman, 1980).

Barney et al. (2012) devised a method of modeling the La Chapelle aux Saints and La Ferrassie Neanderthal vocal tracts using 10 male and 10 female adult humans as the reference model. Their protocol also limited tongue position relative to the hard palate so that no overlap occurred and set a minimum vocal tract area of .2 cm². After the Neanderthal vocal tract was warped to the modern human reference, a series of 35 separate measures were taken between the hyoid bone and other cranial landmarks from a mid-sagittal view. It was found that the reconstructed vertical position of the hyoid bone did not significantly (p<0.05) differ between the Neanderthal and modern human models but that it was located farther anteriorly than among modern humans. This would limit articulation of the [a] vowel, as predicted by Lieberman and Crelin (1971). However, Barney et al. (2012) acknowledge the inherent limitations in modeling a Neanderthal vocal tract after a modern human reference as there may be unaccounted aspects of their anatomy that impacted articulatory abilities in life. One may consider their method a
refinement of the one endorsed by Boe et al (2002) but they omitted details on the manner in which the posterior-most extent of the hard palate was reconstructed on the La Ferrassie cranium. This specimen exhibits heavy damage at the posterior hard palate and appears to be missing the posterior nasal spine. Just as well, the position of the posterior nasal spine on La Chapelle aux Saints likely also requires some amount of reconstruction.

Most recently, Martinez et al. (2013) used the complete set of cervical vertebrae associated with individual XXI from Sima de los Huesos and combined it with the relatively complete Atapuerca 5 cranium to model the SVT proportions Homo heidelbergensis. They used length between C2 and C7 as a proxy for the maximal vertical height of the SVT while prosthion-basion length was used to measure the horizontal SVT length. They found that the former portion was smaller than the vertical SVT dimension, producing a ratio of .8, similar to both the La Ferrassie cranium and the 10 year-old modern human configuration. They concluded that both Neanderthals and H. heidelbergensis were likely more inclined toward speech than earlier hominins but that they were substantially different from the modern human SVT anatomic configuration, which is optimal for articulation of speech sounds, thus supporting the earlier predictions of Laitman and colleagues (1979).

6.3 Hypotheses

The current study will test two hypotheses related to Neanderthal nasopharyngeal morphology: 1) Neanderthals possessed taller and narrower nasopharynges as an adaptation to respiratory function in cold climates; 2) Neanderthals possessed longer, more horizontally oriented cartilaginous Eustachian tubes than human adults. These two hypotheses will be tested independently.
Given the long history of study of the Neanderthal nasal cavity as a potential site for climatic adaptation (see above), Hypothesis 1 was chosen to test whether their nasopharyngeal boundaries were characterized by greater surface area relative to modern humans. As tall, narrow piriform apertures and nasal cavities have been argued to provide greater surface area relative to volume than short, broad ones (e.g., Charles, 1930; Franciscus, 1995; Yokley, 2009), so too should tall, narrow nasopharynges confer greater surface area for postnasal heat and moisture exchange. However, if modern humans (who exhibit little climatic diversity; see Chapter 3) or the Kabwe 1 specimen (from tropical Zambia) overlap extensively in this morphology, the hypothesis will be rejected.

Hypothesis 2 was chosen as the overall cranial morphology of Neanderthals appears anteroposteriorly long and mediolaterally narrow. This cranial shape may have influenced the dimensions of the cartilaginous Eustachian tube (CET), which performs middle ear aeration and is the main route for infectious agents in otitis media or middle ear infection. Human infants tend to exhibit both short and horizontally oriented Eustachian tubes, which have been implicated in their high rates of otitis media (Bluestone 2005 a,b). If similarly horizontal CET morphology were found among Neanderthals, it may have predisposed them to higher rates of otitis media than modern human adults.
6.4 Materials and Methods

6.4.1 Materials

Fossil crania representing Neanderthals (*Homo neanderthalensis*) and those previously attributed to *Homo heidelbergensis sensu lato* (European and African specimens) were selected for this study. As the latter species is not well defined, the taxonomically neutral term “mid-Pleistocene *Homo*” was used (Wang, 2011; Xiao et al., in press). The Neanderthals may be considered a group closely related to *Homo sapiens* that speciated in the cold climate of Middle Pleistocene Europe and Western Asia. European mid-Pleistocene hominins have been argued to be ancestral to the Neanderthals with many exhibiting a mosaic of primitive and derived (i.e., Neanderthal-like) features as per the accretionary hypothesis (Arsuaga et al., 1997; Freidline et al., 2012). Thus any feature of the upper respiratory tract found to differ among Neanderthals or both Neanderthals and European mid-Pleistocene hominins to the exclusion of both humans and the Kabwe 1 cranium (the only African Middle Pleistocene specimen preserved well enough for inclusion in this study) will be considered apomorphic and a potential cold climate adaptation. Below is a list of the fossil specimens which includes description of their respective states of preservation and subjective analysis of their facial and nasopharyngeal boundary morphology.
6.4.1.1 Kabwe 1

The original fossil cranium was examined at the British Museum of Natural History. Presented below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Pycraft et al. (1928), Morant (1928), and Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.

6.4.1.1.1 State of preservation and estimation of landmark locations

Overall, this specimen was extremely well preserved. The only landmark position estimated here was that of basion. As the anterior surface of the foramen magnum exhibited some damage, the location of basion was digitized roughly 1 millimeter posterior to its broken edge on the midline. All bilateral temporal bone landmark locations on the right were missing and had to be statistically mirrored from their intact homologues on the left side (see Section 6.3.2.2). Despite the presence of matrix filling the left osseous Eustachian tube orifice, its borders were clearly visible and digitization of this landmark location was not hindered.
Figure 6.1. Frontal view of the Kabwe 1 cranium from Zambia, Central Africa.
Figure 6.2. Basal view of the Kabwe 1 specimen from Zambia, Central Africa. It is in excellent condition, save for the anterior rim of the foramen magnum.
6.4.1.1.2 Subjective assessment of morphology

Facial morphology

The supraorbital tori are well expressed and lead to a nasofrontal angle medially that appears extremely obtuse, suggesting a platyrrhinic morphology. The shape of the piriform aperture is short and extremely broad, as would be expected of a population inhabiting a tropical climate. Its upper and midface lack projection but this specimen exhibits marked lower facial prognathism (as opposed to the midfacial prognathism of the Neanderthals) as well as an extremely broad dental arcade. In combination with extremely tall maxillary alveolar processes, the hard palate appears broad and superiorly positioned when viewed from norma basilaris. Such a morphologic pattern was found most commonly among adult Inuit males in this study's modern human sample.

Nasopharyngeal morphology

The medial pterygoid plates appear tall and mediolaterally thin with hamuli that are preserved at their proximal-most portions. Their scaphoid fossae were not well expressed but did appear to be within the modern human range of variation. It is unclear whether some amount of damage may have been sustained along aspects of the posterior medial pterygoid plate edge. Choanal orientation is more vertical (acute) than among most Neanderthals or infant modern humans but they take on a tall, narrow appearance. The dimensions of the external surface of the basisphenoid bone (roof of the nasopharynx) appear anteroposteriorly shallow between hormion...
and sphenobasion, as among modern humans. However, the basioccipital element takes on an extremely broad and flat morphology, suggesting the presence of hypertrophied prevertebral musculature. There is a midline depression at the approximate location of sphenobasion that may represent a foramen of Luschka.

The region of anatomy surrounding the osseous Eustachian tube orifice in Kabwe 1 resembles the condition of modern humans. Aside from a moderately hypertrophied sphenoid spine overlying the tympanic plate, the area of origin of the levator veli palatini muscle between the Eustachian tube orifice and carotid canal appears only moderately hypertrophied, as among most of the contemporary human specimens observed in this study. Judging from the distance between these temporal bone structures and the medial pterygoid plate, the cartilaginous Eustachian tube may have been of similar length to those of anatomically modern humans.

6.4.1.2 Petralona 1

The original fossil cranium was examined at Aristotle University in Thessaloniki, Greece. Presented below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Kannellis and Savva (1964), Stringer (1974), Murril (1981), and Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.
6.4.1.2.1 State of preservation and estimation of landmark locations

The face and vault of this specimen are in good condition but the basicranium exhibits some damage. These areas are of a darker hue than the undisturbed facial and vault bones and appear to have undergone erosion, likely from water. The hard palate is also undisturbed in its anterior two thirds but is damaged posteriorly. A narrow midline bony projection is all that remains of the horizontal plates of the palatine bones. However, the position of staphyion is estimable as the preserved portion of palatine bone extends almost as far posteriorly as the pyramidal processes.

The left medial pterygoid plate is well preserved (except for a missing hamulus) and all of the landmarks coordinate data were collected on this structure without issue. This was not the case on the right side, on which an inferior portion of the medial pterygoid plate was missing. Its midpoint was measurable on the preserved edge at the approximate position of the inferior boundary of the scaphoid fossa (as on the opposite side). The superior boundaries of the left and right pterygoid hamuli were also estimable from the preserved bony surfaces.

Heavy wear was exhibited on the petrous temporal bones and quadrate surfaces. Most of tegmen tympani and external auditory tube are both missing. Estimation of the anteromedial boundary of the carotid canal rim was not possible despite the petrous apex and osseous Eustachian tube orifice remaining recognizable. The anteromedial-most portion of the tympanic plate can be seen meeting the alisphenoid where they approximate the floor of the osseous Eustachian tube orifice.
Figure 6.3. Frontal view of the Petralona 1 specimen from Greece.
Figure 6.4. Basal view of the Petralona 1 cranium. Some damage is exhibited on the external surfaces of the temporal bones (particularly on the right side) and the superior-most portions of the medial pterygoid plates. Nonetheless, the landmark locations were estimable.
6.4.1.2.2 Subjective assessment of morphology

Facial morphology

Petralona 1 appears to lack the midfacial prognathism and sagittally oriented zygoma of the Neanderthals. The piriform aperture of this specimen is narrowed superiorly, lacking the frontal contour of the Neanderthals described in this study. It also appears to lack the pronounced subnasal prognathism of Kabwe 1. The facial skeleton is extremely tall and is characterized by extremely robust supraorbital tori. Inferiorly, the dental arcade appears extremely broad and the hard palate is deep or superiorly situated relative to the maxillary alveoli.

Nasopharyngeal morphology

The choanal plane appears more vertical (or flexed) than any of the other fossil specimen studied here. A superioinferiorly short scaphoid fossa may be found along the posterior edges of the left and right medial pterygoid plates. They lead superiorly to a basicranial axis that appears anteroposteriorly short and mediolaterally broad between hormion and basion. Its external basioccipital surface curves inferiorly as in Saccopastore 1. Laterally, a large postglenoid tubercle is observable just lateral to the osseous Eustachian tube orifice. Thus Petralona 1 exhibits some nasopharyngeal morphologic traits in common with Neanderthals despite its choanal orientation, which appears to resemble the condition of modern human adults.
6.4.1.3 Steinheim 1

The original fossil cranium was examined at the Statliches Museum für Naturkunde, Stuttgart, Germany. Presented below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Weinert (1936) and Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.

6.4.1.3.1 State of preservation and estimation of landmark locations

Steinheim 1 exhibits extensive damage to the left side of the facial skeleton. Through this missing portion, the ipsilateral frontal and maxillary sinuses are visible, as is the endocranial cavity. Also damaged is the anterior portion of the dental arcade at the location of prosthion. However, a midline portion of the hard palate surface extends anteriorly, allowing for the estimation of the anterior depth of prosthion. Its vertical height was estimated inferior to the tip of this bony projection, at the transverse plane of the cervicoenamel junctions of the right M$^1$ and left P$^3$.

The hard palate was collapsed on the left side as the articulations of the palatine processes of the maxilla and the horizontal plates of the palatine bones had lost their respective articulations with the alveolar process of the maxilla. The right hard palate lateral edge thus curved superiorly into the nasal cavity, which was filled with matrix. This led to the superior displacement of staphylion, which was estimated at the same transverse plane as the right lateral edge of the hard palate. Nonetheless, its position on the hard palate surface appears to be preserved.
The facial skeleton and medial pterygoid plates are twisted to the left, introducing some distortion to the reconstructed upper respiratory tract boundaries. This may be seen in the compressed, folded over appearance of the left medial pterygoid plate (displaced medially as per Laitman, 1977) and the stretched appearance of the contralateral structure. While such distortion impacts a Geometric Morphometrics Analysis, asymmetry in the dimensions of each choana does not affect the analysis of Procrustes-corrected linear measures as choanal width is here measured between the midpoints of the left and right medial pterygoid plates and choanal height is measured in the midline between staphylion and hormion. Averaging of measures of cartilaginous Eustachian tube length between the osseous Eustachian tube orifice and the medial pterygoid plate also reduces the impact of distortion on this measure (which becomes elongated on the right and shortened on the left).

Unilateral left-side facial damage in Steinheim 1 reached far medially into the nasal cavity, resulting in the absence of the vomer. The location of hormion was thus estimated on the basisphenoid surface between the anterior-most projections of the infratemporal crests, over which the vomeral alae had their articulation. This allows for approximately 3.5 millimeters of anteroposterior depth between hormion and sphenobasion. Nasopharyngeal roof depth was thus reconstructed with some confidence.
Figure 6.5. A frontal view of the Steinheim 1 cranium from Germany. This individual exhibits extensive damage to the left side of the face and most of the nasal cavity is filled by matrix.
Figure 6.6. A basal view of the Steinheim 1 specimen from Germany. The face and anterior basicranium appear to deviate laterally toward the left side.
6.4.1.3.2 Subjective assessment of morphology

Facial morphology

This specimen exhibits robust supraorbital tori that project far anteriorly. Its piriform aperture appears broad relative to its height, attaining tropical proportions. The midface lacks prognathism as exhibited by the Neanderthals and, judging from preserved portions of the lower face, may have resembled Petralona 1 in lacking the strong subnasal prognathism exhibited by Kabwe 1. Within the region of the postcanine alveolae, the palate may be seen deep on the right side but overall breadth and morphology of dental arcade shape is obscured by extensive anterior palatal damage.

Nasopharyngeal morphology

A strong posterior projection (likely a palatal torus) is present on the hard palate and may have served to anchor the central tendon of the soft palate. Some individuals within the Point Hope Inuit sample also exhibited this morphology. The choanae appear extremely tall and narrow with an obtuse angulation as among the Neanderthals. Laterally, the left osseous Eustachian tube orifice appears relatively large and is not abutted by as strong a sphenoid spine as among Neanderthals. A foramen of Luschka is also observable, signifying that Mid-Pleistocene hominins varied in the expression of this trait as do modern humans.
6.4.1.4 Atapuerca 5

A cast of this fossil cranium was examined at the American Museum of Natural History, Division of Anthropology in New York. Presented below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Arsuaga et al., (1997) and Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.

6.4.1.4.1 State of preservation and estimation of landmark locations

This specimen exhibits damage at the location of hormion, in which portions of the basisphenoid and most of the vomer is missing. However, a distal portion of the left vomeral ala remains. Hormion was thus measured slightly medial to this distal tip, in the midline. The rest of the basicranial axis is well preserved.
Figure 6.7. Frontal view of the Atapuerca 5 cast. The specimen represents Mid-Pleistocene *Homo* from Spain.
Figure 6.8. Basal view of the Atapuerca 5 cast.

The pterygoid hamuli are missing on both sides with extensive damage on the right side. Their locations were estimable from the preserved portions of medial pterygoid plate inferiorly.
and the pyramidal processes superiorly and were taken on the same transverse plane as the hard palate, which tends to approximate the level of the superior hamular boundary among humans. Further damage was also exhibited around the middle portions of the posterior medial pterygoid plate margins. Their midpoints were estimable on the same projected planes as their inferior-most points.

6.4.1.4.2 Subjective Assessment

Facial morphology

Atapuerca 5 exhibits some Neanderthal facial features. Its hypertrophied supraorbital tori curve inferiorly and medially into marked midfacial prognathism. The piriform aperture is extremely wide, taking on a "hypertropical" morphology. Its palate is broad and only moderately deep.

Nasopharyngeal morphology

The choanae take on a tall, narrow appearance with an obtuse orientation relative to the midline basisphenoid. Laterally, the course of the cartilaginous Eustachian tube is traced by a raised ridge on the left side. It lacks the hypertrophied sphenoid spine of Neanderthals.
6.4.1.5 Gibraltar Forbes' Quarry 1

The original fossil cranium was examined at the British Museum of Natural History. Presently below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.

6.4.1.5.1 State of preservation and estimation of landmark locations

This specimen exhibited damage in several locations, requiring the estimation of some landmark locations. A portion of the premaxillary alveoli overlapping the midline were missing, necessitating the estimation of prosthion at the horizontal level of adjacent, relatively undisturbed alveoli. The horizontal plates of the palatine bones were also missing in the midline. However, the site of articulation with the maxillary alveolar process on the left side was sufficiently preserved to demarcate the posterior-most extent of the hard palate. Staphylion was thus measured in the midline at the same vertical plane as the broken hard palate edge but at the same transverse plane as the posterior-most preserved portion of the left palatine bone horizontal plate. Such a method allowed for the reconstruction of posterior palatal morphology with some confidence.

The locations of the inferior-most extent of the left and right medial pterygoid plates were damaged with the right side exhibiting more extensive damage than the left. However, the position of the hamulus was estimable on the left side and used to estimate its homologous...
location on the right side, which was taken on the same transverse plane just posterior to the
broken edge. More superior portions of the medial pterygoid plates are better preserved. The
midpoint measured at approximately the inferior boundary of the well expressed, broad scaphoid
fossa. Laterally, the right osseous Eustachian tube orifice is plugged with matrix and its
boundaries are difficult to visualize but definable between the tympanic plate and the overlying
sphenoid spine.

The greatest amount of damage on Gibraltar Forbes' Quarry 1 was to the basioccipital
element, which was incompletely preserved. Laitman (1977) noted that it appeared incorrectly
reconstructed. Without a foramen magnum, the location of basion was estimated as lying
posterior to the broken edge at approximately between the levels of the right porion and the
carotid canal. Laitman (1977) and Laitman et al. (1979) also used the intact right temporal bone
to estimate the position of basion via the acoustic meatus as they observed consistency in this
spatial relationship among human and non-human hominoid crania. Additionally, as per Schaefer
(1999) and Ahern (2005), hominins may typically be characterized as having a relatively shorter
distance between basion and the bilateral line intersecting the posterior carotid canals relative to
Pan. Basion often intersects or anteriorly surpasses this line among contemporary humans and
fossil hominins. Thus, both the relationships of basion with the carotid canals and acoustic meati
informed the choice of estimation method used here.
Figure 6.9. A frontal view of the Forbes' Quarry 1 specimen from Gibraltar. The facial skeleton is well preserved.
**Figure 6.10.** A basal view of the Forbes’ Quarry 1 specimen. The left temporal bone is missing as the area of basiocciput represented at basion. Some estimation was required for this landmark as was staphylion.

6.4.1.5.2 Subjective assessment of morphology

Facial morphology

Forbes’ Quarry 1 exhibits a tall, broad piriform aperture and substantial midfacial prognathism as most Neanderthal specimens have been described (e.g. Rak, 1986, Marquez et al., in press). Its dental arcade appears broad anteriorly with a moderately deep hard palate. The
supraorbital tori are relatively gracile despite far anterior displacement at glabella. Some have described this specimen as female (see discussion by Schwartz and Tattersall, 2002).

Nasopharyngeal morphology

Despite damage at the posterior border of the hard palate, this specimen exhibited marked retroflexion of the reconstructed choanal plane. Its appearance resembles that of infants and young children. The site of the osseous Eustachian tube orifice may be seen bounded inferiorly and laterally by a hypertrophied sphenoid spine. It is relatively inflated in appearance relative to the Kabwe specimen. The quadrate area of the temporal bone is small and angled sagittally relative to the more coronally oriented petrous portion.

6.4.1.6 Monte Circeo 1

The original fossil cranium was examined at the Museo Nazionale Preistorico Etnografico di Luigi Pigorini, Rome, Italy. Presented below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Piperno and Scichilone (1991) and Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.
6.4.1.6.1 State of preservation and estimation of landmark locations

This specimen exhibits damage most noticeably at the right zygoma, nasal and orbital cavity boundaries, maxillae, and lateral nasal aperture. A concentration of calcareous concretions has developed over portions of the anterior maxillary alveoli, which appear to have experienced some erosion. Other preserved portions appear smooth and suggest that this individual was likely edentulous. Prosthion and the lingual surface of the canines at the cervicoenamel junction was estimable from the preserved alveolar edge. However, the horizontal plates of the palatine bones were incompletely preserved. Upon examination of Plate XLIV of Sergi et al. (1972) describing the Monte Circeo 1 specimen, a photograph of an intact posterior hard palate is used to illustrate the areas from which bone fragments were removed for histologic sectioning. These fragments were harvested from most of the posterior portion of the hard palate save the most lateral and posterior articulations between the horizontal palatine plates and the maxillary alveolar process. It was thus possible to reconstruct the original position of staphyion using these lateral articulation sites. It was projected roughly seven millimeters posterior to the broken edge on the same transverse plane.

Some light damage is exhibited at the anterior boundary of the foramen magnum. The location of basion was thus estimated one millimeter posterior to the worn basioccipital edge on the same transverse plane. Further damage can be found at the posterior medial pterygoid plate edges, especially on the right side. The midpoint of this surface was estimated midway between the superior- and inferior-most points on the medial pterygoid plate posterior edge at the same posterior depth as the preserved inferior portion. The left side midpoint was estimated just inferior (by six millimeters) to the inferior boundary of the scaphoid fossa on the preserved edge.
The posterior depth of this landmark location was used to adjust the position of the left side landmark denoting the superior edge of the medial pterygoid hamulus base.

Systemic error may have been introduced into the process of digitizing the landmark coordinates of Monte Circeo 1 as the use of clay was not permitted in anchoring it to the substrate. Thus, any wobble or subtle shift in position between the digitization of separate landmark coordinates could bias the data. Immediately after digitization, the coordinate data was plotted against 19 randomly selected adult modern humans on a Generalized Procrustes Analysis to test for the presence of any grossly observeable errors (i.e., switching of landmark order or the fossil specimen plotting at an unrealistically great distance from the modern human cluster in shape space). When found, the digitization process was repeated and the data was again examined until no errors were observed.
Figure 6.11. Frontal view of the Monte Circeo 1 cranium from Italy. Despite some damage to the alveoli and right orbital cavity boundaries, the boundaries of the nasal and left orbital cavities are in excellent condition.
Figure 6.12. Basal view of the Monte Circeo 1 cranium. The nasopharyngeal boundaries are largely undamaged, except for the posterior boundary of the hard palate. It was removed by Sergi et al. (1972) who studied the microscopic structure of the palatal torus once exhibited by this specimen.
6.4.1.6.2 Subjective assessment of morphology

Facial morphology

The supraorbital tori are robust and project the glabella far anteriorly. The piriform aperture appears broad, especially at its superolateral margins. Projections of the maxilla jut forward here to create a dome-like contour on the superolateral aperture edge. These projections reach far anteriorly relative to the nasal bones. This morphology may be related to the marked midfacial prognathism characterizing Neanderthals and some Middle Pleistocene specimens (Rak, 1986; Klein, 1999; Arsuaga et al., 1997). Overall, its face, palate, and dental arcade take on a long, narrow appearance despite exhibiting damage.

Nasopharyngeal morphology

The choanae of this specimen appear tall and narrow with an obtuse or retroflexed orientation. When combined with a flattened external basioccipital surface, the overall basicranial axis appears flat relative to adult modern humans with the basioccipital element taking on a broad appearance. Laterally, the sphenoid spine is hypertrophied as in the Forbes' Quarry 1 specimen. This structure achieves its greatest size on the left side where it appears to project far inferiorly.
6.4.1.7 Saccopastore 1

A cast of this fossil cranium was examined at the American Museum of Natural History, Division of Anthropology in New York. Presented below are descriptions of the state of preservation and a subjective assessment of the facial and osseous nasopharyngeal boundary morphology. See Condemi (1992) and Schwartz and Tattersall (2003) for a full description of the morphology of this fossil.

6.4.1.7.1 State of preservation and estimation of landmark locations

All data collection was done on a cast of Saccopastore 1 housed in the Department of Anthropology of the American Museum of Natural History in New York. This specimen exhibits damage to the zygomatic arches, left orbital boundaries, and supraorbital tori, which are broken to reveal a frontal sinus. Damage is also present on the anterior aspect of the dental arcade but the location of prosthion was estimable just medial to the preserved portion of alveolus anchoring the left central incisor. The right medial pterygoid plate posterior edge had been partially broken, necessitating estimation of the midpoint six millimeters inferior the preserved edge. Neither medial pterygoid plate exhibited a preserved hamulus but its location was estimable on both sides.

Both the foramina ovale and the spheno-occipital synchondrosis were difficult to visualize on this specimen. Thus the locations of the former were estimated from their spatial relationship with the superior-most edge of the lateral pterygoid plate. Subsequently, the position of sphenobasion was estimated as the midpoint of this bilateral line. Given the reliable
relationship between foramen ovale position and the lateral pterygoid plate observed among the human and non-human primate crania used in this study, some confidence may be placed in the estimation of measures derived from sphenobasion position. Laterally, the osseous Eustachian tube orifices are filled with matrix but estimable.

**Figure 6.13.** Frontal view of the Saccopastore 1 cast.
Figure 6.14. Basal view of the Saccopastore 1 cast.
6.4.1.7.2 Subjective assessment of morphology

Facial morphology

The piriform aperture appears similar in morphology to other Neanderthals (see description of Monte Circeo 1 above). It is tall and broad at both its inferior and superior reaches being positioned anteriorly by marked midfacial prognathism. The palate has a deep and narrow appearance as in other Neanderthals, a feature potentially related to both a relatively long soft palate and robust masticatory apparatus.

Nasopharyngeal morphology

The choanae appear tall and narrow with an obtuse, retroflexed appearance relative to the basicranial axis as in other Neanderthals. The scaphoid fossa is short but broad and deep as in Forbes' Quarry 1. It also shares a hypertrophied sphenoid spine which abuts the osseous Eustachian tube orifice laterally and a diminutive, sagittally oriented quadrate surface with a petrous temporal that appears coronally oriented. The basicranial axis is narrow and the basiocciput appears to curve inferiorly in contrast to the broad, flat morphology of Monte Circeo 1. This may reflect a polymorphism among Neanderthals.
6.4.2 Methods

6.4.2.1 Measurement technique and landmarks

A Microscribe Digitizer was used to collect three-dimensional landmark coordinate data from all fossil specimens (as described in Section 3.3.4 of Chapter 3). The same number of landmark locations were digitized among the fossil crania as in the geometric morphometrics (GM) analysis of the extant hominoid and papionin samples (n=40). However, due to the absence of some bilateral landmarks on some specimens, this number was reduced (n=31; see Table 6.1 and Table 6.2) for comparison of overall relative upper respiratory tract dimensions with modern humans. In the reconstruction and comparison of cartilaginous Eustachian tube dimensions, two landmarks were added for a separate comparative analysis with the subset of modern human crania on which the location of the left and right bony Eustachian tube orifice was digitized (see Chapter 3, Section 3.3.6.7).

Table 6.1 Fossil specimens examined.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Landmarks Missing (numbers in accordance with those from Chapter 3)</th>
<th>Age (Ka)</th>
<th>INST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabwe 1</td>
<td>16, 18, 22, 43</td>
<td>700-400(^1); 202-235(^2); 700-400(^7)</td>
<td>BNHM</td>
</tr>
<tr>
<td>Atapuerca 5</td>
<td>None</td>
<td>~500-600(^7)</td>
<td>Cast</td>
</tr>
<tr>
<td>Monte Circeo 1</td>
<td>8, 10, 24, 29, 33, 35, 37, 39, 41</td>
<td>60-74(^4), ([44±5] – [62.6±6])(^4)</td>
<td>LP</td>
</tr>
<tr>
<td>Petralona 1</td>
<td>8, 20, 21, 24, 37, 39, 41</td>
<td>209-371(^7); 150-250(^n)</td>
<td>AU</td>
</tr>
<tr>
<td>Steinheim 1</td>
<td>9, 10, 20, 24, 30, 34, 36, 40, 42</td>
<td>~225(^b)</td>
<td>SMN</td>
</tr>
<tr>
<td>Saccopastore 1</td>
<td>2, 29, 30, 34, 36, 42</td>
<td>120-130(^b)</td>
<td>Cast</td>
</tr>
</tbody>
</table>

Institution abbreviations: British Natural History Museum (BNHM), London, England; Museo Nazionale Preistorico Etnografico di Luigi Pigorini (LP); Aristotle University of Thessaloniki, Greece (AU); Staatliches Museum für Naturkunde, Stuttgart, Germany (SMNS).

Chronology based on the following and references therein: \(^1\) Klein, 1994; \(^2\) Barham and Smart (1996); \(^3\) Grun and Stringer (1991); \(^4\) Schwarcz et al. (1991); \(^5\) Shen and Yokoyama (1984); \(^6\) Grun (1996); \(^7\) Schwartz and Tattersall (2002); \(^8\) Bischoff et al. (2007)
Table 6.2 Landmarks used in the analysis of fossil morphology.

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prosthion</td>
</tr>
<tr>
<td>2</td>
<td>Staphylion</td>
</tr>
<tr>
<td>3</td>
<td>Hormion</td>
</tr>
<tr>
<td>4</td>
<td>Sphenobasion</td>
</tr>
<tr>
<td>5</td>
<td>The point indicating the midline position of the pharyngeal tubercle: one-third of the distance from exo-basion to hormion</td>
</tr>
<tr>
<td>6</td>
<td>Basion (endo-basion)</td>
</tr>
<tr>
<td>7</td>
<td>Left most posteroinferior point on the alveolar process of the maxilla</td>
</tr>
<tr>
<td>8</td>
<td>Right most posteroinferior point on the alveolar process of maxilla</td>
</tr>
<tr>
<td>9</td>
<td>Left superior-most point on the posterior margin of the medial pterygoid plate</td>
</tr>
<tr>
<td>10</td>
<td>Right superior-most point on the posterior margin of the medial pterygoid plate</td>
</tr>
<tr>
<td>11</td>
<td>Left inferior-most point on the posterior margin of the pterygoid plate</td>
</tr>
<tr>
<td>12</td>
<td>Right inferior-most point on the posterior margin of the pterygoid plate</td>
</tr>
<tr>
<td>13</td>
<td>Left lateral-most midpoint point on the choanal margin (the medial pterygoid plate)</td>
</tr>
<tr>
<td>14</td>
<td>Right lateral-most midpoint point on the choanal margin (the medial pterygoid plate)</td>
</tr>
<tr>
<td>15</td>
<td>Left anteromedial-most point on the petrous pyramid (petrous temporal)</td>
</tr>
<tr>
<td>16</td>
<td>Right anteromedial-most point on the petrous pyramid (petrous temporal)</td>
</tr>
<tr>
<td>17</td>
<td>Left porion</td>
</tr>
<tr>
<td>18</td>
<td>Right porion</td>
</tr>
<tr>
<td>19</td>
<td>Nasospinale</td>
</tr>
<tr>
<td>20</td>
<td>Rhinion</td>
</tr>
<tr>
<td>21</td>
<td>Nasion</td>
</tr>
<tr>
<td>22</td>
<td>Left lateral-most point on the rim of the nasal aperture</td>
</tr>
<tr>
<td>23</td>
<td>Right lateral-most point on the rim of the nasal aperture</td>
</tr>
<tr>
<td>24</td>
<td>Left jugale (the point in the greatest depth of the notch between the temporal and frontal processes of the zygomatic bone)</td>
</tr>
<tr>
<td>25</td>
<td>Right jugale (the point in the greatest depth of the notch between the temporal and frontal processes of the zygomatic bone)</td>
</tr>
<tr>
<td>26</td>
<td>Left ectoconchion (most lateral point on the orbital margin)</td>
</tr>
<tr>
<td>27</td>
<td>Right ectoconchion (most lateral point on the orbital margin)</td>
</tr>
<tr>
<td>28</td>
<td>Left medial-most point on the orbital rim</td>
</tr>
<tr>
<td>29</td>
<td>Right medial-most point on the orbital rim</td>
</tr>
<tr>
<td>30</td>
<td>Left Point on the internal alveolar margin posterior to the maxillary canine</td>
</tr>
<tr>
<td>31</td>
<td>Right Point on the internal alveolar margin posterior to the maxillary canine</td>
</tr>
<tr>
<td>32</td>
<td>Left Eustachian Point</td>
</tr>
<tr>
<td>33</td>
<td>Right Eustachian Point</td>
</tr>
</tbody>
</table>
6.4.2.2 Mirror reflection of missing landmarks

As most of the fossil crania exhibited some degree of unilateral damage, they were missing certain landmarks on either the left or right side. The coordinate values of these unilaterally missing landmarks were estimated via a multi-step process of mirror imaging their contralateral homologues (see McNulty et al., 2006; Baab, 2007):

Step 1) A sample of 20 randomly selected adult human crania preserving all landmark coordinate values was combined with the raw fossil data.

Step 2) Two sets of data are created so that one is comprised of all the raw data and the other has all the coordinates for left and right homologous landmarks switched (i.e. the switched data set).

Step 3) This pooled data was imported into the computer program Morpheus (Slice, 2000), which is among the few morphometrics programs that can perform a Generalized Procrustes Analysis on samples with missing landmark coordinate values. The subsequent superimposition of landmark coordinates allowed for a mirroring of missing values by superimposing unilaterally present landmark coordinates from the "switched" data set with homologous landmark coordinates from the original dataset lying on the opposite side. For example, a missing left landmark value would be mirrored by a Procrustean superimposition of its homologue on the right side to homologous left side values from other individuals in the sample.
Step 4) The remaining bilaterally present landmark coordinates underwent Procrustean superimposition with their reversed homologues so as to reduce asymmetry as is often present among fossils.

6.4.2.3 Generalized Procrustes Analysis

A Generalized Procrustes Analyses was performed on a sample including all fossil crania and the pooled modern human sample. As described earlier (Section 3.3.5 of Chapter 3), Principal Components Analysis was preferred over Canonical Variates Analysis as the latter method assigns individual variation into preset groups. The former technique allows for the free plotting of individual data points on their respective locations in morphospace. This was thus the preferred method as the upper respiratory tract is of sufficiently complicated shape to warrant the least restraining means of visualizing its overall variation. Such visualization was aided by use of wireframe models that connect selected landmark locations with straight lines so as to represent anatomic structures and the ways in which their dimensions change over shape space and among groups of individuals. Note that, for the Principal Components Analysis, only left-side landmarks were used so as not to assign double weighting to bilateral landmarks.

6.4.2.4 Univariate measures

As in Chapter 3, linear distances between pairs of landmark coordinates were calculated from Procrustes-corrected data. The measures used in the analysis of extant hominoids and
papionins were also used here (see Section 3.3.6 of Chapter 3). Group differences in these variables were analyzed via ANOVA and, when the results were significant (p<0.05), unadjusted Bonferroni corrections were applied to multiple t-tests. This method was preferred over other alternatives to the unadjusted Bonferroni correction (e.g., Holland and Copenhaver, 1988; Rice, 1989) as the most stringent testing method was preferred. Being that this chapter is concerned with the estimation of the relative dimensions of the nasopharyngeal boundaries and cartilaginous Eustachian tubes among fossil crania, results that achieve significance under more conservative testing conditions may be more likely to reflect actual biological differences from living humans.

6.4.2.5 Identifying differences between fossil crania and modern humans

Given the low sample size of the fossil sample, three separate statistical analyses were performed to determine whether the fossil samples fell outside the modern human range of variation. Three separate Student's t-tests were performed to compare the pooled modern human sample to the Neanderthals (n=3) and mid-Pleistocene hominins (n=4). Also, to assess individual fossils, Z-scores were calculated for each specimen in each measure so that its relative position within the Stage 5 modern human range of variation could be quantified. A position of at least two standard deviations from the human mean was considered significantly different (i.e., at approximately the 95th percentile of a two-tailed distribution).
6.5 Results

6.5.1 Generalized Procrustes Analysis and multivariate shape diversity

A Generalized Procrustes Analysis was performed on the pooled human and fossil samples. The principal components analysis yielded a first principal component (PC1) comprising 34% of total sample variance while PC2 comprised roughly 9%. None of the other PC’s appeared to represent meaningful shape variation. There was a weak but significant correlation between PC1 and centroid size ($r=-0.4791$, $p<0.05$), implying that a small proportion of the morphologic patterns observed may be related to allometry. PC1 may be seen separating newborns from later growth stages. Stage 1 crania did not overlap with any other group but growth stages between adulthood and Stage 2 graded into each other. Shape change along this axis was characterized by vertical growth of the face and choanae, decreasing anteroposterior nasopharyngeal roof depth, inferior flexion of the palate and basiocciput, and narrowing of the choanae when moving toward negative values (left on the axis). Positive shape change along the PC2 axis occurred in an apparent increase in basicranial flexion, decreased nasopharyngeal depth, decreased superior choanal width (between the medial pterygoid hamuli), and increased palate width. PC2 separated Stage 1 from Stage 2 crania with the former lying higher on the axis. It also separated South East Asians, North Africans, and some Southern European crania from all other human populations (including the fossil crania) except the Stage 1 sample, with whom there was complete overlap on this axis. Whereas PC1 does not separate any populations, PC2 tended to separate Alaskans and Chinese from other groups. They were relegated to the lower range of PC2 whereas other groups tended to plot higher on the axis but with some individuals
from warmer climates (West Africans and Australians) also plotting lower. Northern Europeans were the only cold climate population that spanned nearly the full range of PC2. The principal components plot suggests that climate exerts only a slight influence on overall upper respiratory tract and basicranial morphology, despite the presence of localized differences in external nasal and nasopharyngeal morphology (See Chapter 3).

The Gibraltar Forbes' Quarry1, Monte Circeo 1, and Steinheim1 crania clustered among modern humans and were arguably closest to the average Alaskan. Atapuerca 5 plotted intermediately between cold and warm climate populations while Saccopastore 1 was at the extreme low range of PC2 below all but one modern human individual. Saccopastore 1 was located at the extreme range of all human crania on PC1. The Petralona 1 and Kabwe 1 crania clustered extremely close together and were also at the extreme range of PC 1 but plotted among Gibraltar Forbes' Quarry1 and Steinheim on PC2. These results suggest that Neanderthals and mid-Pleistocene hominins overlapped in some aspects of facial and basicranial morphology. However, the position of Saccopastore 1 outside the human cluster may also suggest that the total range of Neanderthal variation may have only partially overlapped that of modern humans.
**Figure 6.15** Superimposition of wireframes atop the anatomical structures which they represent in Figures 6.16-6.18. This figure is adapted from an image provided courtesy of Jeffrey T. Laitman.
**Figure 6.16** Principal Components plot of total fossil morphology plotted among a modern human developmental series, which includes several, geographically diverse populations. Note that, with respect to facial and upper respiratory tract landmark coordinates, Neanderthals and Mid-Pleistocene hominins fall roughly within (or at the edge of) modern human variation. They tended to cluster on the negative end of PC 1 and PC 2, exhibiting longer palates, taller choanae, and nasopharynges that were long and low in appearance relative to modern humans higher on PC 2. Their longer nasopharynges appear to be the result of inferior rotation of the basicranial midline at hormion and lengthening of the sphenoid bone. The anterior location of the maxillary tubercles relative to contemporary humans on the opposite end of the PC 1 axis also accentuated the long, low appearance of their upper respiratory tracts. Left-side landmarks were used so as not to assign double weighting to bilateral landmarks.
Figure 6.17 Wireframe model used to illustrate palatal (in green), choanal (in red), and cartilaginous Eustachian tube (in blue) morphology. The above image is a mid-sagittally sectioned Stage 3 human.
Figure 6.18. Wireframes representing a modern human developmental series (top row) and the fossil crania. The elongated appearance of the cartilaginous Eustachian tube tract of the fossils appears to exhibit taller choanae and longer hard palates than the average human adult. These may be related to the fossil crania.
6.5.2 Hypothesis #1: Neanderthals possessed taller and narrower nasopharynges as an adaptation to respiration in cold environments

The results of all t-tests underwent unadjusted Bonferroni correction so that, with three orthogonal comparisons of Neanderthals, mid-Pleistocene hominins, and Stage 5 humans, the Bonferroni formula yielded an adjusted level of significance at $P = 0.017$. The relative choanal dimensions of Neanderthals (n=3) and mid-Pleistocene hominins (n=4) appear to differ from those of modern human adults (n=261). Neanderthals and mid-Pleistocene hominins each showed significantly smaller relative choanal width and significantly greater relative choanal height with Steinheim 1 falling outside the modern human range of variation in both dimensions (-3.219 and 5.003 standard deviations from the respective means of relative choanal width and height; see Table 6.3).
Figure 6.19 Box-whisker plots of choanal width (a) and height (b) among Stage 5 modern humans and the fossil crania. Note that the fossils exhibit tall, narrow choanae with Steinheim 1 falling outside the modern human range in both of these measures. Asterisks represent significant differences; $P=0.017$ as per Bonferroni adjustment. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, $25^{th}$ percentile, median value, $75^{th}$ percentile, and maximum value. The Y axes represent units of Procrustes-corrected length and are thus relative dimensions. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. These plots were generated using the program SAS.
Relative choanal dimensions significantly differed between adult modern humans and the Neanderthal crania with each Neanderthal specimen exhibiting narrower and taller choanae than the modern human mean. Mid-Pleistocene hominins also exhibited significantly taller and wider choanae than the modern human sample. The Steinheim 1 specimen had the narrowest choanae, which were -3.219 standard deviations from the modern human mean while Monte Circeo 1 exhibited choanae that were -2.339 standard deviations from the modern human adult mean. Significant results of t-tests of choanal shape index [or (width/height) X 100] confirmed these results. Kabwe 1 exceeded the Neanderthals in all of the above measures, indicating that it had taller, narrower choanae than Gibraltar Forbes’ Quarry 1, Monte Circeo 1, and Saccopastore 1.

Neanderthals were distinguished from modern humans by their significantly more obtuse midline choanal orientation (the staphylion-hormion-sphenobasion angle), falling between 1.345 and 2.14 standard deviations above the adult modern human mean value for the staphylion-hormion-sphenobasion angle. Petralona 1 was the only fossil specimen to possess more acutely angled choanae than the modern human adult mean. It may have contributed to the non-significant difference between modern human adults and mid-Pleistocene hominins. Yet, despite possessing more obtusely angled choanae, the actual vertical height of the nasopharynx may have been roughly similar among modern humans, Neanderthals, and mid-Pleistocene hominins as evidenced by the lack of significant difference in the measure of relative minimum nasopharyngeal height. Relative maximum nasopharyngeal depth was greatest among the Steinheim 1, Atapuerca 5, and Saccopastore 1 crania (between 4.499 and 3.398 standard deviations above the modern human adult mean), which was likely a result of their greater relative choanal height projecting staphylion anteroinferiorly.
**Figure 6.20** Box-whisker plots of choanal shape (a) and orientation (b) and maximum nasopharyngeal depth (staphylion-sphenobasion distance; c) among Stage 5 modern humans and the fossil crania. The large values for maximum nasopharyngeal depth among the fossils is likely due to relatively greater height and obtuse orientation of the choanae. Asterisks represent significant differences; $P=0.017$ as per Bonferroni adjustment. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. The Y axes of (a) and (c) represent relative dimensions while the Y-axis of (b) is in angles. $P$-values listed inside each plot indicate the $P$-value for that respective ANOVA. These plots were generated using the program SAS.
Relative nasopharyngeal width was assessed using the bilateral widths between the left and right Eustachian tube orifices and the apices of the left and right petrous temporal bones. The mid-Pleistocene *Homo* sample was significantly narrower than modern humans in bi-Eustachian tube orifice width than the modern humans but not the Neanderthals, from whom they did not significantly differ. Neanderthals were distinguished from both the mid-Pleistocene fossils and the modern humans in possessing significantly narrower width between the petrous apices. Monte Circeo 1 was the narrowest in this measure at -2.256 standard deviations from the modern human adult mean. Nearly all fossil specimens were narrower than the modern human mean in both width measures with the exception of Petralona 1 and Atapuerca 5, which exhibited greater bi-petrous apex width than the human average. In both measures, Kabwe 1 was nested among the Neanderthals, likely as a result of cranial robusticity.
Figure 6.21 Box-whisker plot of relative bilateral width between the left and right osseous
Eustachian orifices (an estimate of nasopharyngeal/basicranial width; a) and width between the
petrous apices (b) in Stage 5 modern humans and the fossil crania. The fossil crania exhibited
consistently smaller values with Steinheim 1 and Petralona 1 exceeding the modern human range
of variation but the latter measure distinguished Neanderthals. Asterisks represent significant
differences; P=0.017 as per Bonferroni adjustment. The cross represents the mean value, the
horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median
value, 75th percentile, and maximum value. The Y axes represent units of Procrustes-corrected
length and are thus relative dimensions. These plots were generated using the program SAS.
Bilateral Width between the Osseous Eustachian Tube Orifices

\[ P < 0.0001 \]

Stage 5 Modern Humans  Neanderthals  mid-Pleistocene Homo

Width Between Petrous Apices

\[ P = 0.004 \]
Table 6.3 Z-scores of fossils’ values for univariate measures expressed in standard deviations of distance from the pooled Stage 5 modern human mean. A distance greater than two standard deviations was considered a value lying at the extreme range of modern human variation. All of the fossils were distinguished by relatively narrow choanal width, greater choanal height, narrower nasopharynges between the osseous Eustachian tube orifices, and shorter basioccipital bones than the average Stage 5 modern humans.

<table>
<thead>
<tr>
<th></th>
<th>Choanal Width</th>
<th>Choanal Height</th>
<th>Choanal Index</th>
<th>Choanal Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibraltar Forbes’ Quarry 1</td>
<td>-0.649371818</td>
<td>0.685619117</td>
<td>-0.924596321</td>
<td>1.905828642</td>
</tr>
<tr>
<td>Monte Circeo 1</td>
<td>-2.338656069</td>
<td>1.140603964</td>
<td>-2.145967168</td>
<td>1.221665215</td>
</tr>
<tr>
<td>Saccopastore 1</td>
<td>-1.284781819</td>
<td>1.956531514</td>
<td>-1.954801565</td>
<td>1.993223221</td>
</tr>
<tr>
<td>Kabwe 1</td>
<td>-2.119781911</td>
<td>1.126273733</td>
<td>-2.015088481</td>
<td>0.946362429</td>
</tr>
<tr>
<td>Petralona 1</td>
<td>-0.801715946</td>
<td>1.155829835</td>
<td>-1.285616804</td>
<td>-1.549315034</td>
</tr>
<tr>
<td>Atapuerca 5</td>
<td>-0.069885611</td>
<td>2.828884351</td>
<td>-1.73352303</td>
<td>1.43265061</td>
</tr>
<tr>
<td>Steinheim 1</td>
<td>-3.218973718</td>
<td>5.003496966</td>
<td>-3.90513382</td>
<td>1.893301429</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Minimum Nasopharyngeal Height</th>
<th>Maximum Nasopharyngeal Depth</th>
<th>Bilateral Width between osseous Eustachian Tube orifices</th>
<th>Bilateral Width between Petrous Apices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibraltar Forbes’ Quarry 1</td>
<td>-0.485981484</td>
<td>0.231692974</td>
<td>-0.511689739</td>
<td>-1.60502762</td>
</tr>
<tr>
<td>Monte Circeo 1</td>
<td>-0.110258877</td>
<td>1.839693437</td>
<td>-0.501124878</td>
<td>-2.255921088</td>
</tr>
<tr>
<td>Saccopastore 1</td>
<td>-0.381616112</td>
<td>3.398750322</td>
<td>-1.752708719</td>
<td>-0.616373217</td>
</tr>
<tr>
<td>Kabwe 1</td>
<td>1.228727421</td>
<td>0.556963835</td>
<td>-1.293489437</td>
<td>-1.718494184</td>
</tr>
<tr>
<td>Petralona 1</td>
<td>0.276252632</td>
<td>-0.233271016</td>
<td>-3.371949715</td>
<td>0.753141823</td>
</tr>
<tr>
<td>Atapuerca 5</td>
<td>-0.449909499</td>
<td>4.596848321</td>
<td>-1.626634714</td>
<td>0.01604895</td>
</tr>
<tr>
<td>Steinheim 1</td>
<td>0.696692876</td>
<td>4.547904743</td>
<td>-3.68748689</td>
<td>-0.843306345</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Nasopharyngeal Floor Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibraltar Forbes’ Quarry 1</td>
<td>0.121635608</td>
</tr>
<tr>
<td>Monte Circeo 1</td>
<td>1.492806476</td>
</tr>
<tr>
<td>Saccopastore 1</td>
<td>-1.626865785</td>
</tr>
<tr>
<td>Kabwe 1</td>
<td>0.795686342</td>
</tr>
<tr>
<td>Petralona 1</td>
<td>0.950808167</td>
</tr>
<tr>
<td>Atapuerca 5</td>
<td>0.595308639</td>
</tr>
<tr>
<td>Steinheim 1</td>
<td>1.245407052</td>
</tr>
</tbody>
</table>
6.5.3 Hypothesis #2: Neanderthals possessed longer, more horizontally oriented cartilaginous Eustachian tubes than human adults

The below analyses include comparisons of the fossil crania to both Stage 1 and Stage 5 humans. Thus, significance was adjusted to $P=0.0083$ via an unadjusted Bonferroni correction. None of the fossil crania exhibited any relative values of cartilaginous Eustachian tube (CET) length that were beyond two standard deviations from the modern human adult mean except for Petralona 1, which possesses a value for CET floor length that is -2.572 standard deviations from the adult modern human mean. Neither Neanderthals nor mid-Pleistocene hominins were significantly different from the Stage 5 modern humans in relative CET floor length (between its orifice on the temporal bone and the point just superior to the medial pterygoid hamulus) or angulation (between the projected CET floor length and the vertical axis of the medial pterygoid plate posterior edge). There were also no significant differences in distance from the bony Eustachian tube orifice to the superior-most point on the medial pterygoid plate margin or in medial pterygoid plate height between its superior-most boundary and its hamulus. However, the Neanderthal sample exhibited a significantly greater distance between the Eustachian tube orifice and the midpoint on the medial pterygoid plate posterior margin. All Neanderthals exhibited values greater than the modern human mean for this measure while all mid-Pleistocene specimens were below the modern human mean and were not significantly different. Kabwe 1, Steinheim 1, and Atapuerca 5 were all within one standard deviation below the modern human mean while the Petralona 1 cranium was over -2.5 from the mean, outside the modern human range of variation.
**Figure 6.22** Box-whisker plots of CET floor length (a) and angulation (b) among the fossil crania and Stage 1 and Stage 5 modern human crania. Asterisks represent significant differences; P=0.017 as per Bonferroni adjustment. The fossils exhibited no significant differences from Stage 1 or Stage 5 humans in CET length but did resemble Stage 5 modern humans in angulation. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. The Y axes represent units of Procrustes-corrected length and are thus relative dimensions. P-values listed inside each plot indicate the P-value for that respective ANOVA. These plots were generated using the program SAS.
Despite lying well within the Stage 5 modern human range, Neanderthals and mid-Pleistocene hominins did not significantly differ from newborn humans in relative CET floor length (with significance set at $P=0.017$ as per the Bonferroni formula). The non-significant difference between mid-Pleistocene hominins and Stage 1 modern humans was likely a result of the low values of Petralona 1 and Kabwe 1 (which were -0.3415 and 0.2317 standard deviations from the newborn mean value; see Table 6.6). Yet, both fossil samples exhibited significantly greater CET floor angulation than Stage 1 modern humans. It remains unclear if Neanderthal infants may have differed from modern human infants in CET morphology. There are few Neanderthal infant crania well enough preserved to allow a 3D geometric morphometrics comparison of relative CET length and orientation as done here.

The CET morphology of both Neanderthals and mid-Pleistocene hominins fell mostly within the range of anatomically modern human adults. However, as reported above, Neanderthals appeared distinct from mid-Pleistocene hominins in possessing greater relative distance between the osseous Eustachian tube orifice and the midpoint on the medial pterygoid plate posterior edge with no overlap. A t-test between the two groups yielded non-significant results but the Neanderthals were significantly greater in this measure than the Stage 5 modern human average. All Neanderthals exhibited values that were greater than the Stage 5 modern human mean. Nonetheless, neither they nor mid-Pleistocene hominins significantly ($P=0.0036$ as per the unadjusted Bonferroni formula) differed from any individual human population in this measure except for the sample of Australian crania, which was significantly greater than the mid-Pleistocene hominins (Figure 6.23).
**Figure 6.23** Box-whisker plot of CET length (between the Eustachian point and the midpoint on the medial pterygoid plate edge) among Stage 5 modern human crania representing several populations and the fossil crania. Asterisks represent significant differences; $P=0.0036$ as per Bonferroni adjustment. Neanderthals appeared to occupy the upper range of human variation (exhibiting longer distances than most modern humans) while mid-Pleistocene hominins were at the extreme low range (exhibiting shorter distances than most modern humans). Petralona 1 fell below the lower limit of modern human variation. The cross represents the mean value, the horizontal red lines represent (from bottom to top) minimum value, 25th percentile, median value, 75th percentile, and maximum value. The Y axis represent units of Procrustes-corrected length (relative dimensions) and the $P$-value listed inside the plot indicates significance of the ANOVA. These plots were generated using the program SAS.
Table 6.4 Z-scores of fossils’ values for univariate measures expressed in standard deviations of
distance from the pooled Stage 5 modern human mean. A distance greater than two standard
deviations was considered a value lying at the extreme range of modern human variation.
Neanderthals were distinguished by greater CET length (from the Eustachian point to the
midpoint of the medial pterygoid plate edge) than the modern human mean while all of the mid-
Pleistocene specimens exhibited relatively smaller values for this measure. Also, all
Neanderthals and the Kabwe 1 specimen demonstrate more acute CET floor orientation, likely in
relation to their tall choanae.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>CET Floor Length</th>
<th>CET Orientation</th>
<th>Distance from Eustachian Point to Superior-Most Point on Medial Pterygoid Plate Edge</th>
<th>Distance from Eustachian point to midpoint on Medial Pterygoid Plate Edge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibraltar Forbes' Quarry 1</td>
<td>-0.205372691</td>
<td>-1.312422581</td>
<td>-0.592467927</td>
<td>0.653545734</td>
</tr>
<tr>
<td>Monte Circeo 1</td>
<td>0.128827216</td>
<td>-0.67489232</td>
<td>0.117038139</td>
<td>0.473173043</td>
</tr>
<tr>
<td>Saccopastore 1</td>
<td>0.419435831</td>
<td>-0.201284758</td>
<td>0.650666645</td>
<td>0.858863586</td>
</tr>
<tr>
<td>Kabwe 1</td>
<td>-0.857006623</td>
<td>-0.261262305</td>
<td>-0.96121122</td>
<td>-0.599468815</td>
</tr>
<tr>
<td>Petralona 1</td>
<td>-2.571597449</td>
<td>1.055137323</td>
<td>-1.405901641</td>
<td>-2.502016941</td>
</tr>
<tr>
<td>Atapuerca 5</td>
<td>-0.393150565</td>
<td>0.482722128</td>
<td>-0.310664109</td>
<td>-0.798070662</td>
</tr>
<tr>
<td>Steinheim 1</td>
<td>1.367267005</td>
<td>0.55906805</td>
<td>-0.317659239</td>
<td>-0.65415628</td>
</tr>
</tbody>
</table>
6.6 Discussion

6.6.1 Discussion of Principal Components Analysis (PCA) and multivariate shape diversity

As described in Chapter 3 (see section 3.2.4.3), modern human populations were not found to show appreciable climatic gradients in most aspects of nasopharyngeal morphology (i.e., width and height of the choanae and overall osseous nasopharyngeal boundaries). This study sampled specimens from North and South Europe, North Africa, East and West Africa, India, China, South East Asia, Australia, Alaska, and the Aleutian Islands in an attempt to capture clinal variation in upper respiratory tract morphology. The Principal Components plot showed human populations from various climates overlapping extensively as was found by Noback et al. (2011). Some populations appeared more morphologically variable than others. This was especially true for West Africans and Northern Europeans. Despite the presence of well known variation in nasal morphology among human populations from different climates, postnasal morphology does not seem to follow such a relationship when viewed in PCA morphospace.

The Gibraltar Forbes’ Quarry 1, Steinheim 1, and Atapuerca 5 crania all clustered within an area of extensive overlap among several modern human populations. Saccopastore 1 was the only specimen to plot outside the modern human cluster as it possessed taller choanae and an anteroinferiorly located hard palate relative to the basicranial midline (as can be seen from its wireframe model in Figure 6.17). Petralona 1 and Kabwe 1 occupied extremely close points in morphospace at the edge of the modern human distribution, lying close to several Northern
European individuals. The results suggest that both Neanderthal and mid-Pleistocene specimens can overlap with modern humans in aspects of osseous nasopharyngeal boundary morphology despite the clear position of some individuals outside the human range. A similar pattern was observed by Marquez et al. (in press) in the morphology of the Neanderthal “nasal complex” using univariate measures.

The close relationship of Petralona 1 and Kabwe 1 in the PCA corroborates previous observations that the former specimen retains synapomorphies that were likely inherited from a common ancestor with Kabwe 1 (Stringer et al., 1979; Murrill, 1981; Stringer, 1983; Rightmire, 2001; Harvati, 2009; Friess, 2010; Harvati et al., 2010, 2011; Freidlene et al., 2012). It possessed few to none of the derived morphologic features of Neanderthals, instead bearing a closer resemblance to the one African mid-Pleistocene specimen used in this study, Kabwe 1. Petralona 1 is thus an unlikely candidate for possession of upper respiratory tract adaptations to cold climate. Its position on the PCA appears related to a relatively shallower nasopharyngeal roof (between hormion and sphenobasion), relatively taller, narrower choanae, and greater relative facial height with taller maxillae (i.e., inferior location of the maxillary tuberosities relative to the prosthion-staphyion line).

6.6.2 Discussion of Procrustes-corrected (scale-free) univariate measures

The Kabwe 1 cranium appears similar to the modern human sample in the proportions of the external basisphenoid and basioccipital bones, the expression of a sphenoid spine, and the orientation of the petrous temporal bones. Indeed, the analysis of Laitman et al. (1979) could not distinguish the basicranial morphology of this specimen from adult modern humans. However,
Kabwe 1 also exhibited most of the features distinguishing the nasopharyngeal boundaries of Neanderthals and European mid-Pleistocene hominins from modern humans, thus contradicting Hypothesis 1. These included tall and obtusely angled choanae with anterior positioning of the hard palate, greater relative nasopharyngeal floor depth, relatively narrow choanae, and relatively narrow nasopharyngeal width (between the osseous Eustachian tube orifices) compared to adult modern humans. Both Neanderthals and mid-Pleistocene *Homo* are generally characterized by long, narrow nasopharynges. The evidence indicates that these similarities among all of the fossil crania may be due to the common expression of cranial robusticity. The anteroposteriorly short, mediolaterally broad appearance of the modern human nasopharynx is thus likely related to extreme upper and midfacial shortening and overall shortening of the horizontal supralaryngeal vocal tract.

The typical Neanderthal may have been characterized by a relatively long, narrow soft palate with anterior positioning of the hard palate and medial pterygoid plates relative to adult modern humans. Indeed, reconstruction of the Neanderthal vocal tract with more anterior positioning of the hyoid bone relative to modern humans (Barney et al., 2012) is corroborated in this study. Such a configuration would have impacted both speech and respiratory abilities. However, the preponderance of traits in common with European mid-Pleistocene specimens and the one African mid-Pleistocene specimen used here (Kabwe 1) suggest that, as with the modern human sample, craniofacial structure and the physiologic constraints of several functional units (i.e., brain, middle ear, nasal cavity, orbital cavity, the atlanto-occipital articulation) were the predominating factors interacting with nasopharyngeal shape rather than climatic adaptation. The great height (between staphylion and hormion), narrowness, and obtuse angulation of the
Neanderthal choanae appear to be a symplesiomorphy shared with populations represented by the mid-Pleistocene Homo crania.

The results of this study suggest a suite of features not observable among modern human growth series in which adult-like palate length and elongated but narrow postnasal airways are combined with infant-like retroflexion of the hard palate and choanae, which are taller and narrower than among modern human adults. The actual morphology of the upper airways in both Neanderthals and mid-Pleistocene Homo may thus have differed from modern human adults and juveniles in some unexpected ways. Future reconstructions should focus on the range of variation among Neanderthal crania which, despite overlapping with modern humans in some dimensions, may be the result of different sets of functional relationships and biomechanical constraints.

Despite sharing many aspects of nasopharyngeal morphology in common with mid-Pleistocene Homo, Neanderthals are distinguished in the relatively long distance between the midpoint of the medial pterygoid plate posterior edge and Eustachian tube orifice (which may also roughly approximate the location of the levator veli palatini muscle origin), partially supporting Hypothesis 2. Such morphology could increase the length of the cartilaginous Eustachian tube relative to mid-Pleistocene Homo, which plot on the lower end of the Stage 5 human range. Being that this measure spans between the lateral attachment of the CET floor on the temporal bone and a point just inferior to the location of the pharyngeal orifice roof on the medial pterygoid plate, it could represent the diagonal axis of the tube. A longer diagonal axis may thus have translated to a lumen of both greater length and vertical height (as suggested by their choanal dimensions). It may have also necessitated a larger, more laterally positioned origin for the tensor veli palatini on the enlarged Neanderthal sphenoid spine, potentially functioning in
a manner similar to the infratubal process of apes (for a discussion on the condition of great apes, see Dean, 1985).

**Toward a new hypothesis: Cartilaginous Eustachian tube dimensions and the potential role of middle ear disease in the extinction of Neanderthals**

The above data suggests that adult Neanderthals possessed cartilaginous Eustachian tubes of greater length (between the osseous Eustachian tube orifice and the midpoint of the medial pterygoid plate posterior edge) and obtusely oriented choanae (the staphyion-hormion-sphenobasion angle) than adult modern humans. These features also likely characterized Neanderthal infants and children, impacting their susceptibility to middle ear disease (otitis media) via longer, more horizontally oriented CET morphology. When untreated, otitis media can cause hearing loss and even death. It is arguably the most common pediatric condition worldwide among contemporary human populations (Bluestone, 2005a). An increased rate of middle ear disease among Neanderthal populations relative to invading modern humans could have decreased their reproductive fitness.

As reviewed by Bluestone (2005a,b), greater CET length in human adults relative to newborns is one of the factors that may render otitis media a rare disease beyond childhood. However, greater CET length relative to the osseous portion of the Eustachian tube renders the former component patulous and decreases the efficiency with which the middle ear is aerated (Doyle and Rood, 1980; Bluestone, 2008). If the dimensions of the osseous portion of the Neanderthal Eustachian tube were similar to those of modern humans, ventilatory function of the elongated Neanderthal CET may not have been as efficient as among modern humans. Such a
condition would lead to chronic negative middle ear pressure as among modern human children, which leaves them more susceptible to entrapment of mucous in the middle ear and retraction of the tympanic membrane (i.e., atelectasis; see the classic study of Politzer, 1908; Brooke, 1969; Jerger, 1970; Bluestone, 1983, 2005a). These negative ear pressures have been noted among children that are both symptomatic and asymptomatic for otitis media and have been attributed to relatively less efficient ventilatory function of the CET than among adults (Brooke, 1969; Jerger, 1970; Bluestone, 1983, 2005a). Under such a condition, even partial dilation of the Eustachian tube can result in suction of infectious agents from the nasopharynx directly into the middle ear (Bluestone and Beery, 1976; Bluestone, 2005a). Experimental work by Cantekin et al. (1976) on *Macaca* showed that, by transecting a portion of the tensor veli palatini muscle (which is responsible for dilating the CET), non-sterile middle ear effusion developed after bacterial colonies were introduced into the nasopharynx.
Figure 6.24 Three-dimensional reconstructions of the cartilaginous Eustachian tube tracts of a human newborn, adult, and a Neanderthal (Monte Circeo 1). Note the horizontal orientation of the Neanderthal, which likely corresponds with its obtusely oriented choanae.
Developmental changes in Eustachian tube angulation have not yet been described among Neanderthals. As the CET becomes more vertically oriented over modern human development with vertical choanal growth, it is likely that this trend also occurred among Neanderthals whose vertical choanal height (between hormion and staphylion) is great compared to modern humans. However, their choanae remain obtusely angled into adulthood, which may have led to more horizontal CET orientation over a comparatively longer period of developmental time. Thus, Neanderthal children may have remained susceptible to otitis media up until a later relative age than human children whose CET orientation changes with both vertical choanal growth and angulation. Future studies will analyze Neanderthal infants and children (e.g., Pech de l’Aze, MNHM/MH 24.378.1956-6; La Quina, H18; Teshik-Tash 1) to reconstruct their developmental trajectory and its potential impact on susceptibility to middle ear disease. Any differences from the modern human condition may have played a role in their extinction.
Figure 6.25 The cartilaginous Eustachian tubes of a modern human infant (top), adult (middle), and an adult Neanderthal (Monte Circeo 1, bottom) are shown abutting the medial pterygoid plate. The morphology of Neanderthals was likely intermediate between that of adult and infant humans due to oblique choanal orientation and greater choanal height.
It has been argued that modern humans first arriving in European Neanderthal habitats did so at relatively higher population densities (Finlayson et al., 2006, 2008; Mellars and French, 2011). Aside from signifying a higher rate of reproduction, higher population densities may have also fostered increased rates of communicable diseases. Otitis media occurs at higher rates in daycare centers or in home environments with multiple children present (Tos et al., 1978; Visscher et al., 1984; Pukander et al., 1985; Wald et al., 1988; Zielhuis et al., 1989; Paradise et al., 1997; Bluestone, 2005a). Number of children has been found to be positively correlated with the likelihood of developing otitis media (Paradise et al., 1997). Thus, a higher population density could have rendered invading *Homo sapiens* disease vectors for the young children of sympatric Neanderthal populations, reducing rates of survival to reproductive age in these groups.

The fate of the Neanderthals remains one of the greatest enduring controversies in paleoanthropology. Novel data on their cranial morphology may yet contribute to understanding of the factors related to their interaction with- and ecological marginalization by incoming anatomically modern humans. If Neanderthal children suffered from higher rates of morbidity and mortality due to chronic middle ear disease, reproductive fitness would have decreased in this species, contributing to their eventual extinction. Yet, as of the present time, no detailed reconstruction of Neanderthal Eustachian tube physiology has been attempted. There remains much to be learned on the functional integration of facial, basicranial, and middle ear structures within this species. Given their distinct nasopharyngeal boundary morphology, Neanderthal speech, respiratory abilities, and susceptibility to upper airway disease likely differed from *Homo*.
sapiens. This enigmatic group may thus have perplexed the modern humans they encountered in the later Pleistocene as they still do today.

6.7 Conclusions

A sample of fossil crania representing Neanderthals and mid-Pleistocene hominins was analyzed against a geographically diverse sample of modern humans to test for differences in nasopharyngeal airway dimensions and cartilaginous Eustachian tube morphology. Result indicate that all of the fossil crania exhibited tall, narrow nasopharynges relative to modern humans, likely as a consequence of long, low cranial shape. The fact that Kabwe 1, a specimen representing mid-Pleistocene Homo from tropical Africa, exceeds the Neanderthals in relative choanal height and narrowness indicates that there is no discernable climatic adaptation manifested in Neanderthal nasopharyngeal dimensions. However, Neanderthals were distinguished from all other groups in cartilaginous Eustachian tube morphology. Their Eustachian tube length plotted at the upper range of modern human variation while mid-Pleistocene hominins were at the extreme lower range. Angles of Eustachian tube and choanal orientation were also at the extreme range of human adults.

Neanderthals exhibited a unique combination of features that distinguish them consistently from both modern humans and mid-Pleistocene hominins. It appears that the cartilaginous Eustachian tube morphology of both modern humans and Neanderthals diverged independently from the condition of mid-Pleistocene hominins, from whom both groups differ. These strongly suggest species-level distinctions that reflect substantial physiological differences in respiration and middle ear aeration/equilibration. Such differences may have impacted
Neanderthal disease susceptibility and, ultimately, their ability to compete with modern humans for limited ecological resources.
7. Summary and Conclusions

The nasopharynx is an understudied yet vitally important region. It is situated among several important cranial units and morphological change in its boundaries over development and evolution may impact respiration, speech, middle ear aeration, and alimentation among other physiologic functions. Despite its vital importance, this region has been studied mainly from a clinical perspective. Indeed few detailed investigations of its normal development have been performed. Further, there is little information on nasopharyngeal evolution among humans and other primates. To this end, this dissertation has focused on exploring in detail the development, evolution, and functional morphology of a vital nexus of structures within the upper airway.

7.1 Osteology

Morphological relationships of the osseous nasopharyngeal components were analyzed via three-dimensional geometric morphometrics and Procrustes-corrected univariate measures. The relationship of nasopharyngeal boundary shape to the facial skeleton and basicranium was a prime focus. Three independent hypotheses were tested: 1) nasopharyngeal width increases with shortener facial length and greater facial kyphosis (relative to the midline basicranium); 2) External basicranial flexion leads to nasopharyngeal shape that is vertically taller and anteroposteriorly shallower; 3) human newborns and infants exhibit shorter, more horizontally oriented cartilaginous Eustachian tube pathways (measured between the osseous Eustachian tube orifice and the medial pterygoid plate) relative to older children and adults. Additionally,
patterns of human variation were analyzed for climatic differences and other population
differences that may be related to disease susceptibility.

Relative nasopharyngeal width was found to be related to facial length as humans, the
most orthognathic of all primates, exhibited consistently greater relative nasopharyngeal width
than all other non-human primates. This relationship characterized all growth stages of all
respective genera sampled. Nonetheless, the measure of facial kyphosis used here did not
distinguish modern humans from other primates and did not exhibit strong ($r>0.6$) correlations
with measures of relative nasopharyngeal width. Hypothesis 1 was thus only partially supported
by the data.

Over the course of human development, previously documented changes in external
basicranial flexion (e.g., Laitman et al., 1979) coincided with relative increases in the vertical
height of the choanae. Measures of relative nasopharyngeal depth taken on the basicranium also
decreased during this time period. As the larynx descends and the external basicranium flexes, so
too does the nasopharynx increase in vertical height and decrease in anteroposterior depth. Thus
Hypothesis 2 was supported by the data.

The results represent the first three-dimensional geometric morphometric investigation of
the cartilaginous Eustachian tube pathway. Corresponding temporally to human developmental
changes in relative nasopharyngeal dimensions were the orientation and relative length of the
cartilaginous Eustachian tube path. Among newborns, the tube was relatively shorter and more
horizontally oriented than among older children and adults. The choanae were also more
horizontally oriented among newborns and young children, similarly achieving a more vertical
orientation with age. Timing of these growth changes coincide with times of peak incidence of
otitis media (middle ear disease).
Among the functions of the nasopharyngeal space is the conveyance of air that has been conditioned within the nasal cavity to the lower respiratory tract. Few have investigated whether the nasopharyngeal boundaries participate in further postnasal air conditioning. Nasal air conditioning occurs via warming by submucosal blood vessels, humidification via serous cells, and protection of the surface epithelial layer from rapidly moving inspiratory air by mucus produced in mucous cells. Classic studies by Bryant (1916) and Ali (1965) have stated that all of these components necessary for air conditioning reside only within the anterior-most portion of the nasopharynx, abutting the nasal cavity at the medial pterygoid plate. They also argued that non-contractile, immovable surfaces are needed to sustain respiratory epithelium with embedded goblet cells (mucous cells that are distributed among surface epithelia). This study independently tested the hypothesis that all of the components necessary for postnasal air conditioning are restricted to the bony, non-contractile surfaces of the nasopharyngeal boundaries.

To test the basic biology of distribution of the postnasal air conditioning components, a wide range of primate taxa (Homo, Hylobates, Macaca, Ateles, Tarsius, and Otolemur) were utilized. They represent differing patterns of osseous morphology and, by extension, airflow physiology. All specimens were decalcified with formic acid, embedded in paraffin wax, and sectioned histologically between the choanae and posterior nasopharyngeal wall. Every tenth section was mounted and stained with hematoxylin and eosin.

The results indicated that all of the nasopharyngeal surfaces of every taxon exhibited all of the components required for postnasal air conditioning. While there was variation in the
distribution of respiratory epithelium over the posterior pharyngeal wall (the most contractile of nasopharyngeal surfaces), mucous and serous cells were always present within or under the lamina propria. Other studies that have identified a zone of transitional epithelium solely over the torus tubarius were not corroborated here. Rather, this surface of intermediate contractility always exhibited uniform concentrations of respiratory epithelium with large goblet cells.

It appears that all of the nasopharyngeal surfaces are capable of contributing to postnasal air conditioning. Differences in nasopharyngeal surface areas between separate species may thus be translatable to differences in air conditioning capacity. Rather than limiting attention to the surface areas of the medial pterygoid plate or sphenoid body, overall nasopharyngeal surface area appears a better indicator of air conditioning ability. These data may be useful for analyses of populations such as Neanderthals and mid-Pleistocene _Homo_ and comparisons to modern humans from diverse climates.

7.3 Epigenetic influence of climate on nasopharyngeal morphology

As the osseous morphology and histology of nasopharyngeal boundaries have been examined in this study, so too have the overall nasopharyngeal dimensions. CT imaging was utilized to measure the actual airways of two outdoor colonies of _Macaca mulatta_ representing groups reared in cold and warm climates. Local climate was hypothesized to exert influence over the development of the nasopharynx and overall upper airways over an individual’s life history. Such a process may bear upon analyses of genetically heritable climatic adaptations in the osseous upper respiratory tract components. A three-dimensional geometric morphometric analysis of the upper airway was performed and nasopharyngeal surface area and volume were
scaled over centroid size for comparison of relative dimensions. Additionally, univariate measures were derived from Procrustes-corrected coordinate data.

No shape differences were found in the principal components analysis or t-tests of univariate measures. However, there was a pronounced difference in centroid size between the cold-reared Oregon colony and warm-reared California colony with the former exhibiting substantially larger centroid size. This group had upper airway dimensions that were larger than conspecifics raised in a warmer climate. However, the Oregon individuals had lower body mass relative to the California individuals. Thus the Oregon individuals had much larger upper respiratory tract size relative to body size. These results indicate that upper respiratory tract size may be extremely responsive to local climatic factors. As there were no attendant shape differences, the data do not suggest that analyses of genetically heritable shape differences among groups may be influenced by epigenetic forces of ambient climate interacting with an individual’s developmental trajectory.

7.4 Analysis of nasopharyngeal morphology among fossil hominins

The nasopharyngeal morphology (including relative cartilaginous Eustachian tube dimensions) of fossils representing Neanderthals and mid-Pleistocene Homo were reconstructed via three-dimensional coordinate data. Two separate hypotheses were tested:

Hypothesis 1- Neanderthals were hypothesized to exhibit adaptations adaptations in nasopharyngeal morphology to aid the nasal cavity in air conditioning. To this end, it was expected that Neanerthals would exhibit a nasopharyngeal shape that was relatively tall and narrow relative modern humans so that surface area-to-volume ratio could be increased. A
A geographically diverse sample of humans was compared to a sample of Neanderthal crania and several specimens representing mid-Pleistocene hominins. One of these specimens, Kabwe 1, represents a population from tropical Zambia. In the event that this specimen exhibited nasopharyngeal dimensions equally tall and narrow as the Neanderthals, the hypothesis would be rejected.

Hypothesis 2- It was hypothesized that Neanderthals possessed relatively longer, more horizontal cartilaginous Eustachian tube paths than modern humans. Such a configuration was expected based on their long-low overall cranial shape. Differences in these dimensions may have impacted middle ear aeration and disease susceptibility.

Three-dimensional geometric morphometrics were used to analyze overall shape differences. A principal components analysis of Procrustes-fitted coordinate data revealed few global differences in overall osseous upper respiratory tract dimensions between the fossils and extant humans. However, analysis of univariate measures derived from the Procrustes-fitted coordinate data revealed that all of the fossil crania exhibited relatively tall and narrow nasopharynges. The Kabwe 1 specimen exceeded Neanderthals in relative choanal height and narrowness contra Hypothesis 1. It thus appears that tall, narrow nasopharyngeal dimensions are related to a long, low cranial shape rather than climatic adaptation.

Hypothesis 2 was supported by the data as Neanderthals were distinguished from both modern human adults and mid-Pleistocene hominins by relatively greater cartilaginous Eustachian tube tract length. They were also distinguished from modern human adults and the Kabwe 1 and Petralona 1 specimens by possession of more horizontally oriented cartilaginous Eustachian tube tracts and choanae. These two fossils occupied almost identical locations on the principal components analysis and have long been considered to be similar in morphology. They
may more accurately reflect ancestral morphology whereas Neanderthals and the remaining two European mid-Pleistocene hominins (Steinheim 1, Atapuerca 5) may represent a more derived condition.

Overall, Neanderthals are distinguished from modern humans by possession of relatively tall, narrow nasopharynges and long, horizontal cartilaginous Eustachian tube tracts and choanae, which resemble human newborns and young infants in orientation. This likely impacted the efficiency of middle ear aeration and likely disease susceptibility. As human infants experience a peak age of incidence of otitis media corresponding with this study’s Stages 1 and 2, Neanderthals may have experienced relatively longer periods of peak susceptibility over their life histories as a result of their horizontal Eustachian tube orientation and relatively elongated cartilaginous portion. This would have compromised their reproductive success when competing for limited resources with invading anatomically modern humans.

Further investigation of Neanderthal Eustachian tube development is warranted by these results. This study found that Neanderthals tended to overlap modern humans only at the extreme ranges of variation for relative nasopharyngeal dimensions. Their tall, narrow nasopharynges appear to be primitive retentions while their reconstructed cartilaginous Eustachian tube morphology is likely autapomorphic. The consistency with which Neanderthals are distinguished from anatomically modern humans strongly suggests species-level differences.

7.5 Nasopharyngeal morphology within the context of primate evolution

This dissertation has tested hypotheses related to the osteology, soft tissue histology, growth, and evolution of the nasopharynx with the aim of contributing to an understanding of an
understudied but vital region at the center of the upper airway. The nasopharynx has been involved in nearly every major restructuring of the cranium and upper airway over the evolution of terrestrial vertebrates, mammals, primates, and hominins. Nonetheless few have described morphological differences in nasopharyngeal dimensions among fossil hominins. It is the sum of these changes that contribute to disease etiology of nasopharyngeal structures among contemporary humans, rendering upper respiratory tract infection (including otitis media) the most globally prevalent pediatric condition.

The data presented in this dissertation indicate that the nasopharynx is functionally integrated with both the facial skeleton and basicranium. All of its surfaces appear capable of respiratory air conditioning. Nonetheless, differences in surface area-to-volume ratio do not arise, as specified here, among conspecifics raised in different climates, who instead exhibit differences in overall upper airway size. Among our closest extinct relatives, the Neanderthals, species-level differences in nasopharyngeal morphology have been observed. These likely translated to physiological differences in respiration and middle ear aeration, which likely impacted disease susceptibility.

With all of the above results, far greater research attention is warranted on the nasopharynx and its boundaries. This study has been among the first to reconstruct the cartilaginous Eustachian tube dimensions of fossil hominins. Future studies will focus on specimens representing juvenile and adolescent Neanderthals so that their Eustachian tube development may be reconstructed. Additionally, the morphologies of other fossil groups will be analyzed, including those of Plio-Pleistocene age. Such work may ultimately provide a novel perspective on human evolution.
References


Bieger J. 1957. Der Formwandel des Primatenschädel und seine Beziehungen zur
ontogenetischen Entwicklung und den phylogenetischen Spezialisat

Biegert J. 1963. The evaluation of characteristics of the skull, hands and feet for primate
taxonomy. In Washburn SL, editor. Classification and Human Evolution. Chicago:
Aldine. p 116-145.

Leipzig: Leopold Voss.

G. 2007. High-resolution U-series dates from the Sima de los Huesos hominids yields:

Variations in Growth Occurring Between the Ages of 12 and 20 Years and Its Relation to

Bluestone CD. 1983. Eustachian tube function: physiology, pathophysiology, and role of allergy

Ontario: BC Decker.


527.


Bluestone CD, Swarts JD. 2010. Human evolutionary history: Consequences for the

rhinosinusitis, like otitis media, a unique disease of humans? Otolaryngol Head Neck

Boe L-J, Heim J-L, Honda K, Maeda A. 2002. The potential of Neanderthal vowel space was as
large as the of modern humans. J Phon 30:465-484.

humans and Neanderthals: Acoustic capabilities and consequences for the debate on the


Brooks D. 1969. The age of the electro-acoustic impedance bridge in the assessment of middle
ear pressures. Audiol 8: 563-569.


Brink AS. 1957. Speculations on some advanced mammalian characteristics in the higher mammal-like reptiles. Palaeontol Afr 4: 77-96.


Dean MC. 1985. Comparative myology of the hominoid cranial base II the muscles of the prevertebral and upper pharyngeal regions. Folia Primatol 44: 40-51.


Haak W, Balanovsky O, Sanchez JJ, Koshel S, Zaporozhchenko V, Adler CJ, Der Sarkissian


Krogman WM. 1930. The problem of growth changes in the face and skull as viewed from a comparative study of anthropoids and man. Dental Cosmos 72: 624-630.


and perception of speech: essential elements in analysis of the evolution of human
speech.


Lugaresi E, Coccagna G, Berti Ceroni G, Petrella A, Mantovani M. 1968. La "maledizione di
Ondine" il disturbo del respiro del sonno nell’ipoventilazione alveolare primaria. Sisto
Nerv 20: 27-37.


MacDonald G. 1889. On the respiratory functions of the nose: and their relation to certain

Maier, W. 2000. Ontogeny of the nasal capsule in the cercopithecoiods: a contribution to the
comparative and evolutionary morphology of catarrhines. In: Whitehead PF, Jolly CJ.

Maier W. 2013. The levator veli palatini muscle in artiodactyls- A comparative ontogenetic

69. p 69-82.

Maier W, van den Heever J, Druand F. 1996. New therapsid specimens and the origin of the

OM in Unselected Children Aged 1 to 8 Years Followed by Weekly Otoscopy through

Press.

Márquez S. 2002. The human nasal comlex: A study of its anatomy, function, and evolution by
CT, comparative and morphometrics methods. Doctoral Dissertation. City University of
New York.


Micham BM, Laitman JT. 2013. Developmental horizons in the 2-4 month old infant aerodigestive tract. FASEB J 27: 9650


O’Higgins P, Jones N. 2006. Program Morphologika2, version 2.5 was downloaded from <http://www.york.ac.uk/res/fme/index.htm>


Rightmire GP. 2001. Patterns of hominid evolution and dispersal in the Middle Pleistocene. Quatern Int 75: 77-84.


Schneider CV. 1662. De catarrhis. Wittenberg.


Strand Viðarsdóttir U, Cobb S. 2004. Inter- and intra-specific variation in the ontogeny of the


Page 406 of 410


Xiao D, Bae CJ, Shen G, Delson E, Jin JHJ, Webb NM, Qiu L. In press. Metric and geometric morphometric analysis of new hominin fossils from Maba (Guandong, China). J Hum Evol


