

Microscopic Variation in Feathers of Birds of Prey

CHARLES CODDINGTON

Why Identify Bird Remains?





Law Enforcement & Anthropological Artifacts



Prey Remains Identification

SHORT COMMUNICATIONS

503

The Wilson Journal of Ornithology 127(3):503–506, 2015

Forensic Techniques Identify the First Record of Snowy Owl (*Bubo scandiacus*) Feeding on a Razorbill (*Alca torda*)

Carla J. Dove^{1,2} and Charles P. J. Coddington¹

ABSTRACT.—Stomach contents were analyzed using microscopic feather characters and osteological comparisons to document the first record of a Razorbill (*Alca torda*) being eaten by a Snowy Owl (*Bubo scandiacus*). Careful comparisons of fragmented morphological evidence in combination with geographic location and other circumstantial data can enhance dietary analyses, especially when it is difficult to obtain DNA sequences from the degraded and digested stomach contents. *Received 4 December 2014. Accepted 26 February 2015.*

Key words: food items, microscopy, osteological comparisons, Razorbill, Snowy Owl.

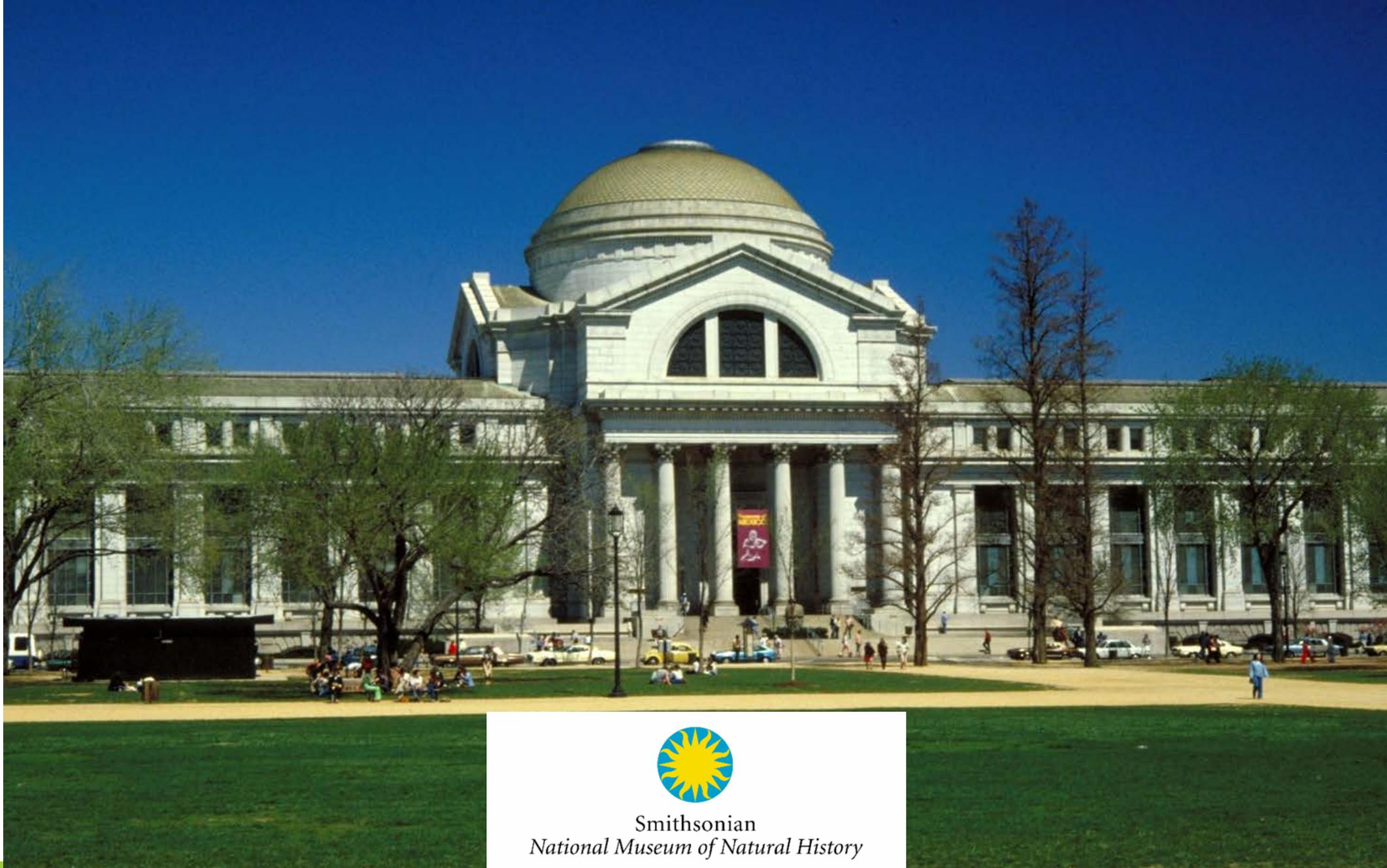
A Snowy Owl (*Bubo scandiacus*) carcass was found during a routine surface inspection of runway 22L at John F. Kennedy (JFK) International Airport in New York (40.6397° N, –73.7789° W) on 3 February 2013. The specimen was in good condition, and was sent to the Smithsonian Institution's Feather Identification Lab to be preserved as a museum specimen (USNM 627903). During preparation procedures, several avian bones, feathers, a mammal skull, and hair were discovered in the stomach of the specimen. Detailed examination procedures using microscopy together with osteological comparisons were conducted on the food items to

reported that Snowy Owls fed on birds when other sources were less available, and Campbell and MacColl (1978) found heavy representation of waterfowl and grebes in the winter diets of Snowy Owls from British Columbia.

Studies of prey remains in predatory birds often rely on identification of osteological material regurgitated as pellets, but many times feathers are also present in the samples and can be identified to obtain more complete dietary information (Woodman et al. 2005). Careful comparisons using trace evidence techniques and morphological comparisons enhance studies of prey items, especially when it is difficult to obtain DNA sequences from the degraded stomach contents. These combined methods can also be used to discover food items that may be overlooked when focusing only on osteological material. Here, we describe a combination of methods used to identify a previously undescribed food item of the Snowy Owl.

METHODS

We first cleaned the food items (bones and feathers) following methods for preparing prey remains in Sabo and Laybourne (1994). This involved gently agitating the material in hot water with liquid soap. Then we dried it by using short, soft bursts of compressed air. Microscopic samples



Smithsonian
National Museum of Natural History



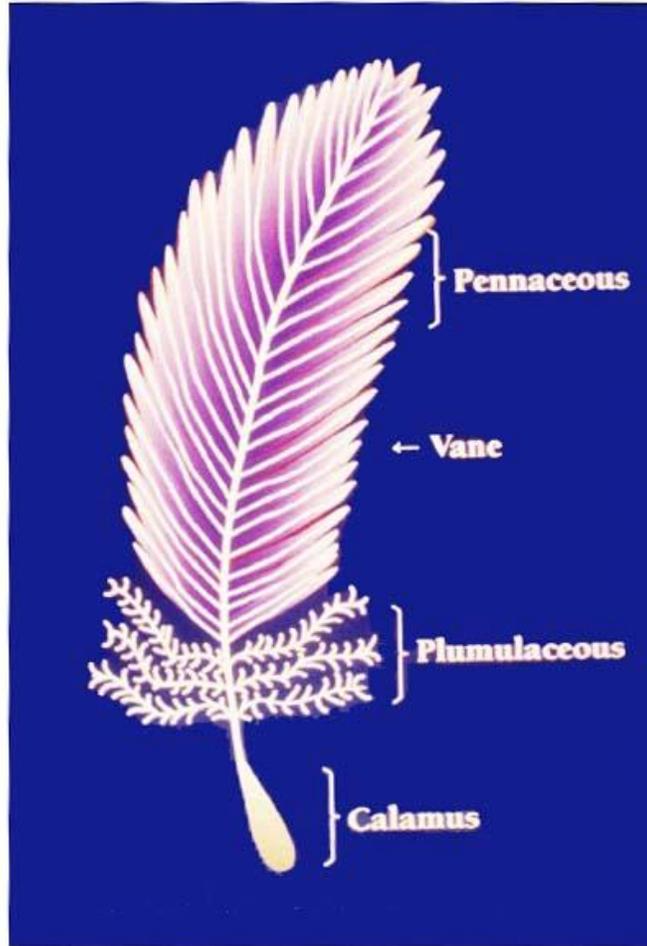
Division of Birds

Whole Feather Identification

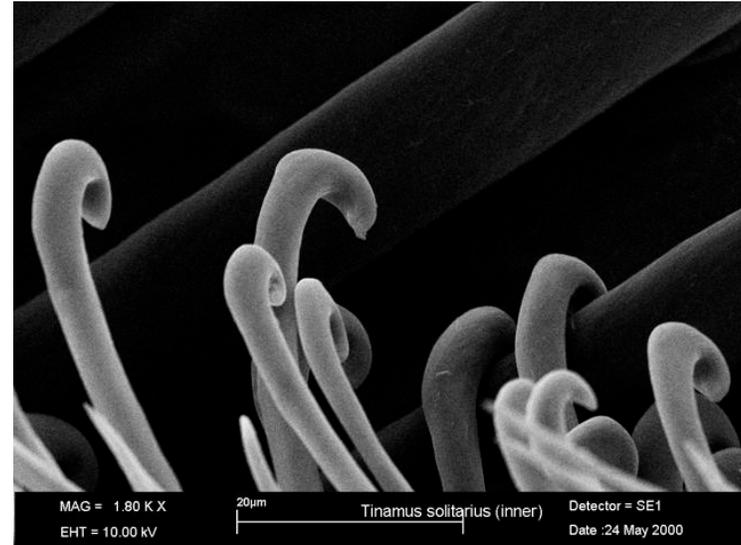


Museum Specimen: Mourning Dove

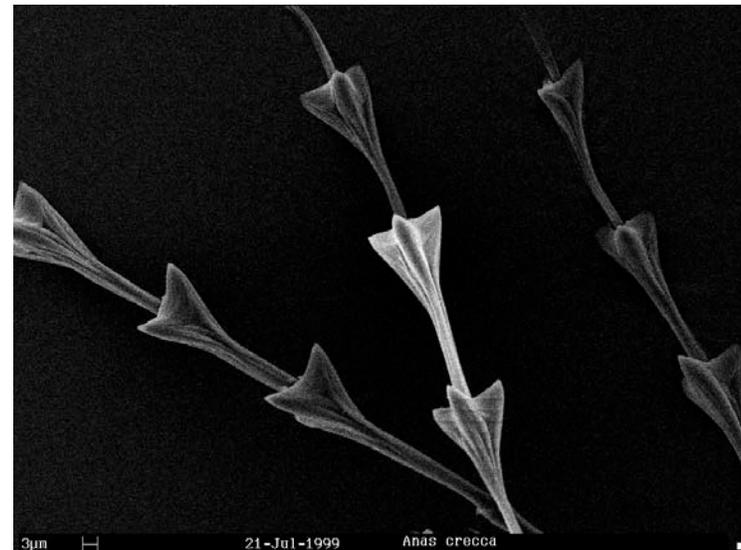
Feather Microstructure

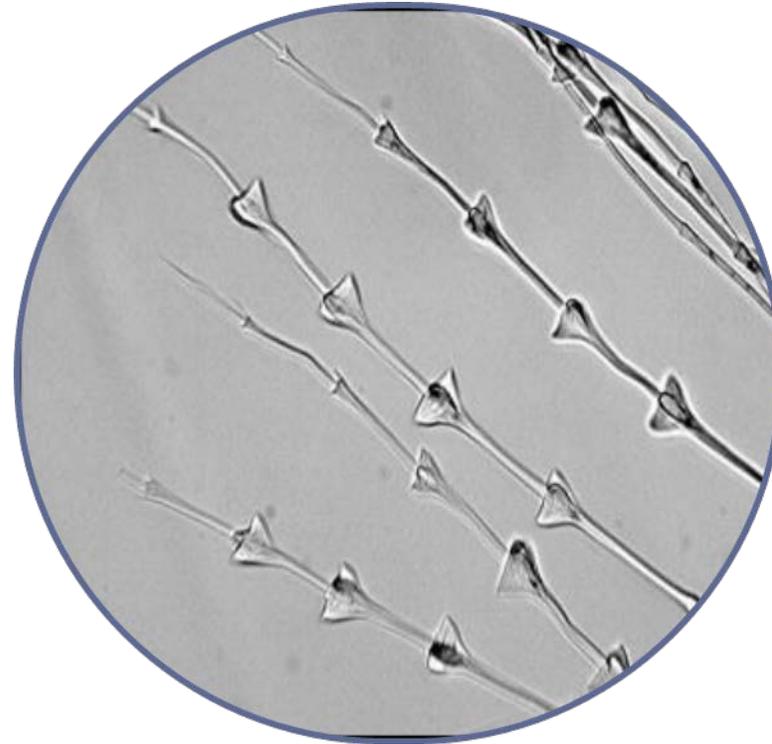
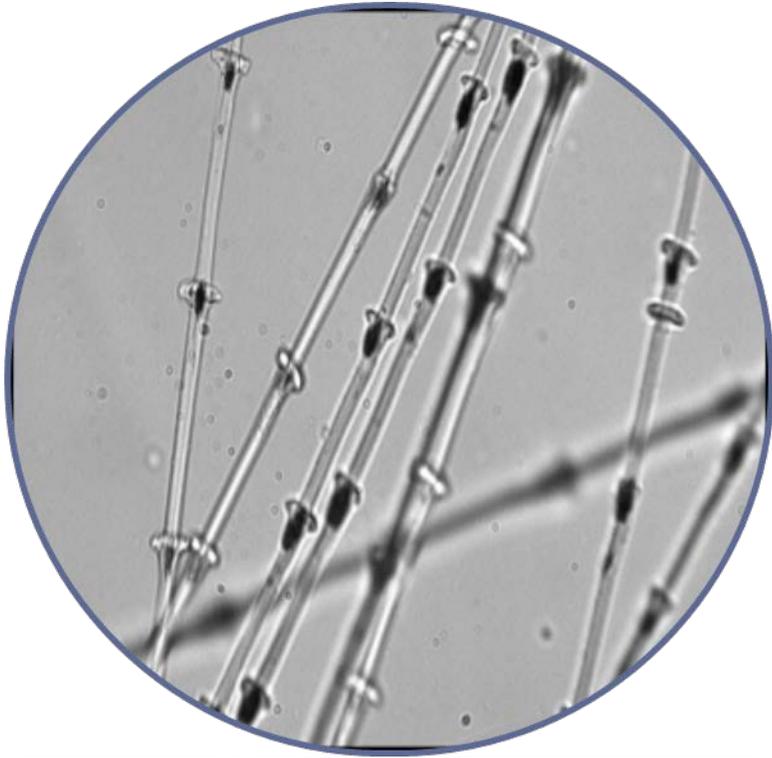


Pennaceous



Plumulaceous







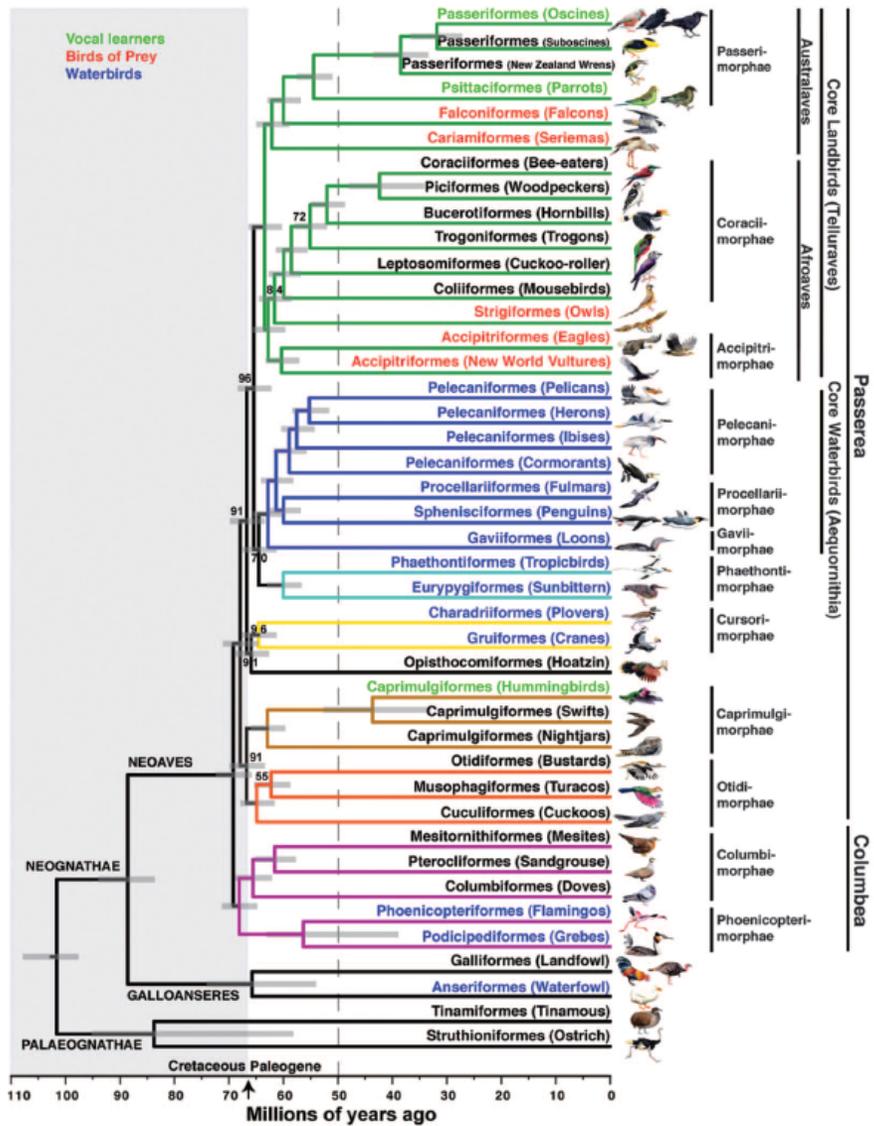
Why Accipitriformes?

- Responsible for over \$30,000 in damage per strike (2015)
- Frequently used in anthropological artifacts
- Often found in illegally imported tourist trade items



T-38 – White-tailed Hawk





Microstructure

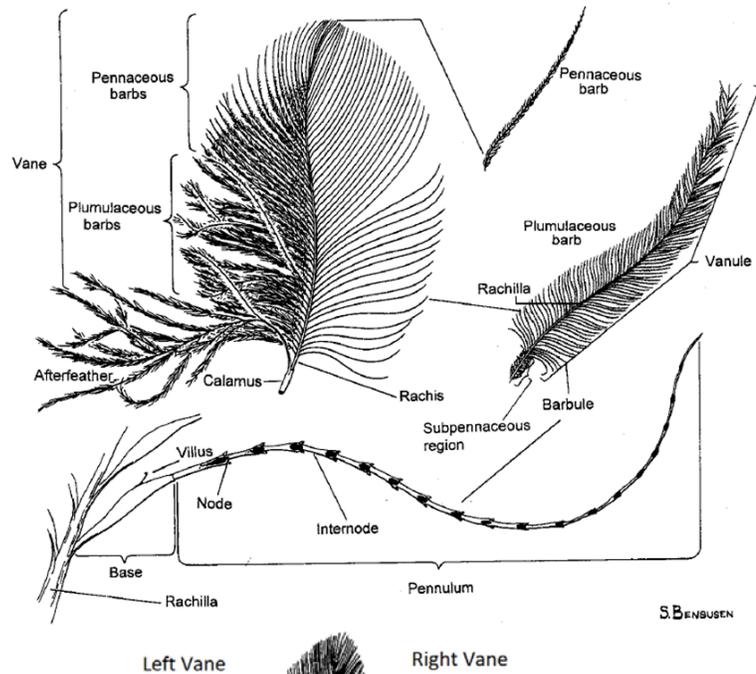
- 16 species of Accipitriformes within 3 families that occur within the United States
- 3 species of Falconiformes and 1 species of Psittaciformes to investigate recent taxonomic rearrangement



Order	Family	Species
Accipitriformes	New World Vultures (Cathartidae)	Turkey Vulture (<i>Cathartes aura</i>) Black Vulture (<i>Coragyps atratus</i>)
Accipitriformes	Osprey (Pandionidae)	Western Osprey (<i>Pandion haliaetus</i>)
Accipitriformes	Hawks, Eagles, Kites, Harriers (Accipitridae)	Red-tailed Hawk (<i>Buteo jamaicensis</i>) Swainson's Hawk (<i>Buteo swainsoni</i>) Cooper's Hawk (<i>Accipiter cooperii</i>) Sharp-shinned Hawk (<i>Accipiter striatus</i>) Common Black-Hawk (<i>Buteogallus anthracinus</i>) Harris's Hawk (<i>Parabuteo unicinctus</i>) Northern Harrier (<i>Circus cyaneus</i>) Bald Eagle (<i>Haliaeetus leucocephalus</i>) Golden Eagle (<i>Aquila chrysaetos</i>) Mississippi Kite (<i>Ictinia mississippiensis</i>) Swallow-tailed Kite (<i>Elanoides forficatus</i>) White-tailed Kite (<i>Elanus leucurus</i>) Snail Kite (<i>Rostrhamus sociabilis</i>)
Falconiformes	Falcons (Falconidae)	Peregrine Falcon (<i>Falco peregrinus</i>) American Kestrel (<i>Falco sparverius</i>) Crested Caracara (<i>Caracara cheriway</i>)
Psittaciformes	Parrots (Psittacidae)	Monk Parakeet (<i>Myopsitta monachus</i>)

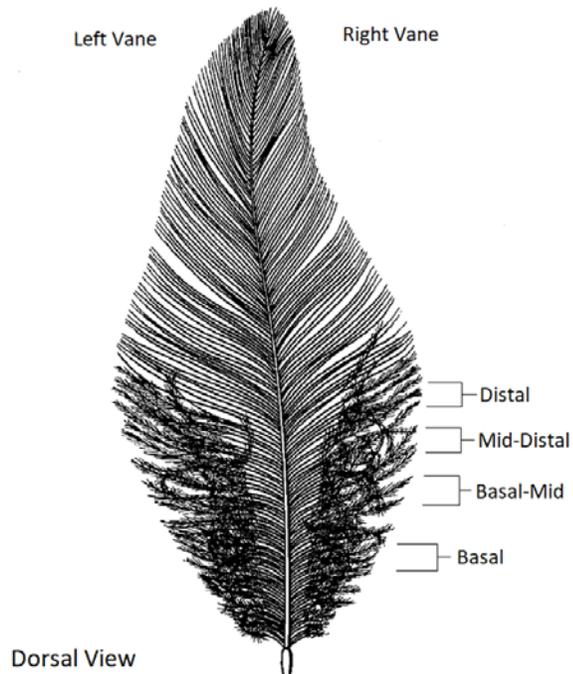
Materials and Methods

- Barbs sampled from upper-left breast feathers
- 20 total species
- 3 individuals per species
- 4 barbs per individual
- 40 measurements per barb
- 9,600 total measurements



Left Vane

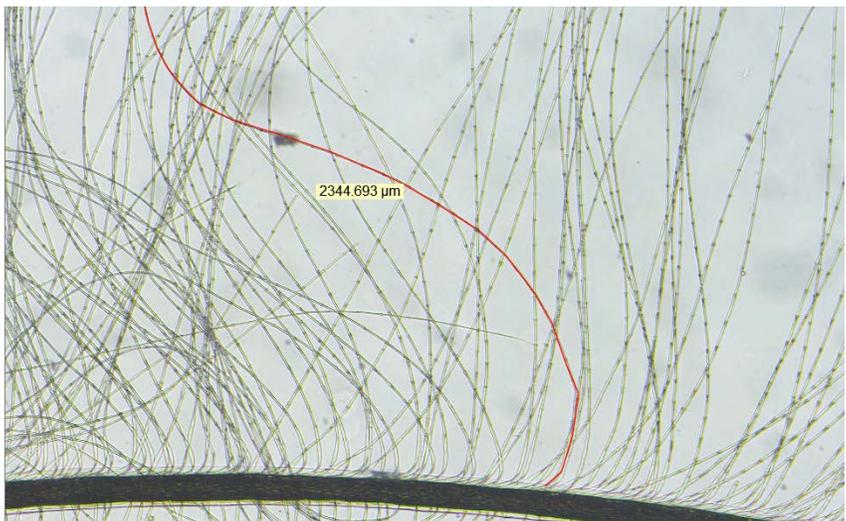
Right Vane

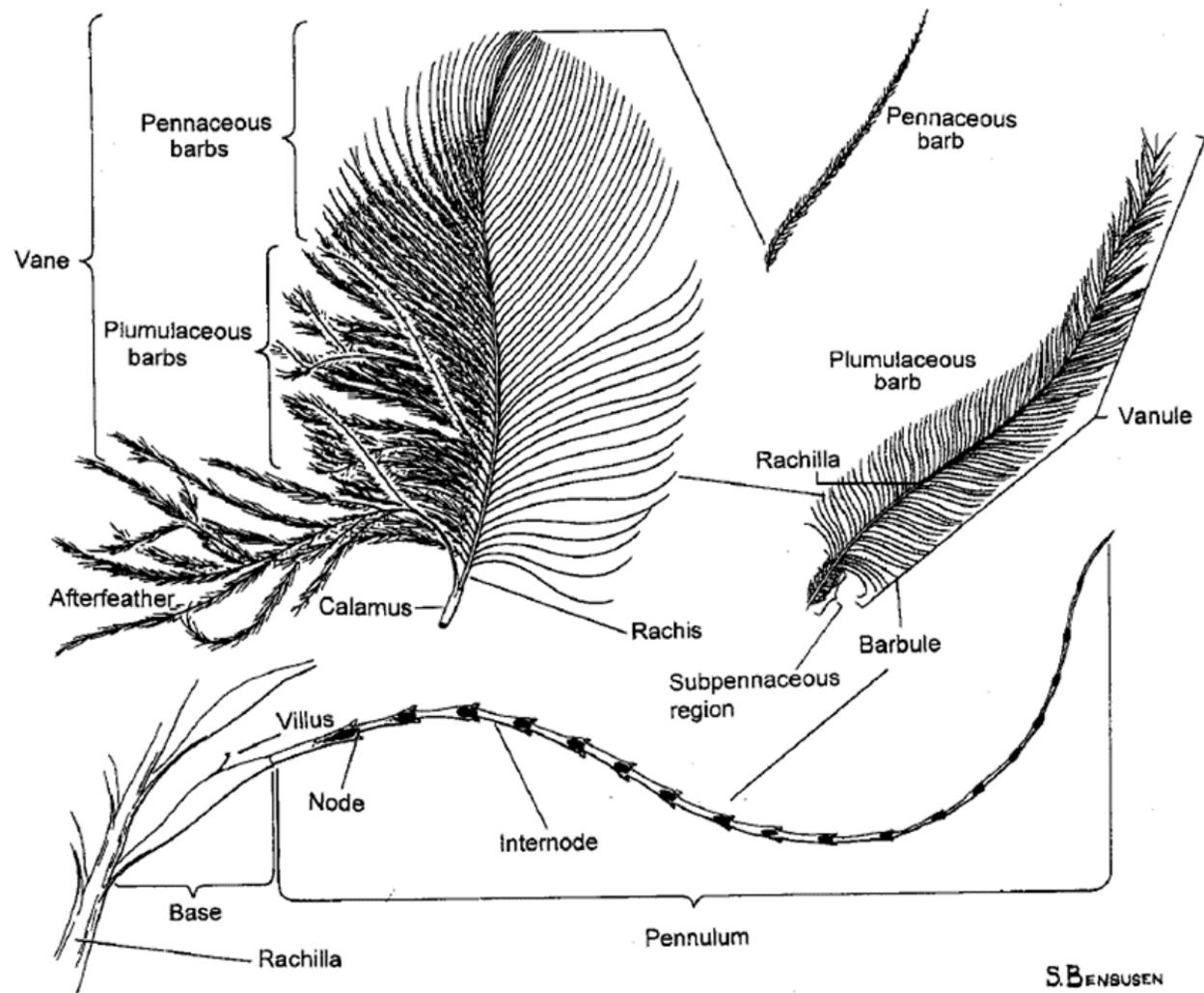


Dorsal View

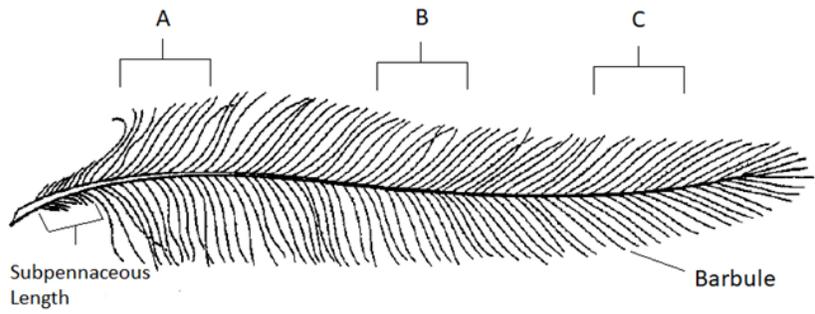
Microslide Preparation and Analysis

- Permanent microslides prepared following methods by Laybourne and Dove (1994)
- Microslides studied using a Leica[®] DM750 comparison light microscope at 50x, 100x, 200x, 400x
- Photomicrographs of barbules were taken with a Leica[®] DFC290 HD camera
- Measurements (μm) were made on photomicrographs using the 'Manual Measurements' module in Leica Application Suite[®]

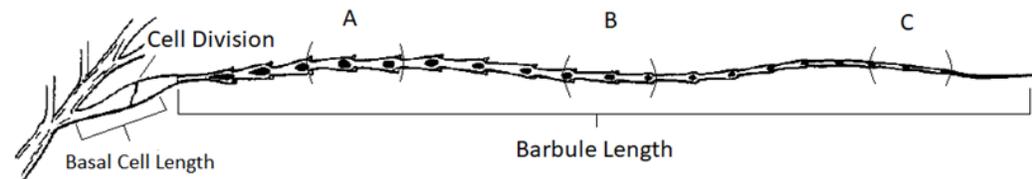




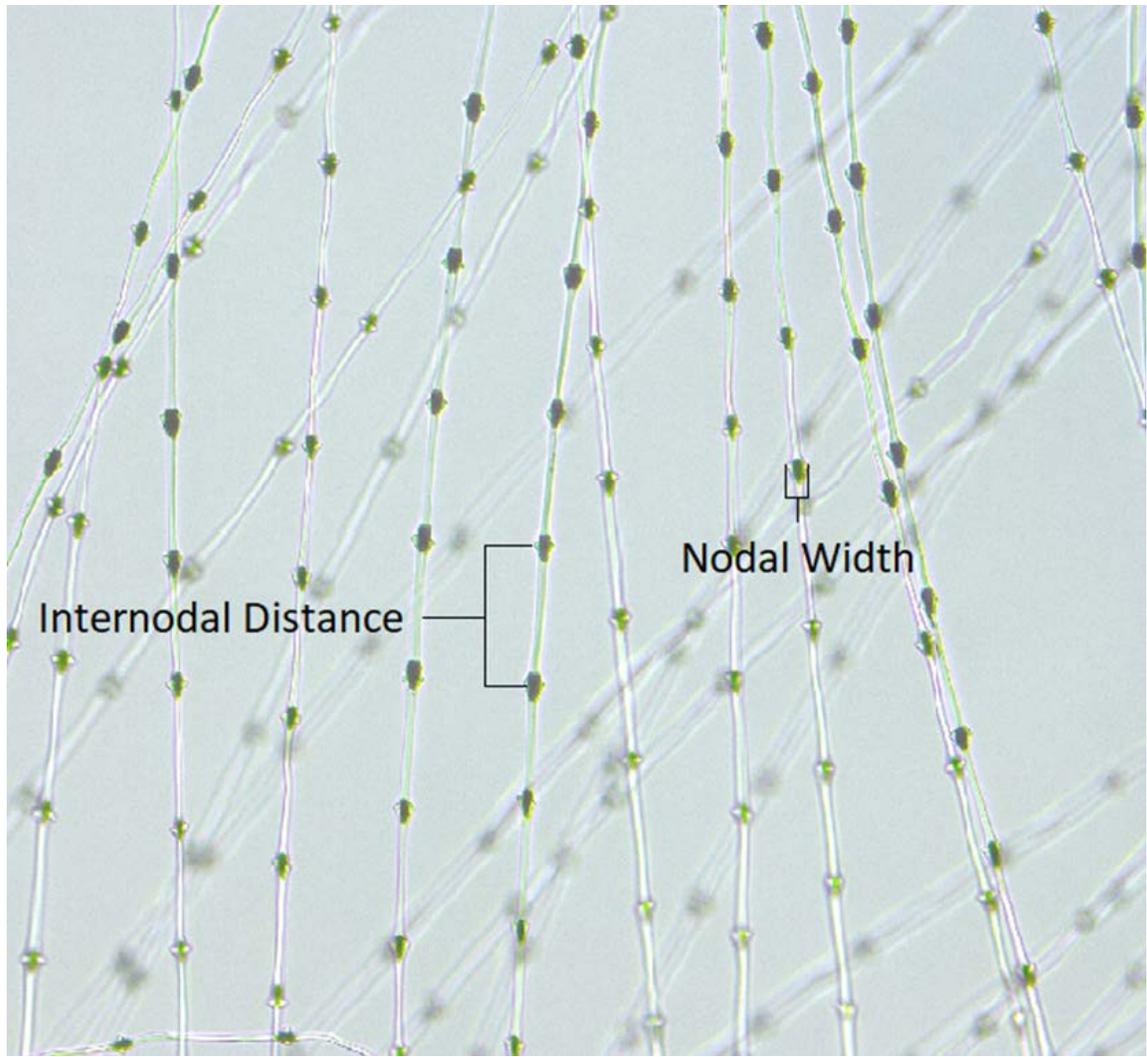
S.B. ENSUSEN



Barb - Subpennaceous length. Barb section: Basal (A), Mid (B), and Distal (C).



Barbule - Barbule length, Basal cell length, Barbule sections: Basal (A), Mid (B), and Distal (C).



Quantitative Analysis

Principal Component Analysis (PCA)

Characters tested for significance using analysis of variance (ANOVA)

P values adjusted using a Holm-Bonferroni stat correction

Tukey's test of honest significant differences used to identify significant differences in groups with multiple comparisons

Qualitative Analysis



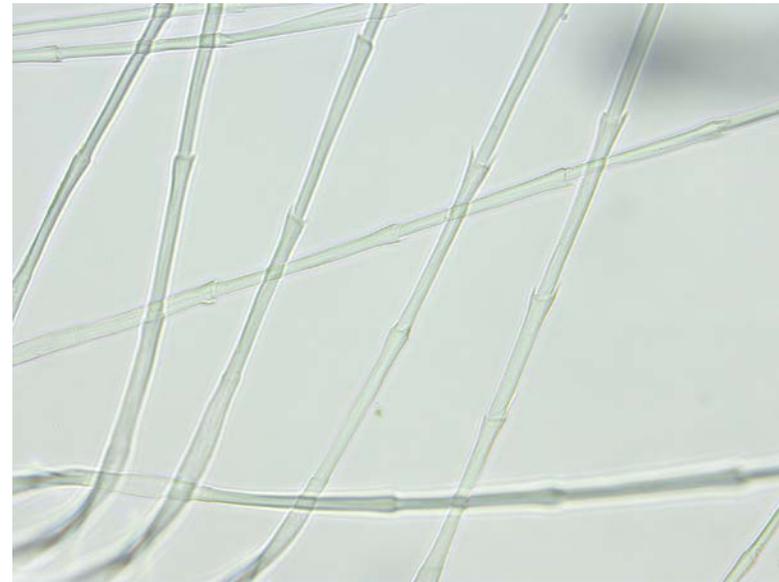
Stippled – Common Black Hawk (*Buteogallus anthracinus*)



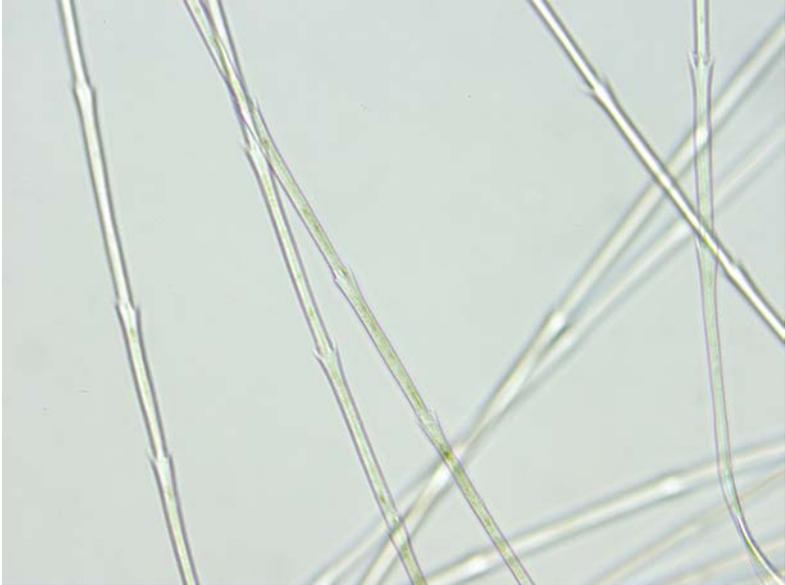
Spotted – White-tailed Kite (*Elanus leucurus*)



Oval – American Kestrel (*Falco sparverius*)



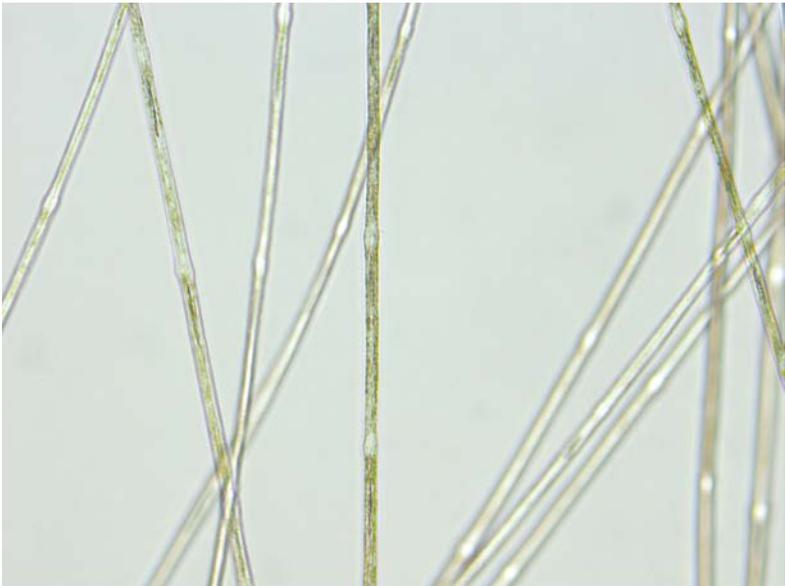
Absent – Swallow tailed Kite (*Elanoides forficatus*)



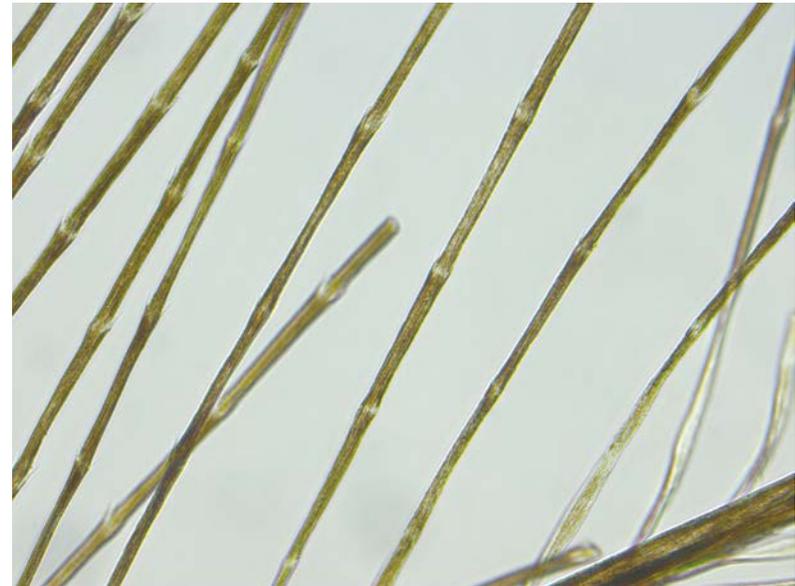
Intensity 1 (0-25%) – Red-tailed Hawk (*Buteo jamaicensis*)



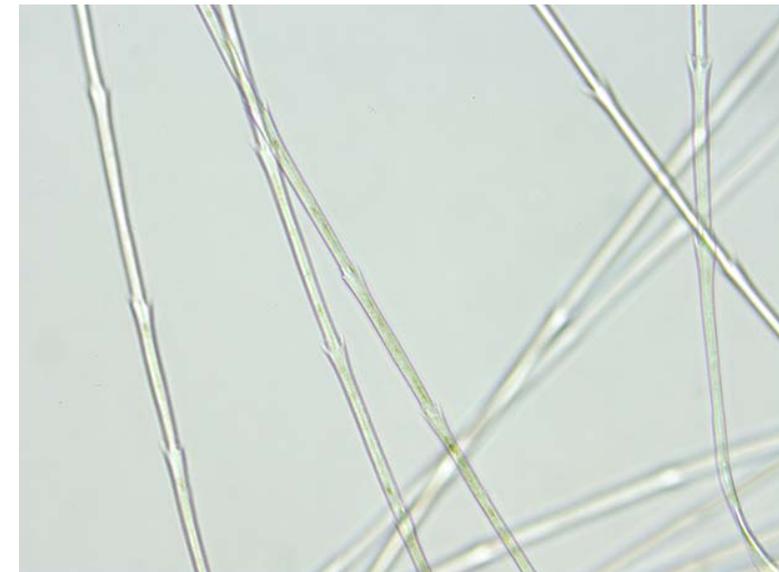
Intensity 2 (25-50%) – Turkey Vulture (*Cathartes aura*)



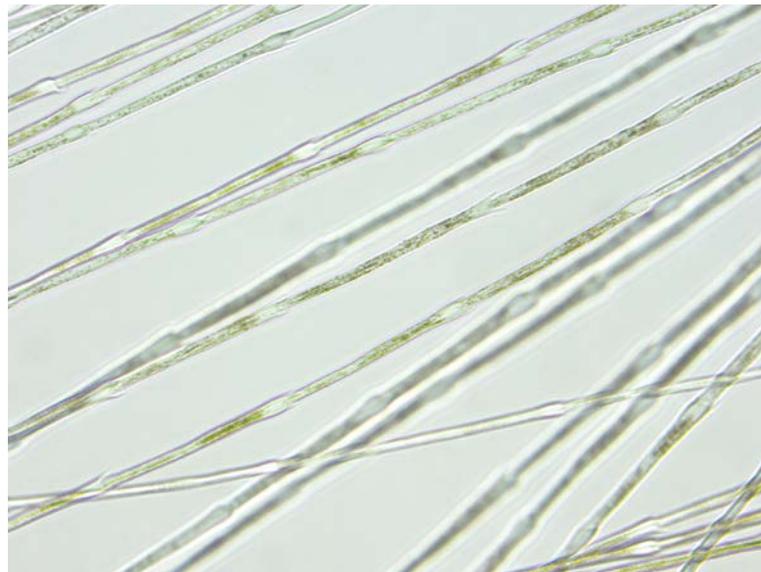
Intensity 3 (50-75%) – Bald Eagle (*Haliaeetus leucocephalus*)



Intensity 4 (75-100%) – Black Vulture (*Coragyps atratus*)



Light – Red-tailed Hawk (*Buteo jamaicensis*)



Medium – Mississippi Kite (*Ictinia mississippiensis*)



Heavy – Bald Eagle (*Haliaeetus leucocephalus*)

Quantitative Results – Hawks, Eagles, Vultures, Kites

- Two principal components that account for 41.66% of observed interspecific variation

PC1 – 24.64% Eigenvalue: 2.67	PC2 – 17.02% Eigenvalue: 2.22
Average # Nodes/Barbule = -0.919	Nodal Abundance (Mid) = 0.676
Barbule Length (Mid) = 0.817	Nodal Abundance (Base) = 0.645
Basal Nodal Width (Distal) = -0.775	Subpennaceous Length = 0.576
Basal Nodal Width (Mid) = -0.730	Mid Nodal Width (Distal) = 0.550
Barbule Length (Base) = 0.698	Nodal Abundance (Distal) = 0.424
Mid Internodal Distance (Mid) = 0.683	Basal Nodal Width (Base) = 0.515
Barbule Length (Distal) = 0.682	Barbule Length (Distal) = 0.510
Distal Internodal Distance (Mid) = 0.631	Mid Nodal Width (Mid) = 0.509
Nodal Abundance (Base) = -0.617	Basal Nodal Width (Mid) = 0.495
Basal Internodal Distance (Distal) = -0.560	Basal Internodal Distance (Mid) = 0.477
Basal Internodal Distance (Mid) = -0.512	Basal Nodal Width (Distal) = 0.404
Distal Internodal Distance (Distal) = 0.505	Distal Nodal Width (Base) = 0.403
Mid Nodal Width (Distal) = -0.473	Barbule Length (Mid) = 0.402
Subpennaceous Length = -0.445	Distal Nodal Width (Mid) = 0.377
Nodal Abundance (Distal) = 0.424	Distal Internodal Distance (Base) = 0.366
Distal Internodal Distance (Base) = 0.389	Barbule Length (Base) = 0.364
Basal Internodal Distance (Base) = 0.381	
Mid Internodal Distance (Distal) = 0.369	
Mid Nodal Width (Base) = 0.361	
Mid Nodal Width (Mid) = -0.319	

Quantitative Results - Falcons/Parrots

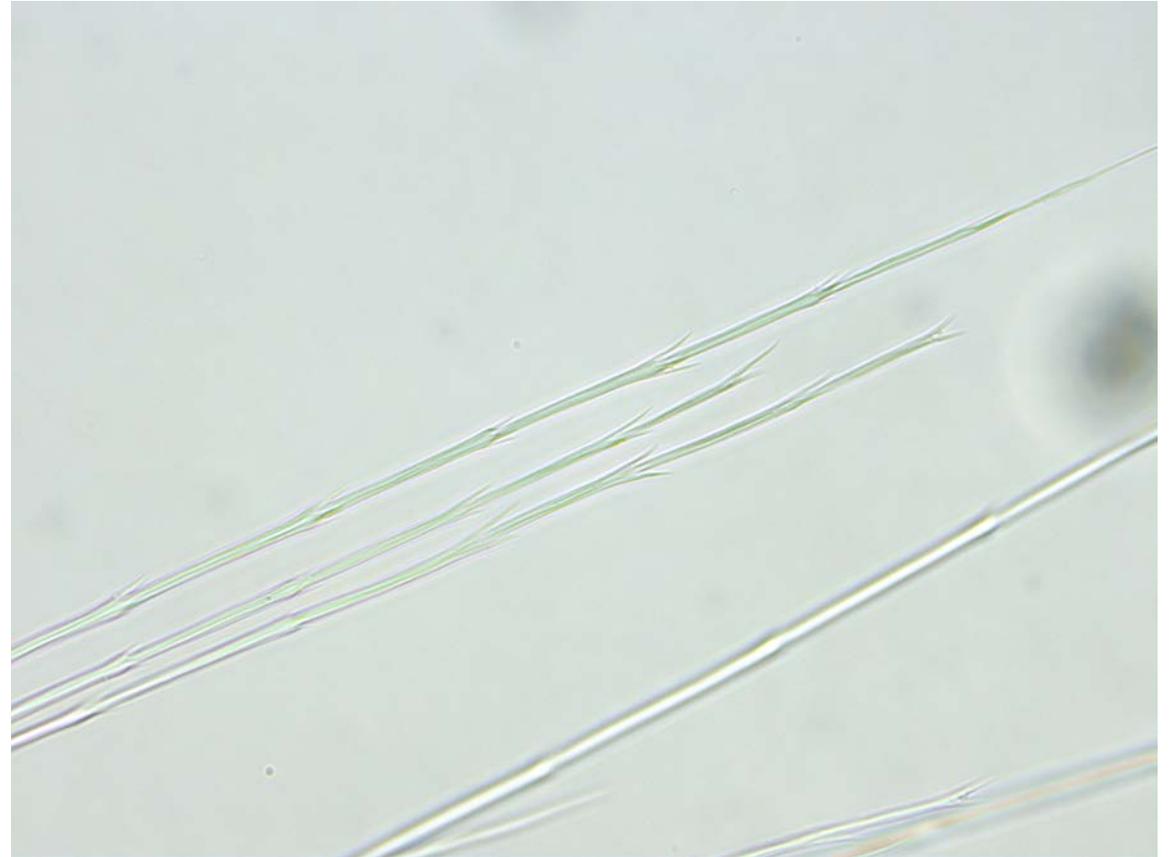
- Two principal components that account for 56.2% of the observed interspecific variation

PC1 – 34.3% Eigenvalue: 3.15	PC2 – 21.9% Eigenvalue: 2.52
Nodal Abundance (Mid) = 0.891	Basal Nodal Width (Base) = -0.776
Barbule Length (Mid) = 0.857	Basal Nodal Width (Distal) = -0.759
Barbule Length (Distal) = 0.805	Distal Internodal Distance (Mid) = 0.733
Nodal Abundance (Distal) = 0.786	Basal Nodal Width (Mid) = -0.704
Subpennaceous Length = -0.760	Distal Internodal Distance (Distal) = 0.616
Barbule Length (Base) = 0.720	Basal Cell (Distal) = 0.615
Mid Internodal Distance (Distal) = 0.709	Mid Nodal Width (Base) = -0.604
Distal Nodal Width (Mid) = 0.680	Average # Nodes/Barbule = -0.557
Basal Internodal Distance (Distal) = 0.644	Basal Cell (Mid) = 0.544
Distal Nodal Width (Distal) = 0.622	Basal Cell (Base) = 0.517
Distal Nodal Width (Base) = 0.620	Mid Nodal Width (Mid) = -0.517
Mid Nodal Width (Distal) = 0.613	Mid Internodal Distance (Mid) = 0.503
Nodal Abundance (Base) = 0.600	Distal Internodal Distance (Base) = 0.443
Mid Nodal Width (Mid) = 0.595	Mid Internodal Distance (Distal) = 0.440
Average # Nodes/Barbule = -0.588	Mid Nodal Width (Distal) = -0.414
Basal Nodal Width (Mid) = 0.577	Subpennaceous Length = 0.396
Mid Nodal Width (Base) = 0.550	Basal Internodal Distance (Mid) = 0.358
Basal Internodal Distance (Mid) = 0.541	Barbule Length (Mid) = 0.345
Mid Internodal Distance (Mid) = 0.529	Barbule Length (Distal) = 0.338
Basal Internodal Distance (Base) = 0.522	Barbule Length (Base) = 0.322
Mid Internodal Distance (Base) = 0.503	
Basal Nodal Width (Base) = 0.476	
Distal Internodal Distance (Distal) = 0.371	
Basal Cell (Mid) = -0.367	

Results - Qualitative Analysis - Osprey

Osprey Characteristics:

1. Internodal pigment is heavily stippled at base of barbule, absent towards distal end
 2. Pigment intensity 1
 3. Short barbule length
 4. Spines at nodes present on more than 75% of each barbule
- Osprey have shortest barbules and most spines of any species studied



Accipitridae - Northern Harrier

Harrier Characteristics:

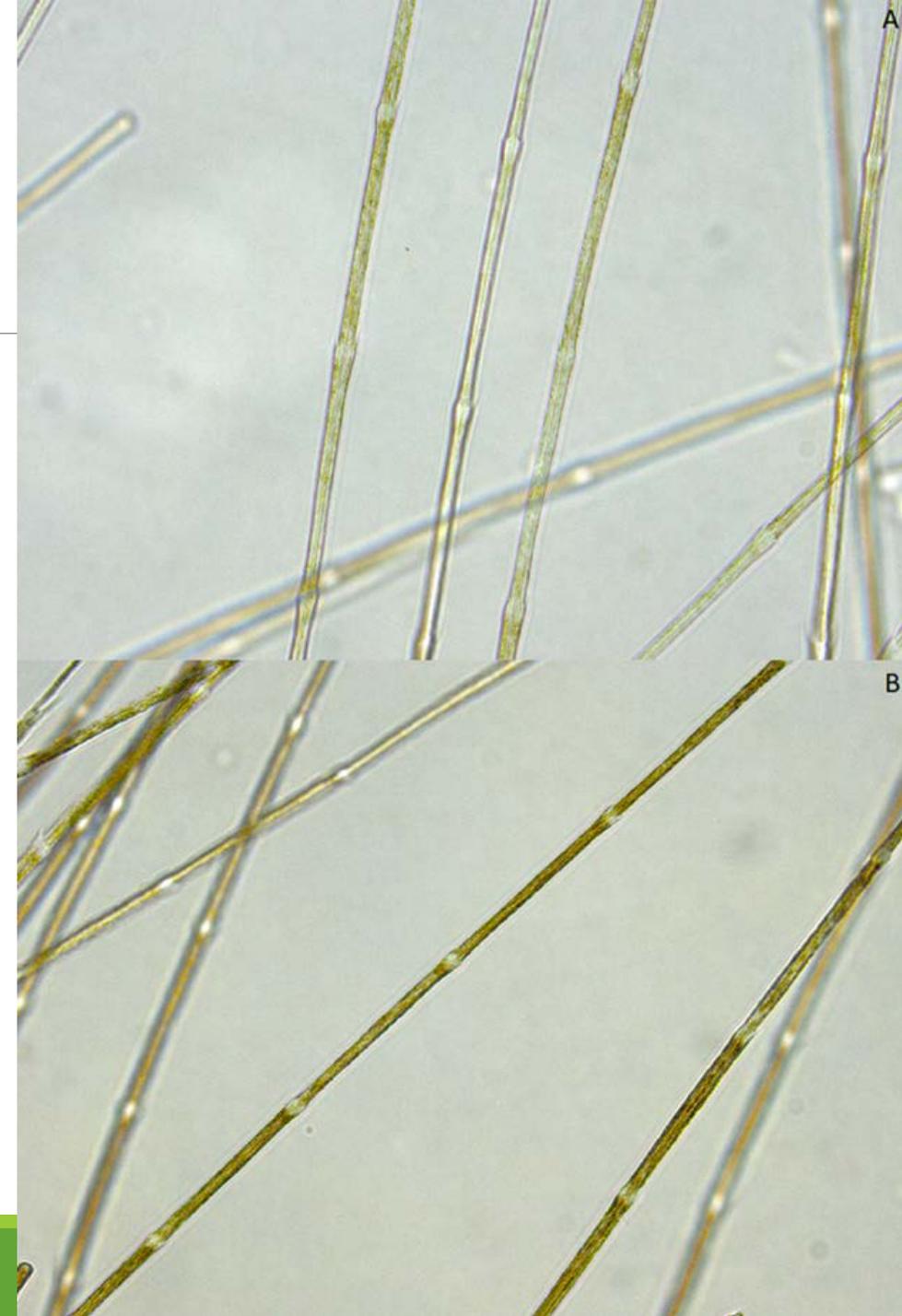
1. Internodal pigment is medium at base of barb, becomes lighter towards distal end
 2. Pigment intensity 1-2
 3. Long barbule length
 4. Low # nodes/barbule
- Northern Harrier (bottom) has longest barbules and lowest # nodes/barbule of any species studied



Cathartidae - Vultures

Vulture Characteristics:

1. Heavy stippled internodal pigment, evenly distributed along barbules
 2. Pigment intensity is light at base (intensity 1-2) and becomes darker towards distal end (intensity 2-4)
- Black Vulture (bottom) has much darker pigment than Turkey Vulture (top)



Accipiter Hawks

Accipiter Characteristics:

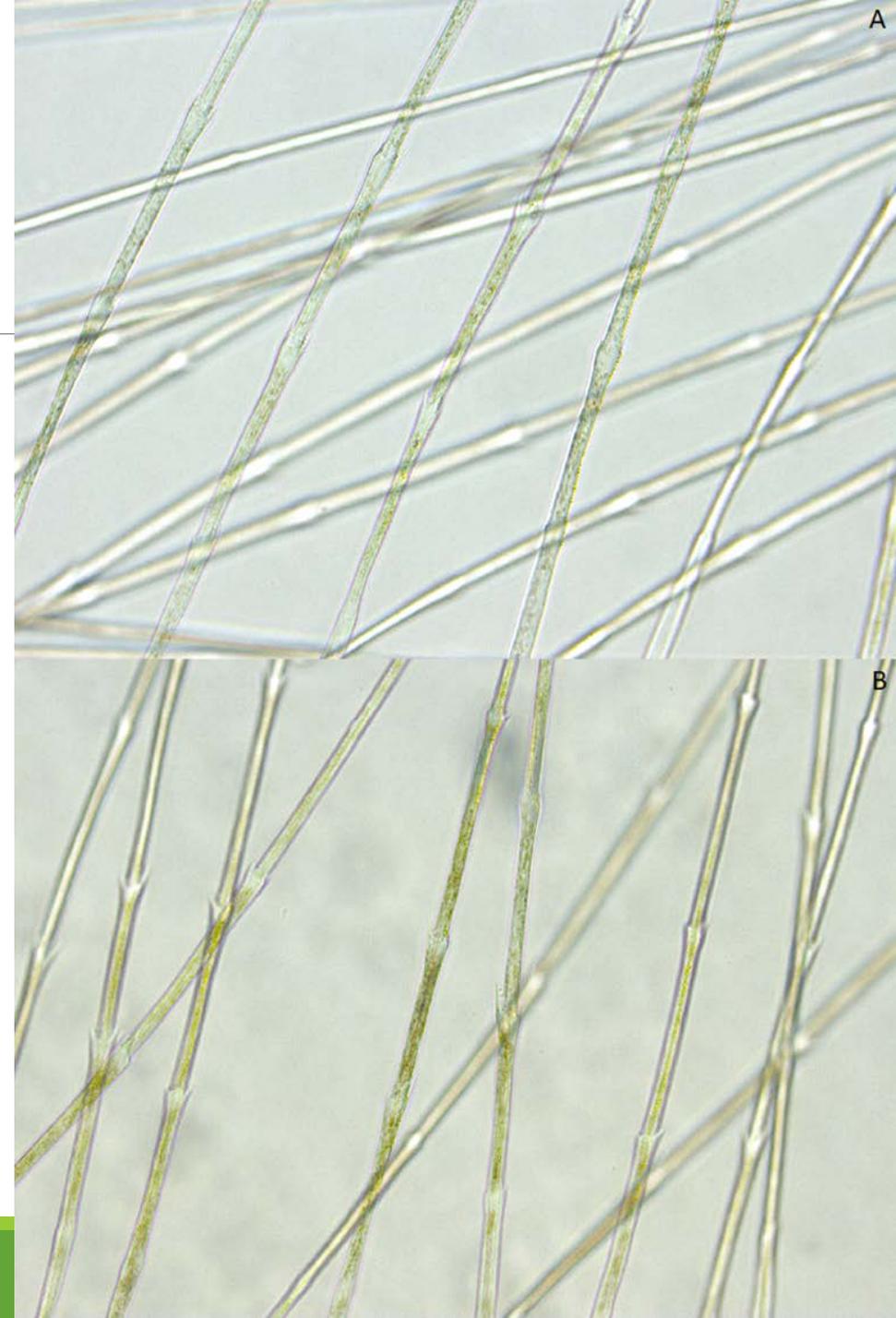
1. Heavily stippled internodal pigment is dark at base of barb (intensity 2-3) and becomes light towards distal end (intensity 1-2)
 2. Barbule length shorter than all other hawk genera studied
 3. Highest # nodes/barbule of all hawk genera studied
- *A. striatus* (bottom) has darker internodal pigment than *A. cooperii* (top) at base of barb



Buteo Hawks

Buteo Characteristics:

1. Heavily stippled internodal pigment dark at base (intensity 2-3) becomes lighter towards distal end (intensity 1-2)
 2. Longest barbule length of all hawk genera studied
 3. Lower # nodes/barbule than *Accipiter* hawks
- *B. swainsoni* (bottom) has slightly darker pigment than *B. jamaicensis* (top). *B. swainsoni* has lower # nodes/barbule at basal barbule than *B. jamaicensis*.

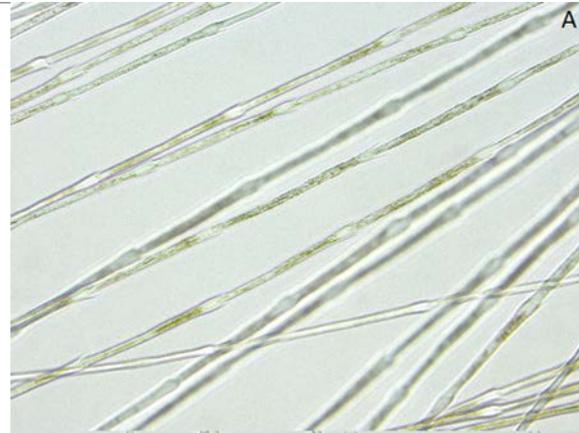


Kites

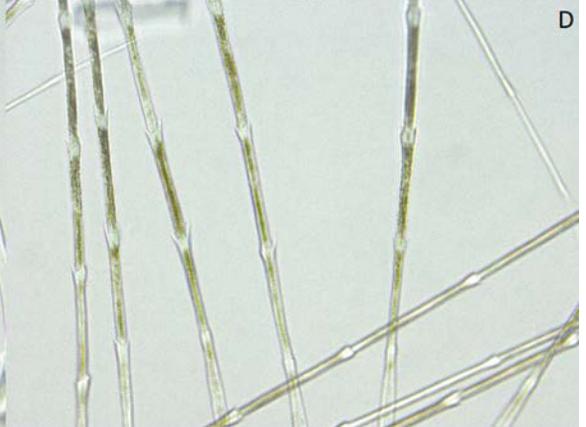
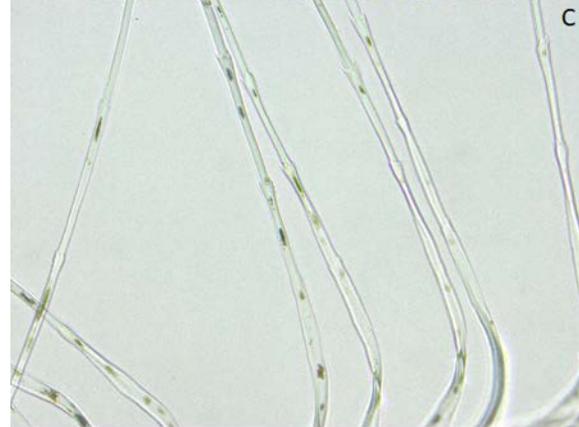
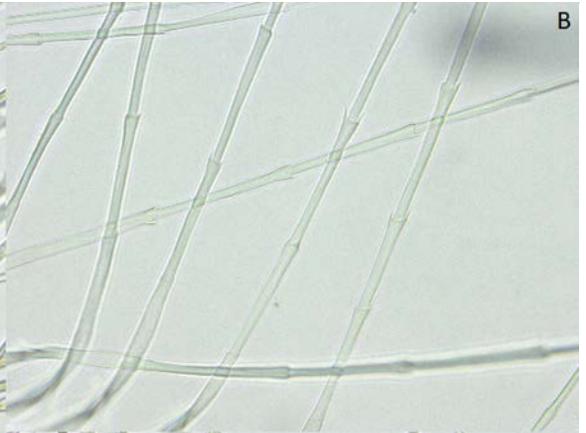
Kite Characteristics:

1. All kite species were distinguishable by pigment pattern
 2. Kites were indistinguishable from other groups based on measurements alone
- *Elanus* was distinguishable from other kite species by long barbules

Mississippi Kite (*Ictinia mississippiensis*)



Swallow-tailed Kite (*Elanoides forficatus*)



White-tailed Kite (*Elanus leucurus*)

Snail Kite (*Rostrhamus sociabilis*)

Eagles

Eagle Characteristics:

1. Pigment is medium/heavy in internode, evenly distributed along barb (intensity 2-3)
 2. High nodal abundance on all barbules
 3. Long barbule length
 4. Long subpennaceous region length
- *H. leucocephalus* (top) has darker pigment at base of the barb than *A. chrysaetos* (bottom)



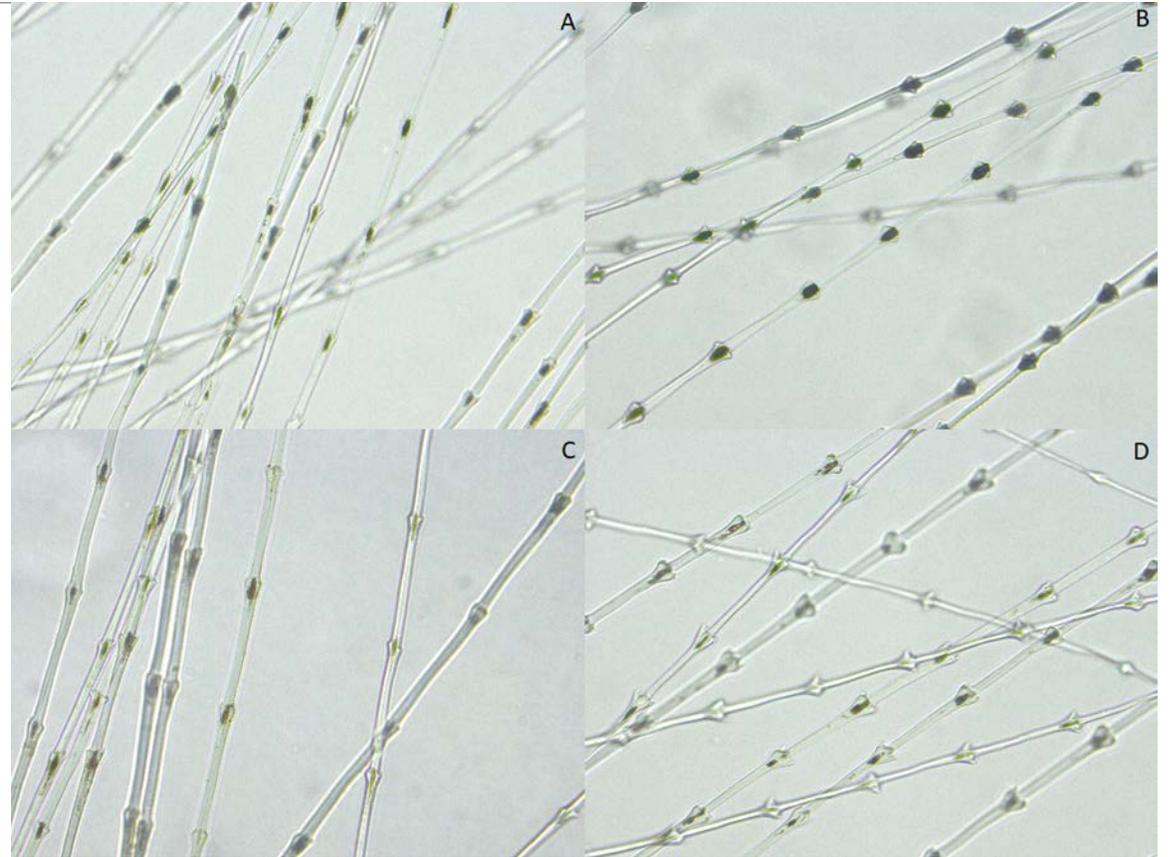
Falcons/Parakeet

Characteristics of Falcons/Parakeet:

1. Oval pigment at nodes, evenly distributed along barb
 2. Internodal pigment medium/heavy when present (intensity 4)
 3. Wide nodes
 4. Short barbules
- Falcon genera are all distinguishable by pigment pattern
 - *M. monachus* has significantly wider nodes at basal and mid barbules than *Falco* species, but are otherwise indistinguishable

Peregrine Falcon (*Falco peregrinus*)

American Kestrel (*Falco sparverius*)



Crested Caracara (*Caracara cheriway*)

Monk Parakeet (*Myiopsitta monachus*)

Discussion

- Overlap in measurements of many microscopic characters
- Microscopic differences at family level agreed with taxonomic placement (i.e. Falcon/Parakeet)
- Microscopic characters were useful, should continue to be used for plumulaceous microstructure studies
- Average # nodes/barbule, barbule length, basal barbule internodal distance and nodal width were strongest characters found for separating Accipitriformes
- Nodal abundance, barbule length and nodal width were strongest characters found for separating Falconiformes and Psittaciformes
- Future studies should continue to use Leica Application Suite[®] software for precise measurements

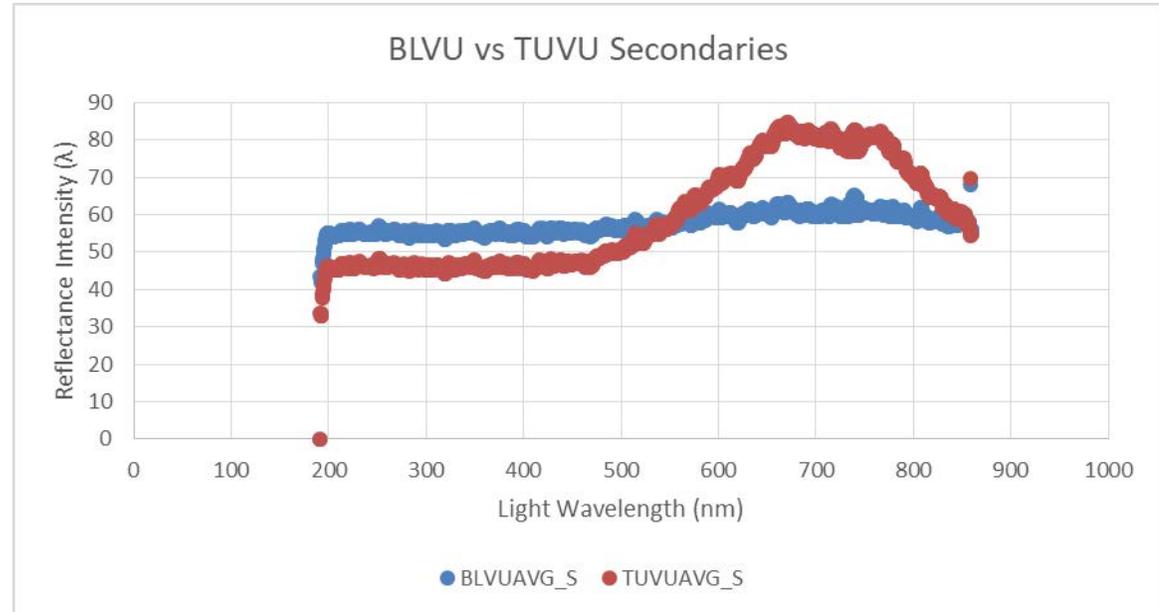
Spectrophotometry

- Feather microstructure is rarely useful for identifying at the species level
- Flight and body feathers can be used to identify birdstrike remains when a nearly complete feather is available, but accurate identification is challenging with partial feather fragments
- I explored spectrophotometry as a method for identifying species from the same genus that are indistinguishable based on microstructure



What is Spectrophotometry?

- Quantitatively measure color
- Measures which wavelengths of light are absorbed and reflected by a surface
- Reflectance peaks directly correlate to the brightness or intensity of color displayed by bird feathers



Materials and Methods - Species Pairs

Top – Black Vulture (*Coragyps atratus*) (left) & Turkey Vulture (*Cathartes aura*) (right)

Middle – Red-tailed Hawk (*Buteo jamaicensis*) (left) & Swainson's Hawk (*Buteo swainsoni*) (right)

Bottom – Cooper's Hawk (*Accipiter cooperii*) (left) & Sharp-shinned Hawk (*Accipiter striatus*) (right)





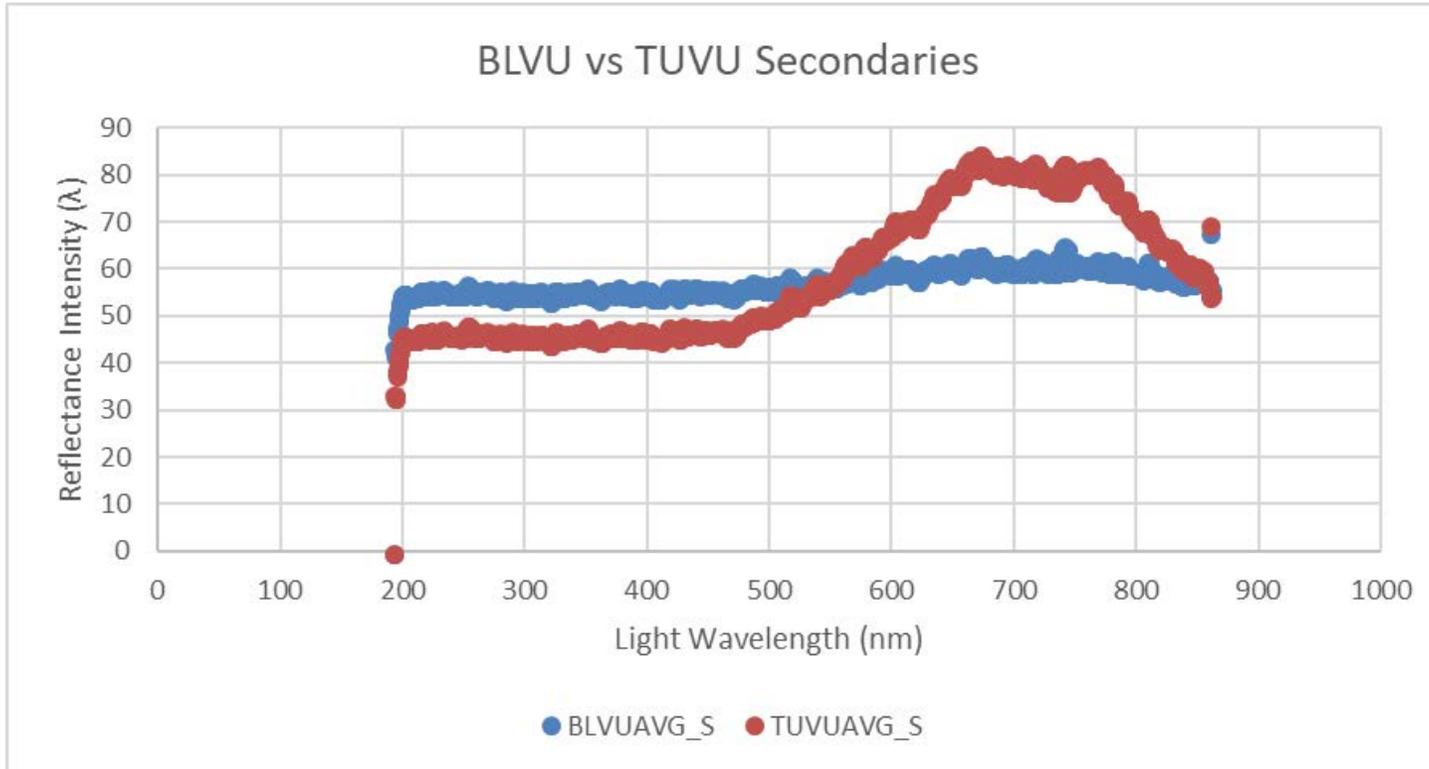
Materials and Methods

- Reflectance spectra were measured with an Ocean Optics[®] S2000 spectrophotometer with an AIS[®] Model DT 1000 fiber optic halogen light source
- Black housing box used to standardize probe measurements at a 90° from a fixed distance (~1mm) on each feather

Materials and Methods

- Light intensity readings were taken at 3 standardized locations along primary feather 7, and secondary feather 3 of each specimen examined



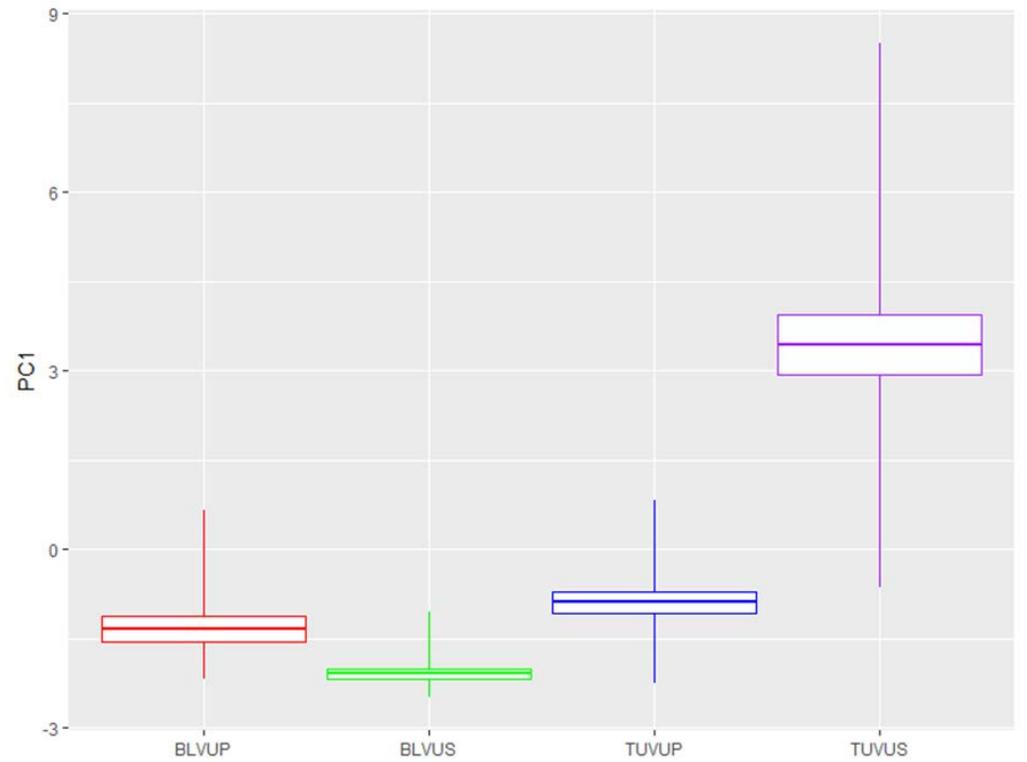


Analysis

- Intensity readings were adjusted in terms of change in intensity at certain wavelengths
- Principal Component Analysis (PCA)
- Characters tested for significance using analysis of variance (ANOVA)
- P values adjusted using a Holm-Bonferroni stat correction

Results – Vultures

PC1 – 51.58%
Eigenvalue = 2.59
550-600nm = 0.984
750-800nm = 0.982
800-850nm = -0.976
600-650nm = 0.965
500-550nm = 0.964
450-500nm = 0.898
700-750nm = -0.774
650-700nm = 0.481
300-350nm = 0.363
250-300nm = -0.321

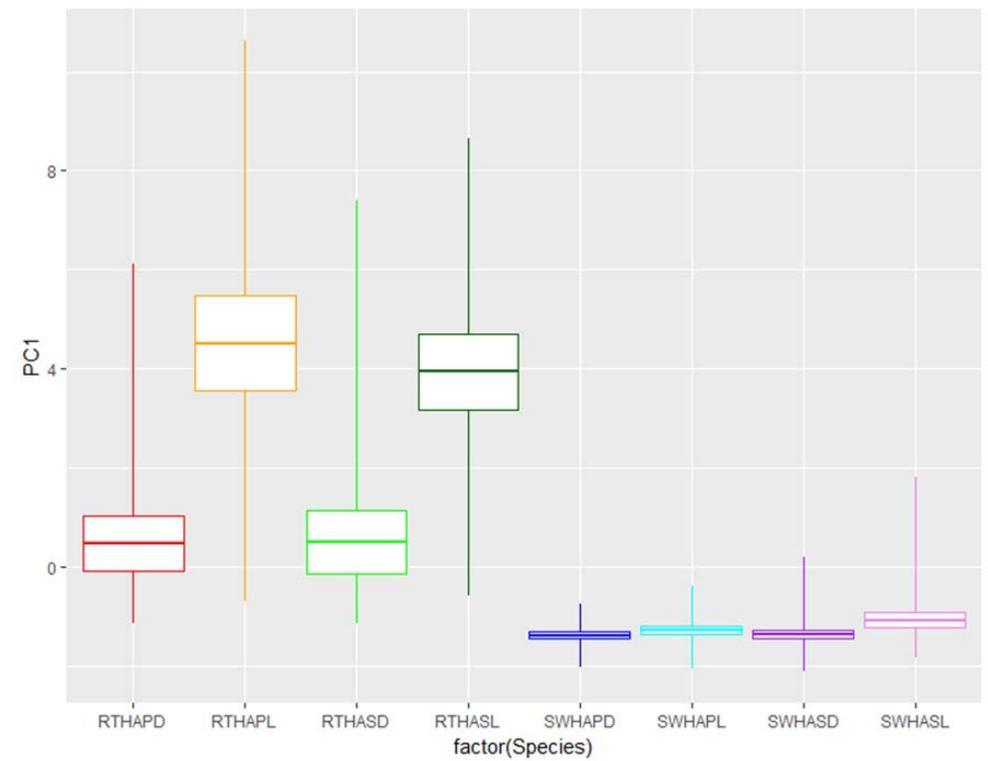


Comparison	Adjusted P Value	F Value	Degrees of Freedom
Vulture Primaries PC1	0.384	2.442	1
Vulture Secondaries PC1	< 0.001*	86.07	1



Results – *Buteo* Hawks

PC1 – 52.79%
Eigenvalue = 2.61
500-550nm = 0.983
750-800nm = -0.980
550-600nm = 0.970
800-850nm = -0.965
600-650nm = 0.961
450-500nm = 0.933
700-750nm = -0.912
400-500nm = 0.607

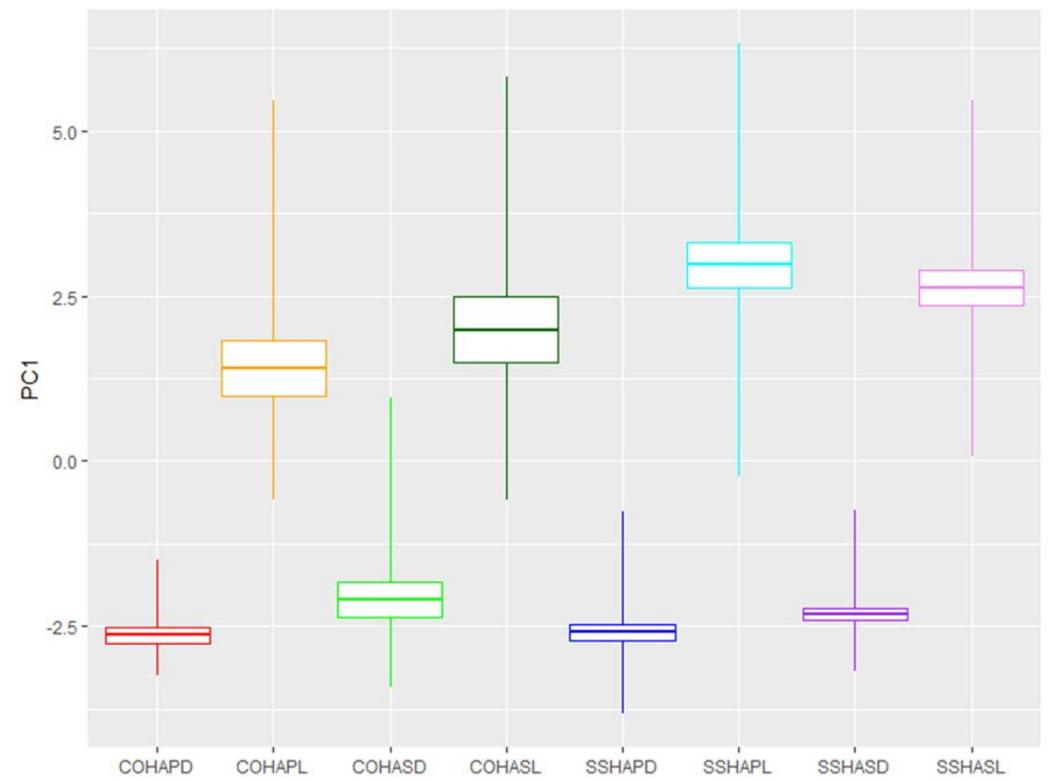


Comparison	Adjusted P Value	F Value	Degrees of Freedom
Buteo Dark Primaries PC1	< 0.001*	19.56	1
Buteo Light Primaries PC1	< 0.001*	65.76	1
Buteo Dark Secondaries PC1	0.001*	15.26	1
Buteo Light Secondaries PC1	< 0.001*	71.27	1



Results – *Accipiter* Hawks

PC1 – 59.16%
Eigenvalue = 2.77
500-550nm = 0.991
750-800nm = -0.972
700-750nm = -0.969
450-500nm = 0.964
550-600nm = 0.955
800-850nm = -0.916
600-650nm = 0.894
400-450nm = 0.836
650-700nm = -0.738



Comparison	Adjusted P Value	F Value	Degrees of Freedom
Accipiter Dark Primaries PC1	1	0.061	1
Accipiter Light Primaries PC1	0.023*	7.815	1
Accipiter Dark Secondaries PC1	0.999	0.96	1
Accipiter Light Secondaries PC1	0.657	1.553	1



Discussion

- Spectrophotometry failed to adequately differentiate subtle color differences among species pairs
- Excessive overlap in reflectance profiles makes it unlikely that these species with melanin-based feathers can be distinguished confidently using this technique
- It is possible however, that there are differences in the reflectance profiles in geographically distinct morphs of a species
- This method may be useful in identifying species with carotenoid-based plumage where reflectance peaks are more dramatic

Conclusion

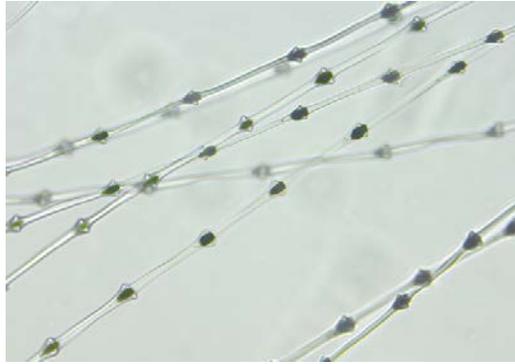
- Plumulaceous microstructure can be used to make various levels of taxonomic identifications, when enough information is available
- This study shows that microscopic study can be used to verify other forms of identification such as DNA or whole feather identification
- Future microstructure studies in these groups should include all species that occur in the US
- The morphological differences between *Accipitriformes* and *Falconiformes* support their recent phylogenetic rearrangement
- Spectrophotometry is not useful in identifying melanin-based feathers, but future studies should consider exploring its use for carotenoid-based feather identification



Smithsonian
National Museum of Natural History

Acknowledgements

- Feather Identification Lab - C. Dove, M. Heacker-Skeans, J. Whatton, N.F. Dahlan, S. Luttrell
- Smithsonian Institution - C. Milensky, G. Graves, J. Coddington
- George Mason University - D. Luther, L. Rockwood, A. Baranova, J. Clark
- Luther/Freeman Lab Members



Questions?

