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Microscopy of Feathers: A Practical Guide for Forensic Feather Identification

ABSTRACT: The identification of bird species from feather fragments is useful in many disciplines including human forensics. Feather evidence can be helpful to criminal investigations in demonstrating physical contact between clothing manufactured with down feathers, or may provide specific links to the crime scene by identifying the species or group of birds from which the feather evidence came. This guide describes the potential importance of feather evidence to criminal investigations and introduces the basic techniques of approaching the identification of birds from feather evidence in criminal cases. Photomicrographs and descriptions are provided for eight (8) Orders of birds that are commonly involved in human criminal cases with emphasis on the diagnostic microscopic characters for each Order. Characteristics of feather barbs, barbules, nodes and pigmentation patterns are described in detail with cautionary notes for similar species in each group. Details of feather topography, microslide preparation for downy (plumulaceous) feather barbs, and information on report writing, testimony, and the significance of feathers in forensic cases is discussed.

KEYWORDS: Forensics, Feathers, Microscopy, Identification, Downy Barbs

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Introduction

Interspecific variation in the microscopic characters of plumulaceous (downy) feathers was first investigated by Chandler (1916). Although his work demonstrated the importance of microscopic feather characters to avian systematics, the process of identifying birds from feather fragments has since been applied to a wide variety of studies. Some of the current applications of feather identification include: identifying birds that collide with aircraft (birdstrikes); ecological studies of prey remains; food contamination investigations; and law enforcement cases for agencies such as U.S. Customs, U.S. Fish and Wildlife Service, the National Park Service and the Federal Bureau of Investigation (FBI).

During the course of a criminal investigation, the feather evidence may be helpful in demonstrating physical contact between clothing manufactured with down feathers, or may provide specific links to the crime scene by identifying the species or group of birds from which the feather evidence came.

OBJECTIVES:

The objectives of *"Microscopy of Feathers: A Practical Guide for Forensic Feather Identification"* are two-fold:

1. To increase awareness of the potential importance of feather evidence to criminal investigations.
2. To introduce the basic techniques of approaching the identification of birds from feather evidence in criminal cases.

CAUTIONS, CONVERGENCES AND DISCLAIMERS

This manual is intended to focus on the preparation, examination, and comparison of microscopic feather evidence in a forensic setting. Photomicrographs and microscopic feather characters for selected species that may be or have been involved in criminal cases are presented and described here to provide guidance for the identification of feather fragments but are not meant to be used as a sole source of identification. Due to the great amount of variation in feather structures between species, within species and even within the same feathers, users of this manual should consult an ornithologist before assigning positive species identifications to feather samples when using only microscopic evidence.

The diagnostic microscopic characteristics described in this guide are helpful to identify groups, or Orders, of birds. Not all members of a group, nor all of the feather types or even all barbs, will have the diagnostic characters needed for identification.

Also, when incomplete feathers or small samples are all that is available for examination, it may not be possible to determine any information about the feather sample. It is always best to have multiple barbs, with many diagnostic features before confidently assigning an unknown feather sample to a group of birds.

Finally, morphological convergences do occur in plumulaceous (downy) feather structures. The microscopic structures of downy feathers of birds that occupy the same environments may have 'converged' to appear similar even though the birds are not closely related. An example of this occurs in grebes, loons, and alcids (Dove 2000). All of these birds live in aquatic environments, dive for food and have similar microscopic downy feather characteristics even though they are not closely related to each other, nor do they resemble one another in physical appearance. Convergences may also occur in other groups but are not typical of birds that are involved in criminal investigations.

LAWS PROTECTING BIRDS

All species of birds native to North America are considered migratory and therefore are protected by the Migratory Bird Treaty Act (MBTA). This treaty also **prohibits the possession** of feathers and other bird parts (i.e. nests, talons, feathers, etc.) of native species without permission. Therefore, any feathers or bird parts found in a criminal investigation are probably illegal and should be suspect. The only exceptions involve feathers taken from introduced non-protected species or legally-hunted waterfowl or other migratory gamebirds, which may be possessed by hunters. This prohibition extends to molted feathers and to feathers taken from road- or window-killed birds. Individuals or institutions wishing to use bird feathers, bones, bird parts, or whole specimens for educational or research purposes must apply for permits from the U.S. Fish and Wildlife Service and their state wildlife or natural resource agency. For more information see http://www.fws.gov/migratorybirds/mbpermits.html

Some species are also protected by additional statutes, such as the Endangered Species Act (ESA) and the Bald and Golden Eagle Protection Act (BGEPA). The list of North American birds protected by the MBTA can be found at http://www.fws.gov/migratorybirds/RegulationsPolicies/mbta/mbtintro.html

Bird Classification

Birds are vertebrates (Kingdom: Chordata) within the Class Aves. There are approximately 23 Orders, 142 Families and 10,000 species of birds. Birds are classified and named using the Linnaean system of binomials (*Genus species*) to assign Latin names to each species. Although common names are more familiar to the general public, it is best to use scientific names in addition to common names for clarity and consistency. When a bird group ends with *-formes*, the word is an indicator that it is the

bird Order while if it ends with *–dae*, it is an indicator that it is the bird Family. Figure 1 explains the hierarchical classification scheme for naming birds using the Peregrine Falcon (*Falco peregrinus*) as an example.

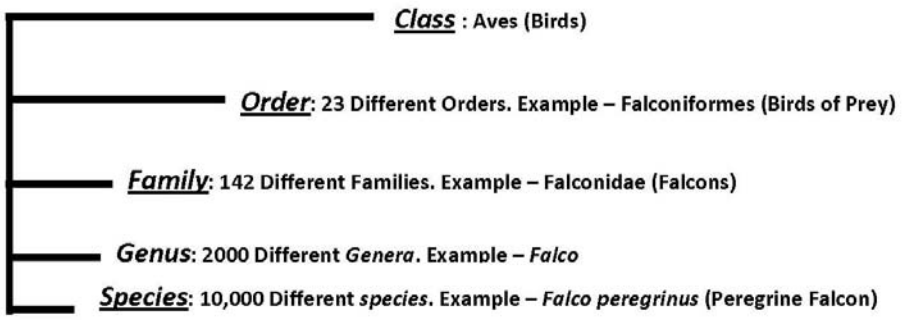

Figure 1. Hierarchy of bird taxonomy using the Peregrine Falcon (Falco peregrines) as an example of how birds are classified.

The goal of identifying feather fragments is to reach the lowest possible taxonomic level (species). Typically, birds that are closely related to each other have similar microscopic feather structures. For example, most members within the same Family Falconidae (falcons) will have similar microscopic feather characteristics. Therefore, it is very difficult to identify birds to the species level using only microscopic characters. It is best to use microscopic characteristics in combination with whole feathers that have color, texture and patterns that can be matched to reference specimens in a museum collection, and to corroborate the final identification with geographic distributions.

Feather Topography

Some of the different types of feathers are illustrated in Figure 2 and include: contour and flight feathers, semi-plumes, and true down. The afterfeather, or secondary structure, is found in some species and is attached to some contour feathers.

It is important to know what type of feather you are examining when you compare it to a reference collection. Contour feather down is fluffy in appearance and is located at the base of the feather. Rectricies and remiges (wing and tail, or flight feathers) also tend to have a small amount of down at the very base of the feather but these barbs may not always have the diagnostic microscopic features that are readily observed in body feathers.

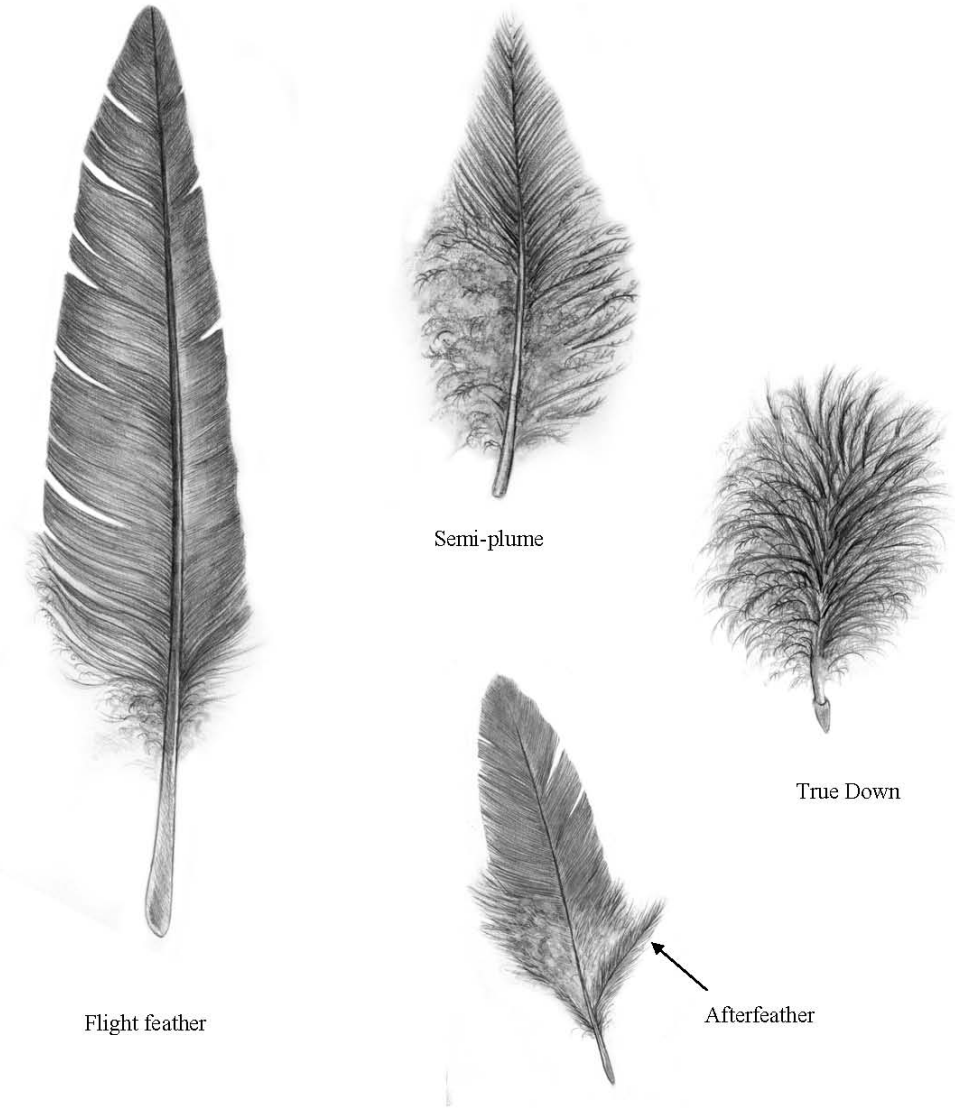

Figure 2. Illustrations of types of contour feathers with downy barbs (illustrated by Lisa Bailey).

PARTS OF A FEATHER

Barbs of all plumulaceous (down) types consist of a central **rachilla** (ramus) with **vanules** on either side, which, in turn, are made up of barbules (Figure 3). **Barbules**, branching from the rachilla of barbs, are the smallest division of the feather and consist of a **base** and a **pennulum.** Many cells make up the barbules' pennulum which typically telescope or taper distally. Cumulatively, many barbules make up the vanules of barbs. The **nodes** are located along the barbule and are usually the distal most junction of single cells that connect in a filament to form the barbule. Certain groups of birds (e.g. songbirds,

hummingbirds, woodpeckers, and some shorebirds) have transparent fringe-like projections on base cells called **villi (villus – singular)**. When conducting microscopic identifications of feathers, the morphology, pigmentation, and location of the nodes on the downy barbules aid in the identification of groups of birds.

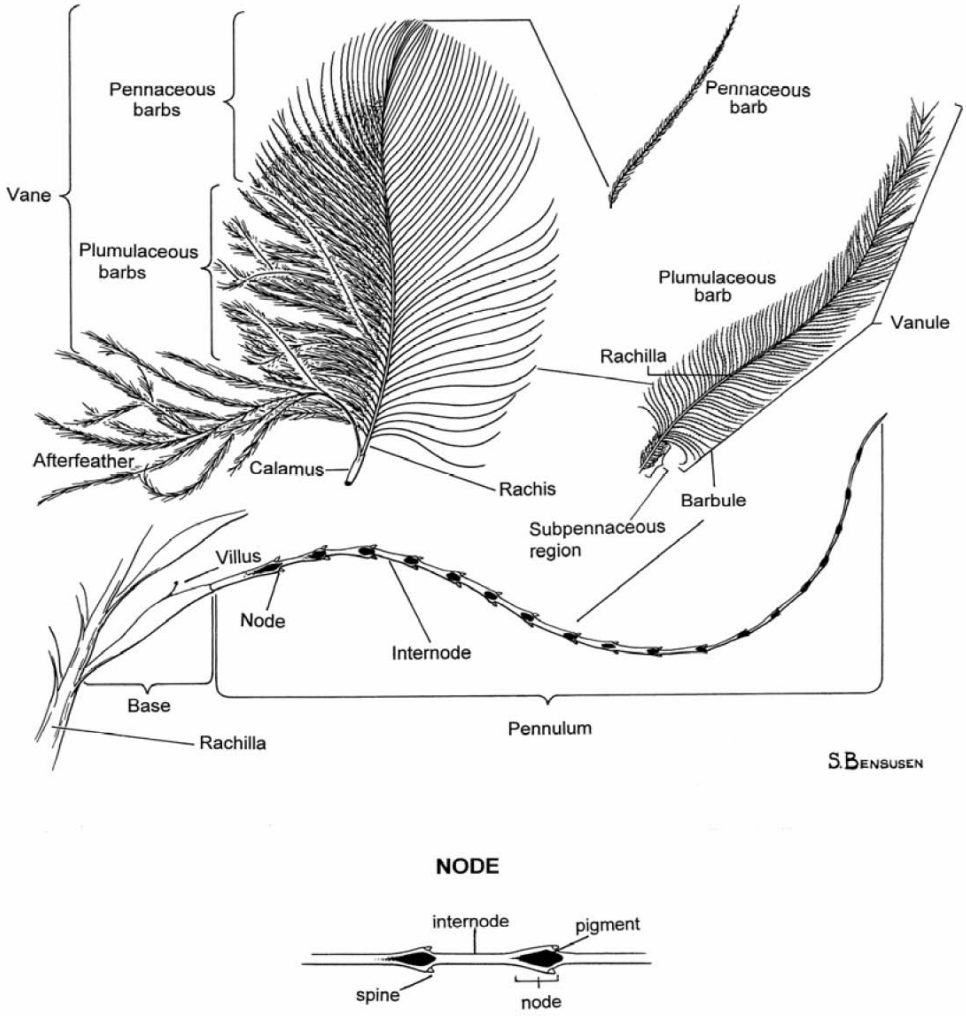

Figure 3. Topography of a contour feather with details of barb, barbules, villi, and node (from Dove 2000).

PENNACEOUS AND PLUMULACEOUS BARBS

Pennaceous and plumulaceous barbs appear quite different when viewed microscopically. Feather vanes are composed of both **pennaceous barbs** (Figures 4 & 5) that interlock and make up the surface of a feather and **plumulaceous barbs** (Figures 6 & 7) which are commonly referred to as downy **barbs** which function to aid in insulation. The pennaceous barbs have tiny hooklets on barbules that help interlock the feather barbs. Figure 4 is a scanning electron photomicrograph showing the detail of the

hooklet structures while Figure 5 is a photograph of the same species taken with light microscopy (LM) showing the same structures. The downy (plumulaceous) barbs are quite different in appearance from the pennaceous barbs. The microscopic characteristics of pennaceous barbs are generally not used for identification purposes.

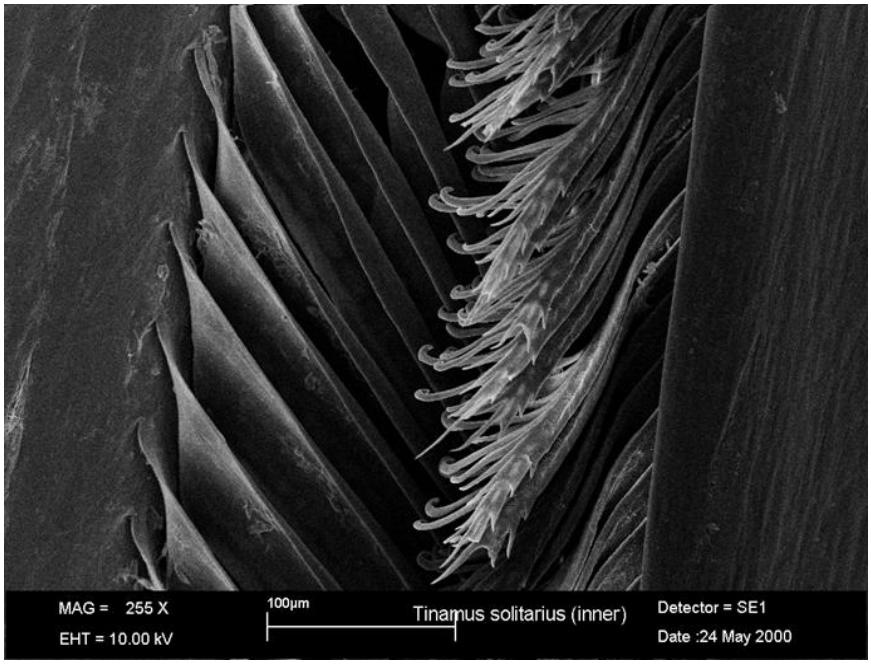

Figure 4. Scanning Electron Microscopy (SEM) image showing pennaceous feather barbs with hooklets.

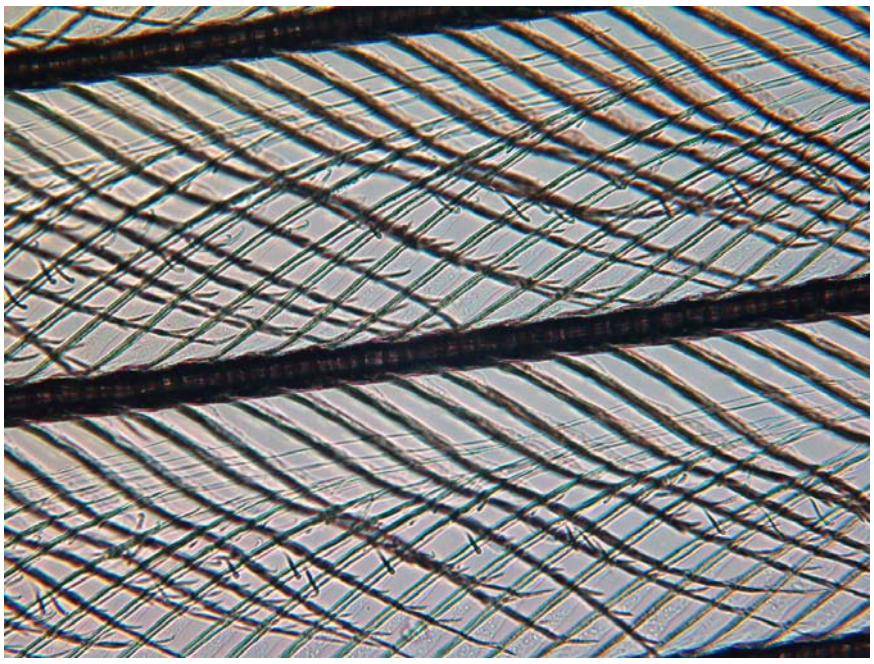

Figure 5. Light Microscopy (LM) image of pennaceous hooklets.

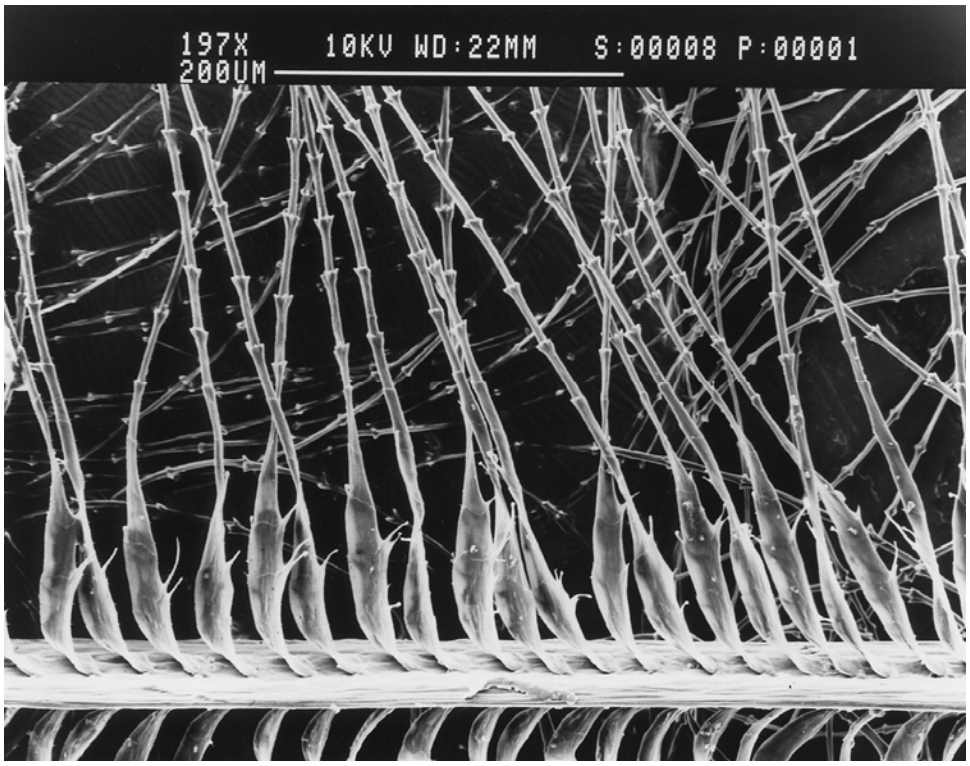

Figure 6. SEM photomicrograph showing plumulaceous (downy) barbules with distinct nodes and knobbed villi on base cells (American Crow – Corvus brachrhynchos).

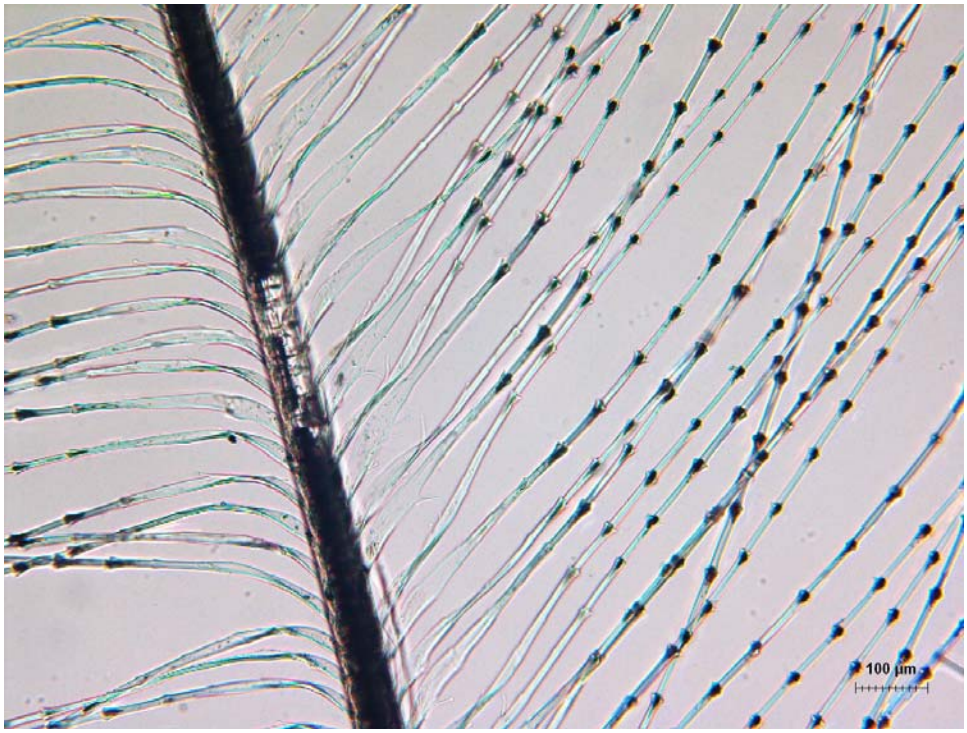

Figure 7. Light photomicrograph showing nodes and villi of American Crow. Because pigment is visible with LM but not SEM; identifications are best done with a comparison light microscope.

Microscope Materials and Preparation

Feathers should be examined using a compound light microscope or a comparison light microscope together with a reference collection of microslides made from downy feathers of positively identified birds. Typically, microslides are viewed at low power (40X-100X) for general overview and then examined at higher powers (200X-400X) for more detailed views of nodes and pigment patterns. Known feather samples of many of the species described in this guide (and often found in criminal cases) may be obtained from craft shops or fly-tying vendors (i.e. chicken, duck, turkey). If specific species are needed to build a reference collection, contact a local museum or university for possible assistance. It is important to build the reference collection from known, properly identified birds.

SUPPLIES FOR PREPARING FEATHER MICROSLIDES

- Flo-texx® (Lerner Laboratories, Pittsburgh, PA USA) mounting medium (methacrylate polymer).
  Any mounting medium with a similar refractive index to water is acceptable, but Flo-texx® does not "yellow" over time. This is important for making reference samples that you will store for long periods of time. Non-permanent slides of unknown samples can be made by using water and a coverslip.
- Microslides
  25x75mm slides with frosted edges which allow easy labeling by pencil.
- Cover Glass
  Either rectangular (no. 1, 24 x 50 mm) for whole slide specimens, or square (no. 1½, 22 mm) to mount two samples on a single microslide.
- Histosolve™ (Xylene substitute) or Xylene
  These products are used as a liquid base to allow downy barbules to spread or float onto the microslide. Xylene dries fast and acts as a solvent if there is foreign sticky substances on the feather. Xylene is also recommended for use when creating quality, permanent reference slides with Flo-texx® for long term storage.
- Micro-Forceps
  Fine, or super-fine tipped forceps such as Dumont #5 allow for removing fine, delicate downy barbs.

DOWNY BARB REMOVAL

The location of the downy barbs are shown in Figure 8 and defines the position of the *barb* and *barbules* to the *feather*.

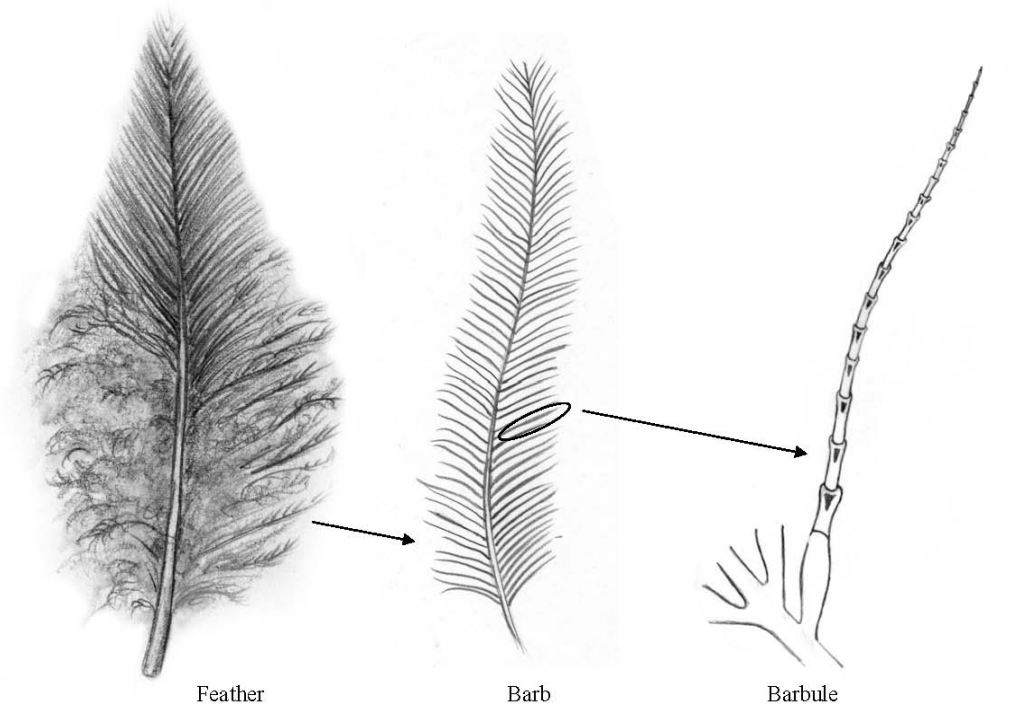

Figure 8. Illustration of the position of the barb and barbule to the feather (illustrated by Lisa Bailey).

MICROSLIDE PREPARATION

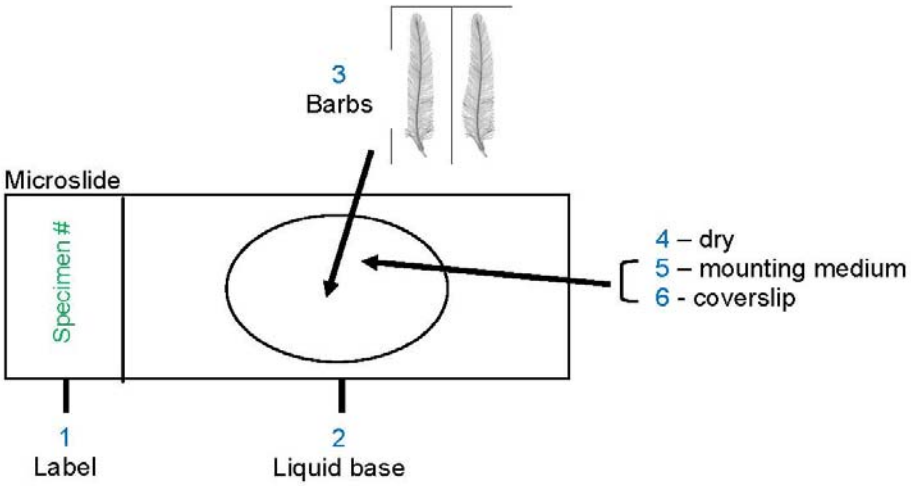

Figure 9. Steps for microslide preparation of feather barbs (illustrated by Marcy Heacker).

1) Label the microslide with the specimen number/identifier.

2) Place a small drop of liquid (Histosolve™, Xylene, or water) on the microslide to act as a base to float feather barbs. (Review Material Safety Data Sheets for handling precautions if using chemicals).
3) 'Float' downy feather barbs in the liquid. The liquid holds the barbs in place and allows the barbules to spread apart and provides better viewing. Let liquid air dry or tilt slide onto absorbant paper to drain excess liquid from slide. (When making permanent slides, allow the liquid to completely dry leaving feather barbs frimly attached to the microslide).
4) Apply a few drops of Flo-Texx® or other mounting medium onto dried feather barbs.
5) Place the coverslip over the feather barbs.

Allow microslide to "set" or dry before viewing. View slides using a standard compound light microscope or comparison microscope with magnification range of 40 – 400X. View first at low power to get an overview of barb, barbule length and pigment patterns. View at high power for node morphology details and diagnostic characteristics.

ILLUSTRATED DIAGNOSTIC FEATURES

Figure 10 illustrates some typical examples of node morphology that will be described in the various bird Orders in this guide. Figure 11 shows examples of pigment location and pigment distribution in barbules.

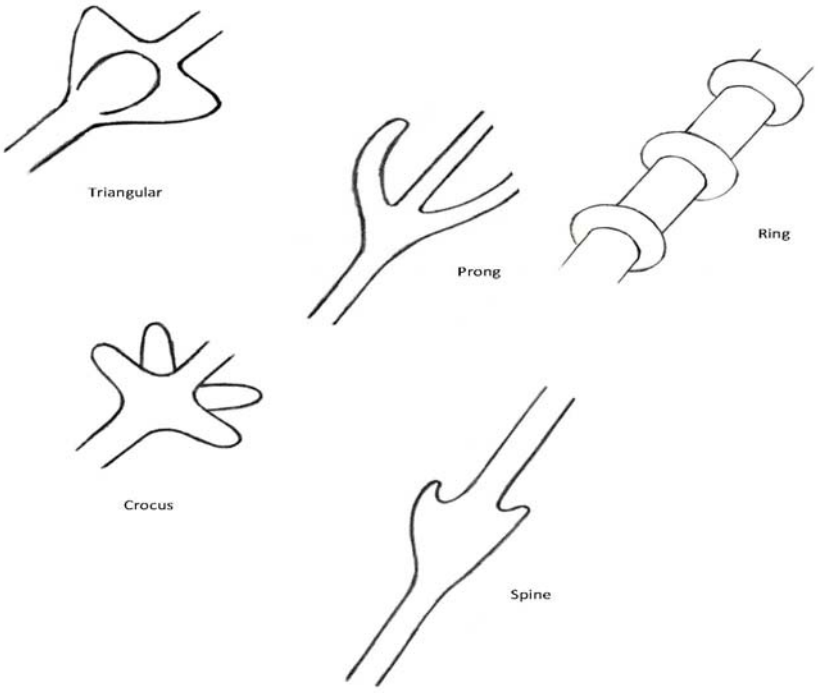

Figure 10. Illustrations of Node Morphology (illustrated by Lisa Bailey).

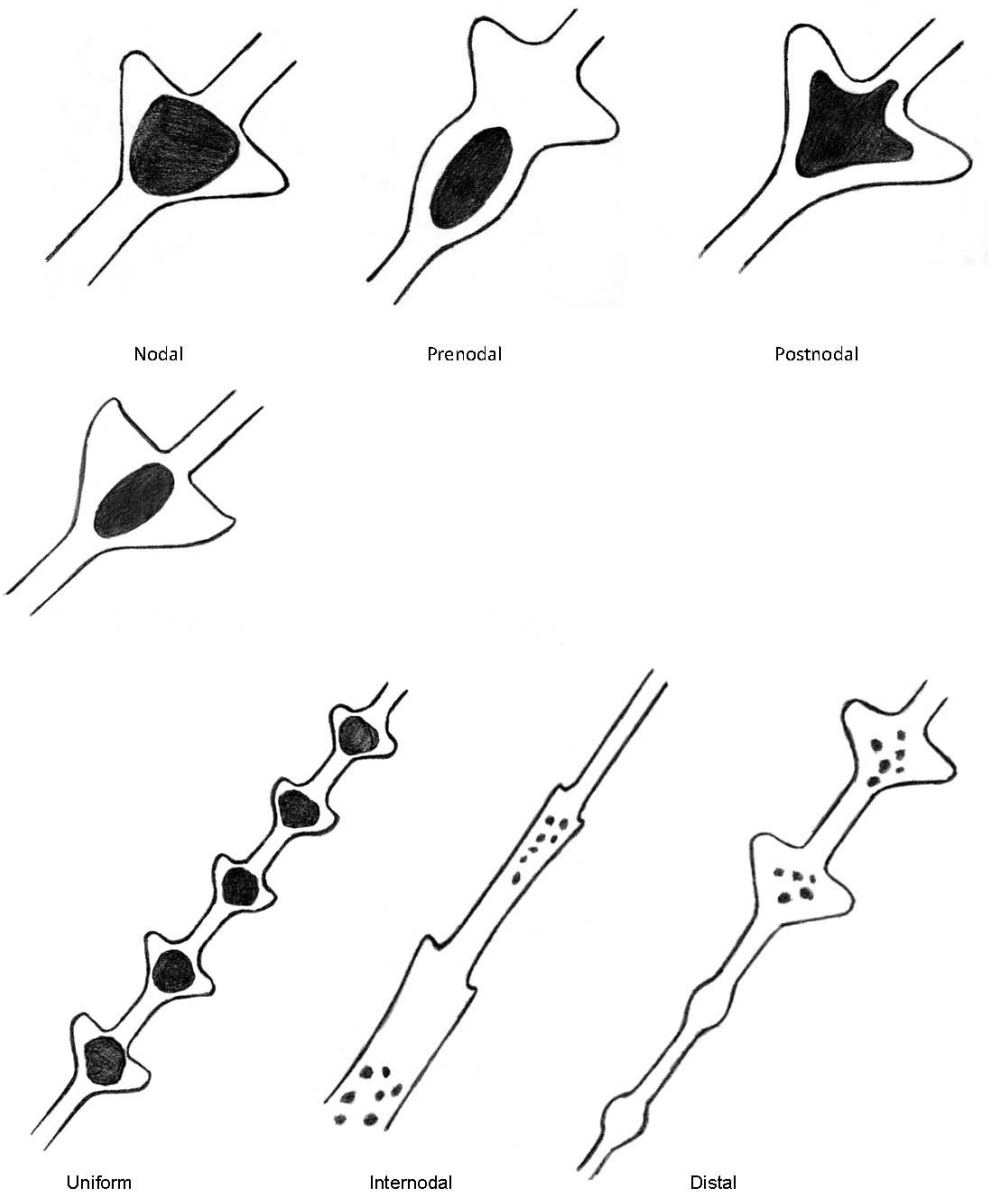

Figure 11. Illustrations of pigment location and distribution (illustrated by Lisa Bailey)

Special Feather Features

1. Villi

Villi (villus singular) were first described in the downy barbules of passerines in 1916 (Chandler 1916) as "… a constant and peculiar character in the presence of lobate or finger-like villi on the ventral edge or on the side of the base [of plumulaceous barbules]." The tiny structures appear as transparent projections on the base cells of downy barbules usually proximal on barbs, and are most visible at 400X using light

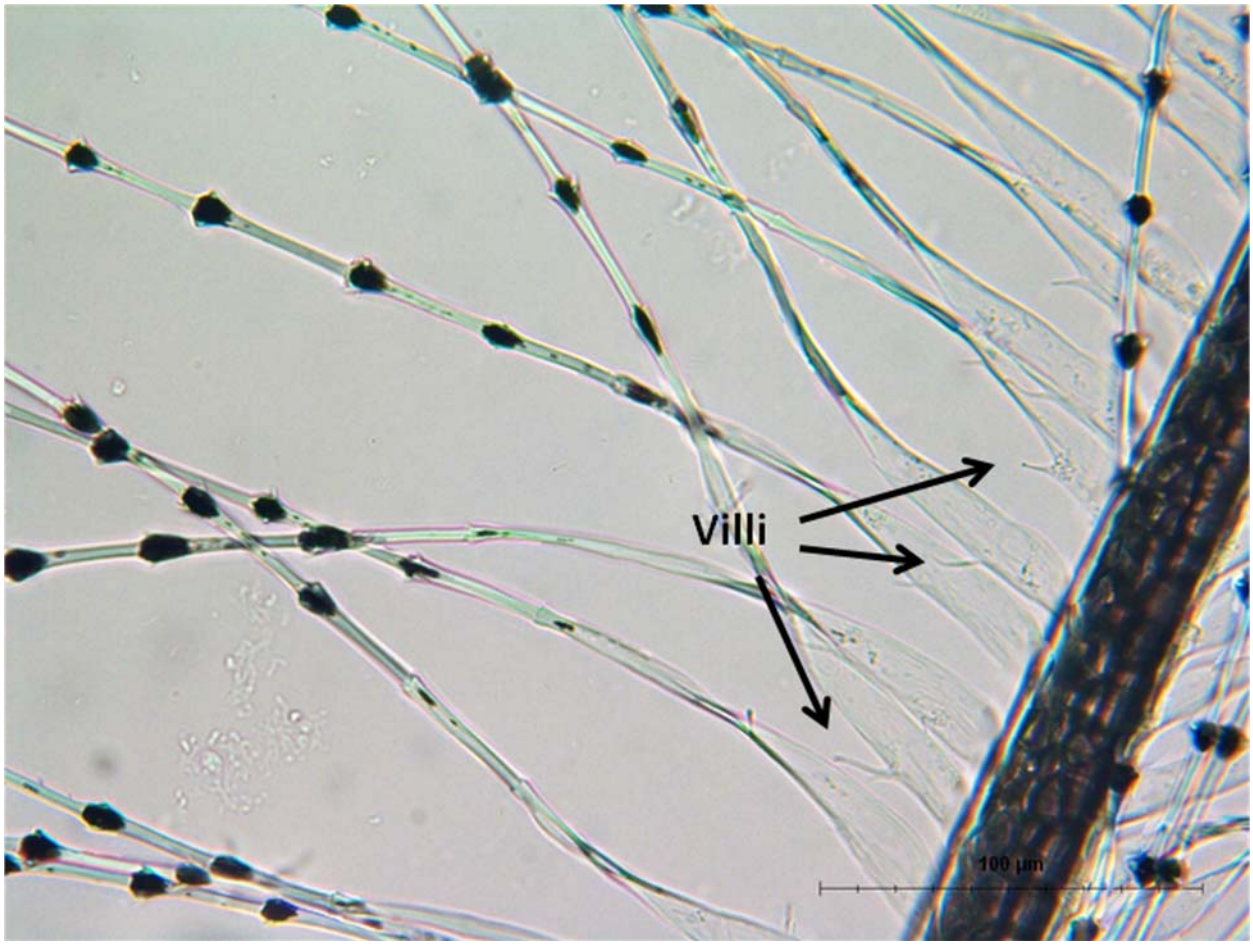

Figure 12. Image of Western Kingbird (Tyrannus verticalis) showing many villi on base cells (400X).

microscopy (Figure 12). Villi are mainly noted to occur in hummingbirds, woodpeckers and songbirds but these structures have recently also been noted in shorebirds (Dove 2000) although much less common in this group of birds. The villi, if present, are usually observed on the proximal part of the barb and decrease in frequency toward the tip of the barb. The function of villi remains unknown. In forensic studies, villi are very important features that can assist in the identification of groups of birds. One of the first diagnostic features to search for in a microscopic examination of a downy feather sample is the presence of villi. This feature can help eliminate some groups quickly if you do indeed observe it in the sample. If villi are observed on a downy feather sample then the feathers came from one of the four groups where it is known to occur (Songbirds, Hummingbirds, Woodpeckers, Shorebirds). Villi may be morphologically different in some groups (Figures 13, 14, 15), and can be used to identify songbirds (knobbed villi) from woodpeckers (curved back). If villi are not observed, the sample may or may not be from one of the groups listed above.

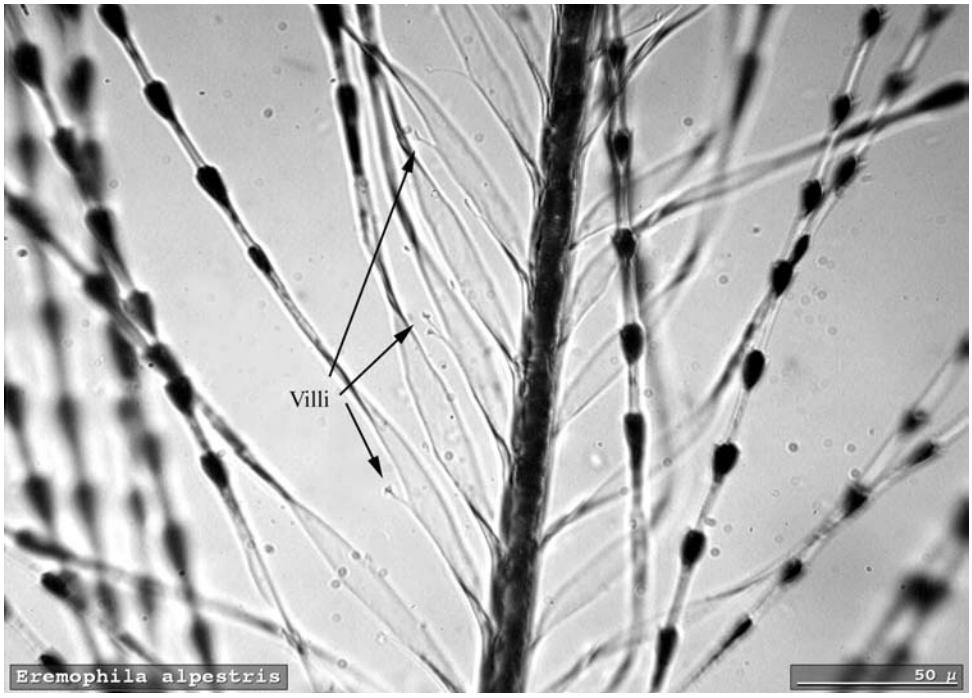

Figure 13. Image of songbird (Passeriformes) villi. These villi are knobbed or pointed and are commonly observed on base cells near the base of the barb.

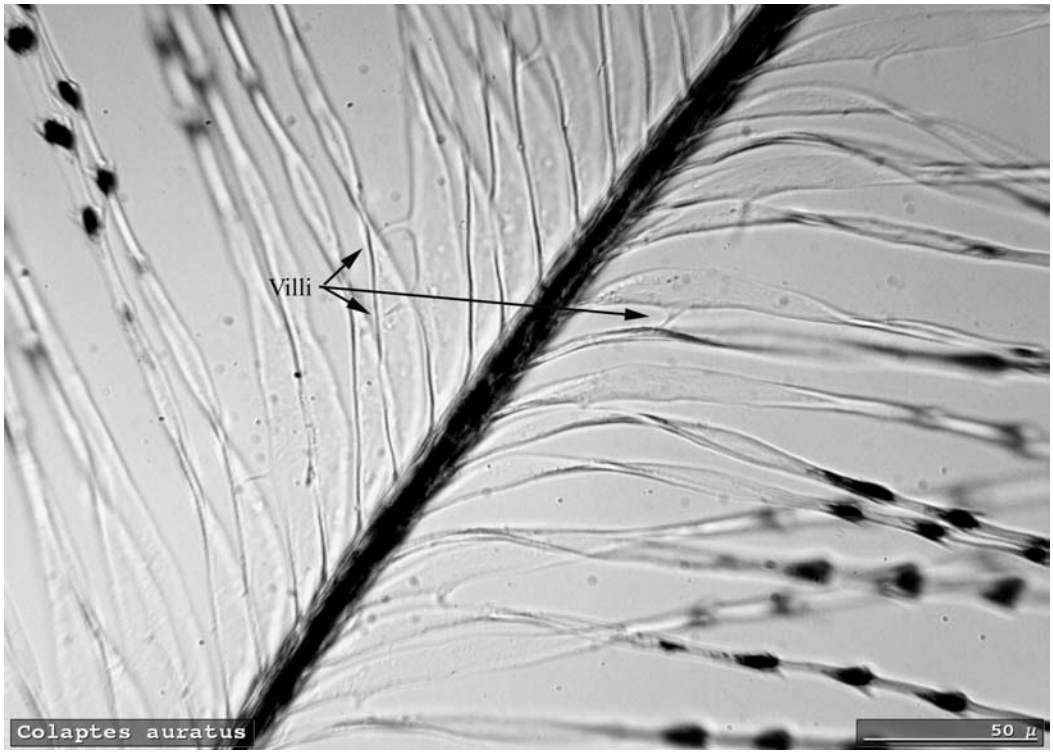

Figure 14. Image of Woodpecker (Piciformes) villi that are typically curved backward toward the rachilla.

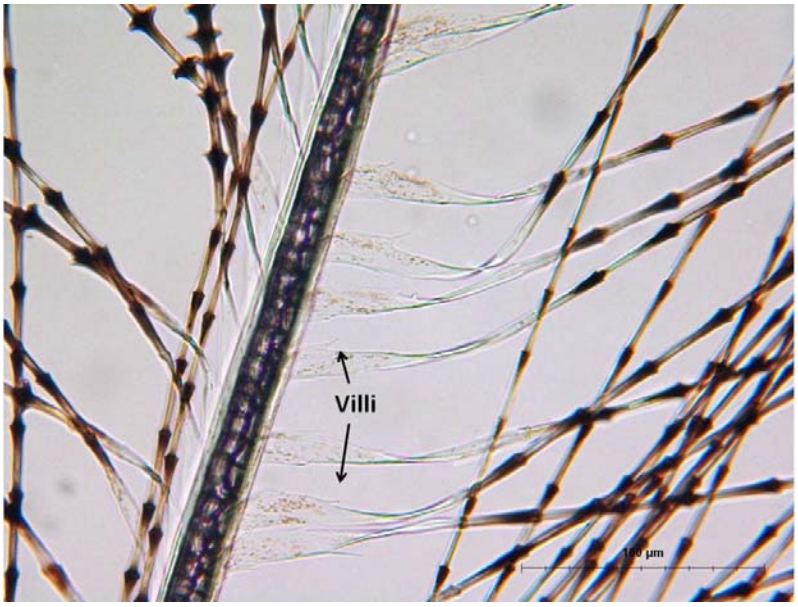

Figure 15. Image of Hummingbird (Family: Trochillidae) villi that numerous and knobbed like songbirds but asymmetric vanules set hummingbirds apart.

2. Asymmetry

Asymmetrical vanules, or the occurrence of barbules with nodes that are significantly more expanded on opposing vanules (see feather topography) of barbs have been observed in some groups of birds (Figures 16 & 17).

Figure 16. Photomicrograph (100X) showing asymmetry that is sometimes observed in Doves (Mourning Dove – Zenaida macroura).

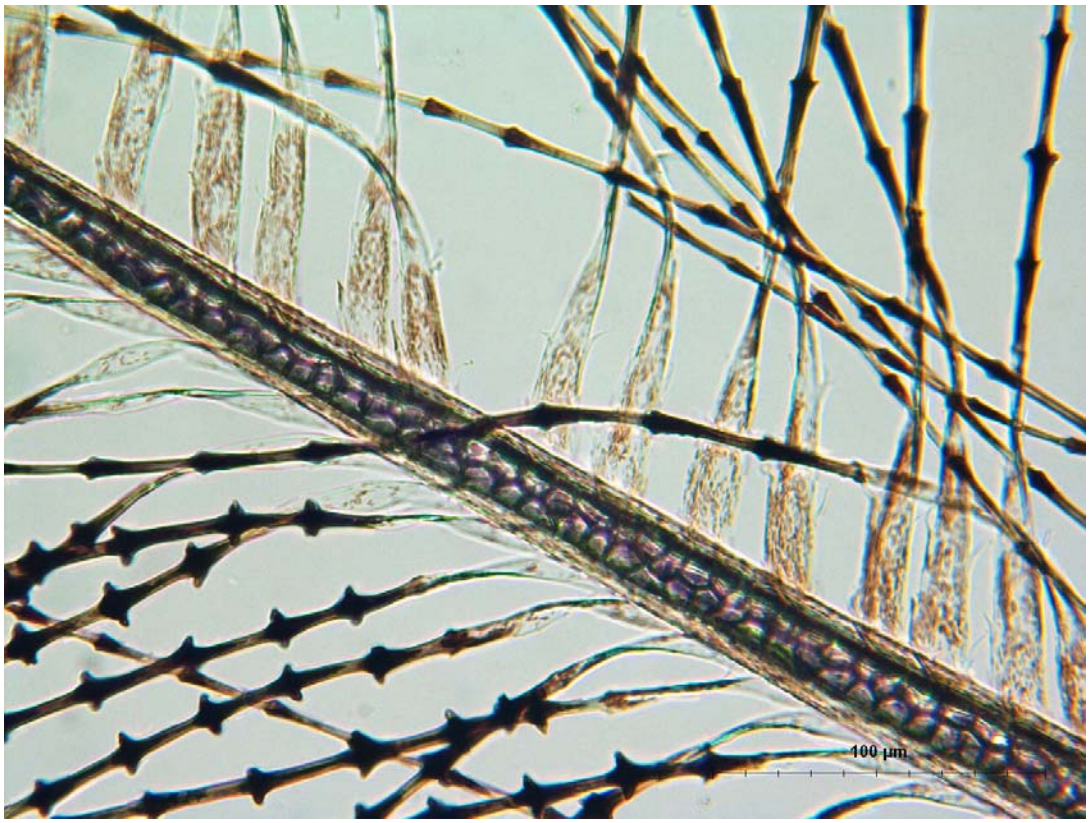

Figure 17. Photomicrograph (400X) showing expanded nodes of Ruby-throated Hummingbird (Archilochus colubris) on the left vanule and less expanded nodes on the right. The asymmetry in conjunction with the presences of villi makes identifying hummingbirds (Trochillidae) fairly straight-forward.

The most consistent examples of this feature occurs in Hummingbirds (Order: Apodiformes, Family: Trochillidae), some Rails (Order: Gruiiformes, Family: Rallidae), and some Doves (Order: Columbiformes). Asymmetry can be a diagnostic feature if sufficient feathers are available for a thorough search, but are not typically observed in the types of birds that are involved in human forensic cases (with the exception of doves). It is described here for information purposes. Because asymmetry may also simply be a factor of the type of feather sampled, this feature is not always observed.

Descriptive Notes for Selected Bird Orders:

Descriptive notes for diagnostic feather characteristics are included here for eight Orders of birds that might be involved in human criminal cases. These descriptions are provided as a guide to feather examination and only include the most diagnostic features. First, determine if the sample is downy or pennaceous, and if the diagnostic features are present. Characteristics described here are the most typical, or normal features used to identify groups of birds and may not be present in every sample.

Feather characteristics, generally referred to as 'characters' are described in a general way, and not in formal morphological terms. Feather characters may exhibit a range of variation depending on the feather type, and location of the sample on a single feather. It is only by examination and comparison of reference collections that a full understanding of the range of variation is achieved.

Struthioniformes (Ostriches)

Ostriches are part of a group of birds called "Ratites" which includes Emus, Cassowaries, Kiwis, and Rheas. Ostrich feathers are used commercially for feather dusters, feather boas, and other trinkets and decorative items.

Barb length (100X): Long to very long.

Barbule length (100X): Short.

Node shape: Nodes are simple, not expanded and are often difficult to distinguish because there is no notable difference between the node junction and the barbule (Figure 18).

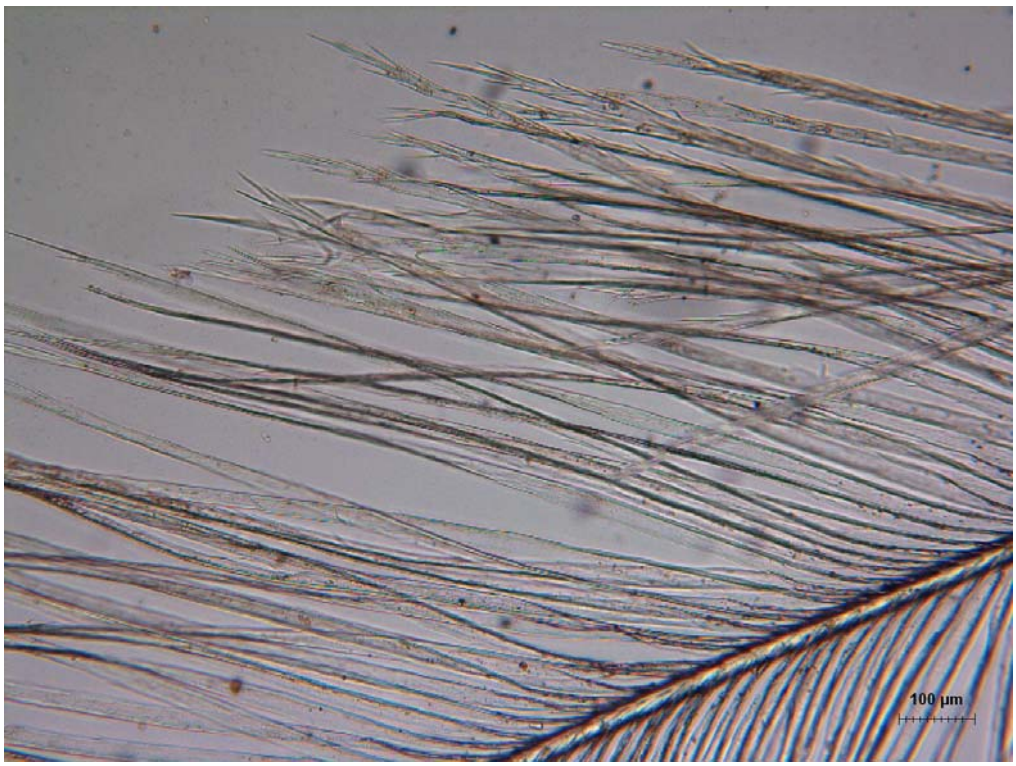

Figure 18. The microstructure of Ostrich (Struthio camelus) down is simple in structure without diagnostic characteristics. Pigment is lightly stippled throughout downy barbules (100X).

Node distribution: Nodes are not diagnostic so distribution is not relevant in species of this group. Long prongs are often present at distal cell junctions on barbules (Figure 19).

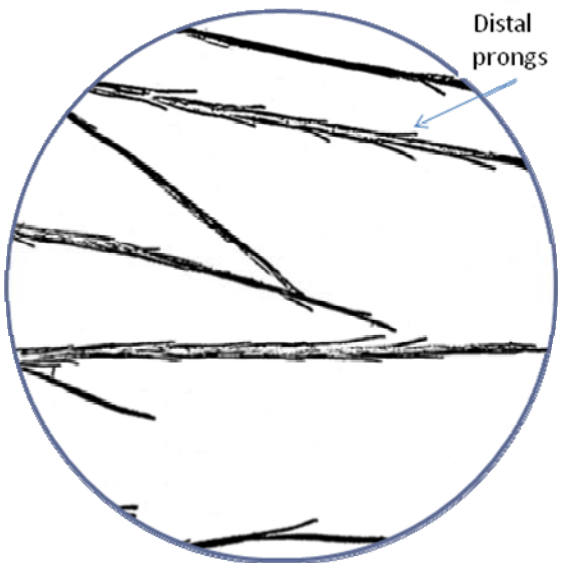

Figure 19. Schematic of distal prongs typical of Ostrich (100X).

Pigment pattern: Downy barbs are typically not uniformly pigmented but rather the barbules are lightly stippled with pigment throughout (Figure 18); pennaceous barbs are normally pigmented brown throughout and appear structurally similar to downy barbs (Figure 20).

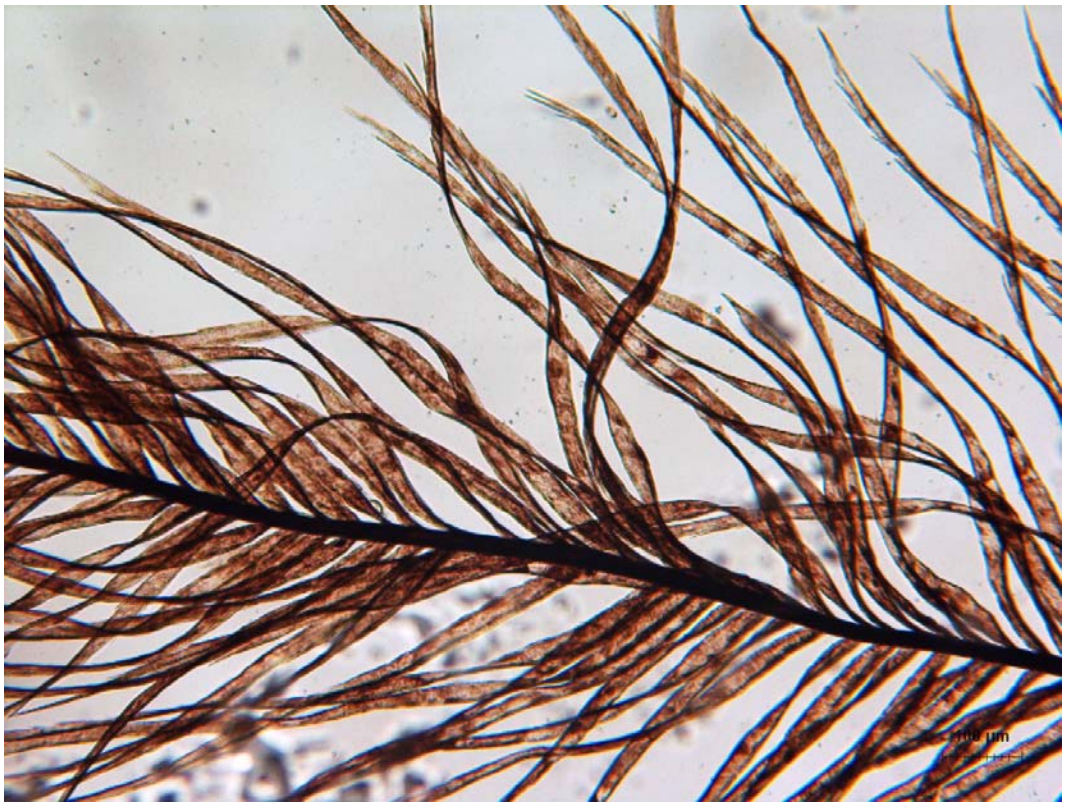

Figure 20. Pennaceous barbs of Ostrich (Struthio camelus) are similar to downy barbs but are much darker. The darkness of the pigment can be used to distinguish the pennaceous feather type in this group.

Diagnostic features: Microscopically, Ostrich feathers appear simple; the entire barbule is uniformly wide and appears flattened throughout the length of the barbules instead of narrowing toward the tip (telescoping) as in most birds. The appearance is "grass-like," without expanded nodes or diagnostic pigment patterns. The downy barbules usually have long prongs (Figure19) located distally on the barbules. There is no apparent distinction between the base cell and the barbule. Pennaceous barbules (Figure 20) appear similar to downy barbules but are usually pigmented dark brown. Ostriches do not fly and therefore do not have well developed 'hooklets' on the pennaceous feather barbs. This feature gives the whole bird a 'downy' appearance.
Similar species: All "Ratites" have similar microscopic feather characteristics. Some diving birds have microscopic structures that at first glance appear similar to Ratites with the long distal prongs but Ratites have uniformly wide, flattened barbules unlike the rounded, telescoping barbules of other groups.

Anseriformes (Waterfowl)

There are over 158 species of waterfowl worldwide; 62 occur in North America. Feathers, especially downy types, are used in the manufacture of clothing, sleeping bags, pillows, and furniture cushions. Because of the frequency of use in household and commercial items, waterfowl feathers can be associated with criminal cases. Some examples include downy feathers from torn jackets, pillows, and feathers from clothing found attached to broken windows during robberies or assaults. The microscopic characteristics of ducks, geese and swans, although similar to each other, can be diagnostic if appropriate material is available. High quality clothing is typically manufactured from the finest down of Eider Ducks, whereas cheap fillers can include both downy and chopped whole feathers from domestic duck and chicken or turkey.

Barb length (100X): Medium.
Barbule length (100X): Short (Figure 21) to medium (Geese).
Node shape: Waterfowl typically have two characteristic node shapes; triangular-shaped expanded nodes and pronged nodes. Both types are located on the distal portion of barbules (Figure 22). Because prongs are not always apparent at the tips of barbules, and the down may not always have the diagnostic triangular-shaped nodes, it is best to examine multiple samples for these characteristics.
Node distribution: Typically, the diagnostic triangular-shaped nodes are found proximally on the barbs, and distally on the barbules of most down types. These are unique in shape. There is some distinction between the location of the nodes in ducks and geese; ducks typically have fewer nodes that are located toward the distal 1/3 of the barbule and have a shorter distances between the diagnostic nodes (Figure 23). Geese have more numerous nodes that are narrower in width, are located about half way on

the barbules, and the distance between diagnostic nodes is greater than in ducks (Figure 24).

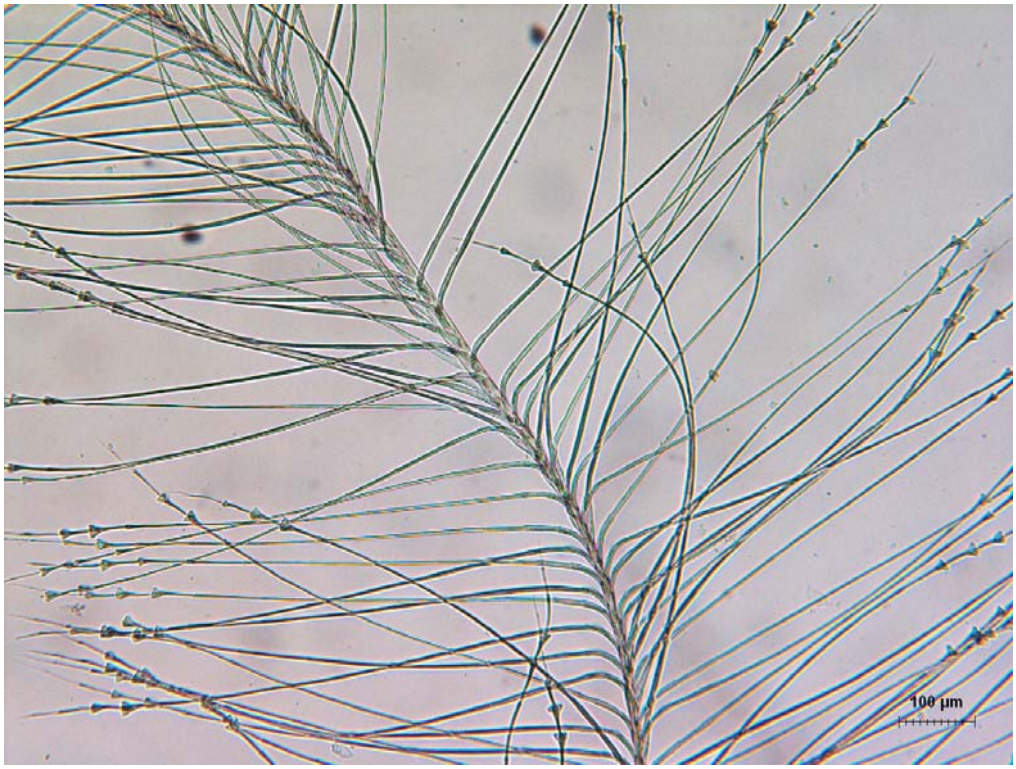

Figure 21. Image of American Black Duck (Anas rubripes) showing typical short barbules that are within the field of view with LM at 100X.

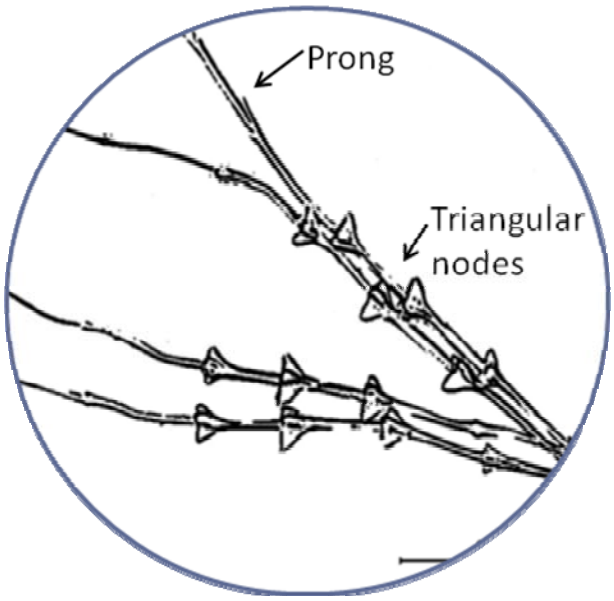

Figure 22. Schematic showing distal triangular-shaped nodes and distal prongs of waterfowl (Anseriformes).

Pigment pattern: Nodes may be pigmented or un-pigmented and sometimes the pigment is stippled in the internode space. Typically, however, nodes do not contain dark, or diagnostic shaped pigment.

Diagnostic features: The most diagnostic features for waterfowl includes the combination of triangular-shaped nodes and short to medium barbule length. The

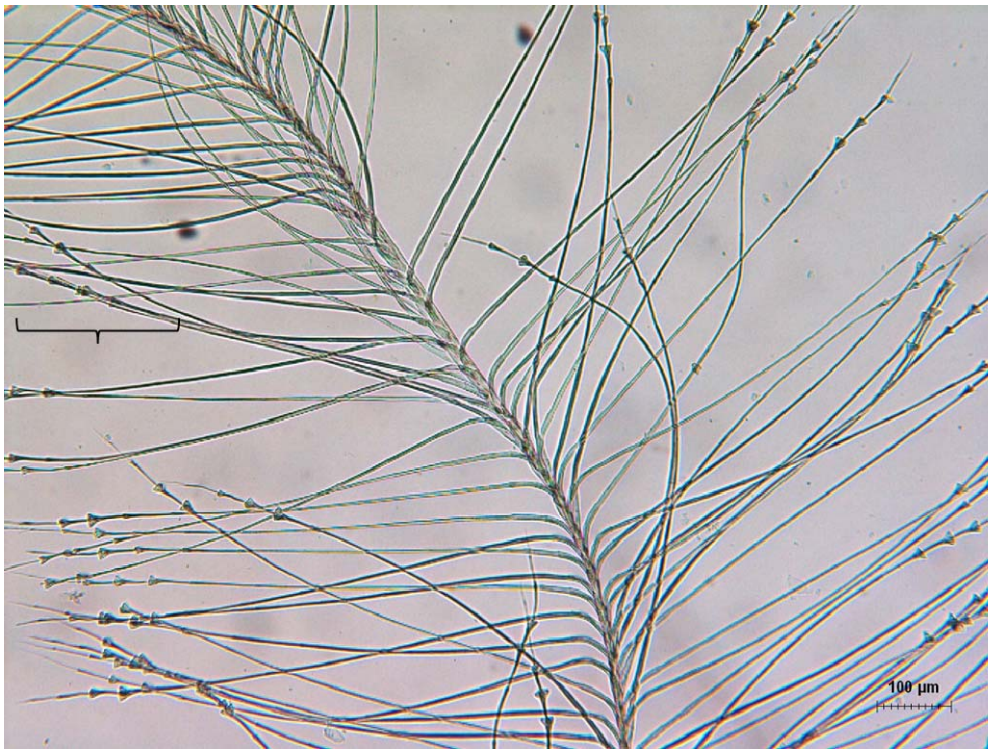

Figure 23. Ducks (i.e. Mallard – Anas platyrhynchos) usually have wide, triangular-shaped nodes that begin more distally on barbules and have shorter distance between nodes than in geese (100X).

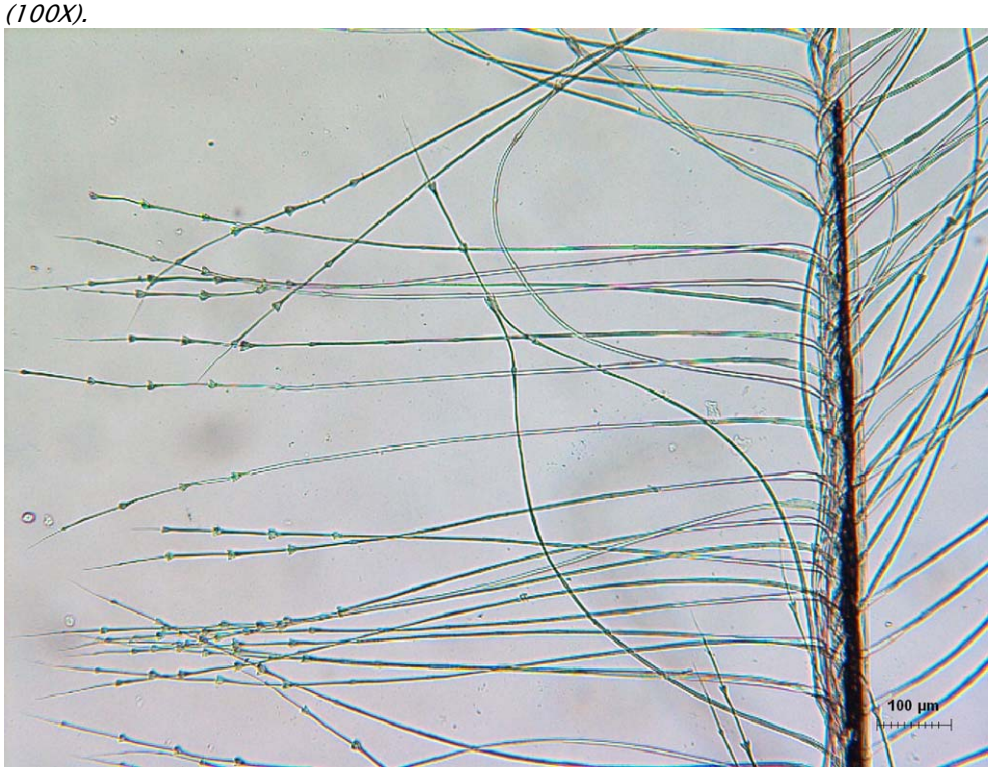

Figure 24. Triangular-shaped nodes usually begin more medially on barbules of geese (i.e. Canada Goose – Branta canadensis) and have greater distance between nodes than in ducks (100X).

location of the nodes, relative to the entire barbule, can aid in the separation of ducks and geese. The nodes of ducks are generally located more toward the distal portion of the barbules (Figure 23). Eider Duck (5 species) down is thought to provide the best insulation due to its denseness and is sometimes used in high-end, more expensive products. Eider Duck down typically has few triangular-shaped nodes distal on barbules (Figure 25), but some other diving waterfowl also exhibit this pattern so use caution when identifying this group.

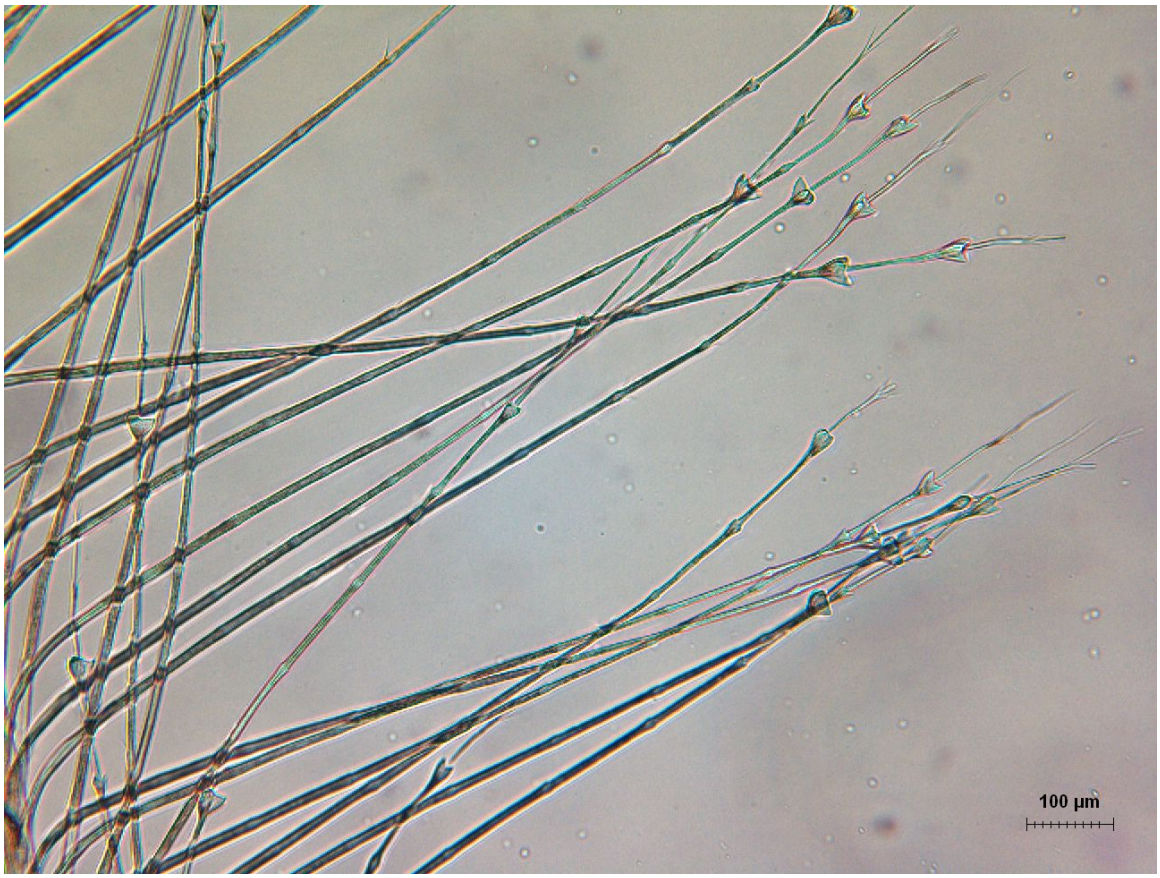

Figure 25. Common Eider (Somateria mollissima) showing few triangular-shaped nodes on barbules (200X).

Similar species: When the triangular-shaped nodes are not present in a feather sample, waterfowl down is difficult to identify. The long prongs at the tips of barbs and barbules in waterfowl may be confused with other diving birds that lack expanded nodes (Figure 26). Examination of multiple barbs, barbules and feathers may be necessary to find the diagnostic features for identification.

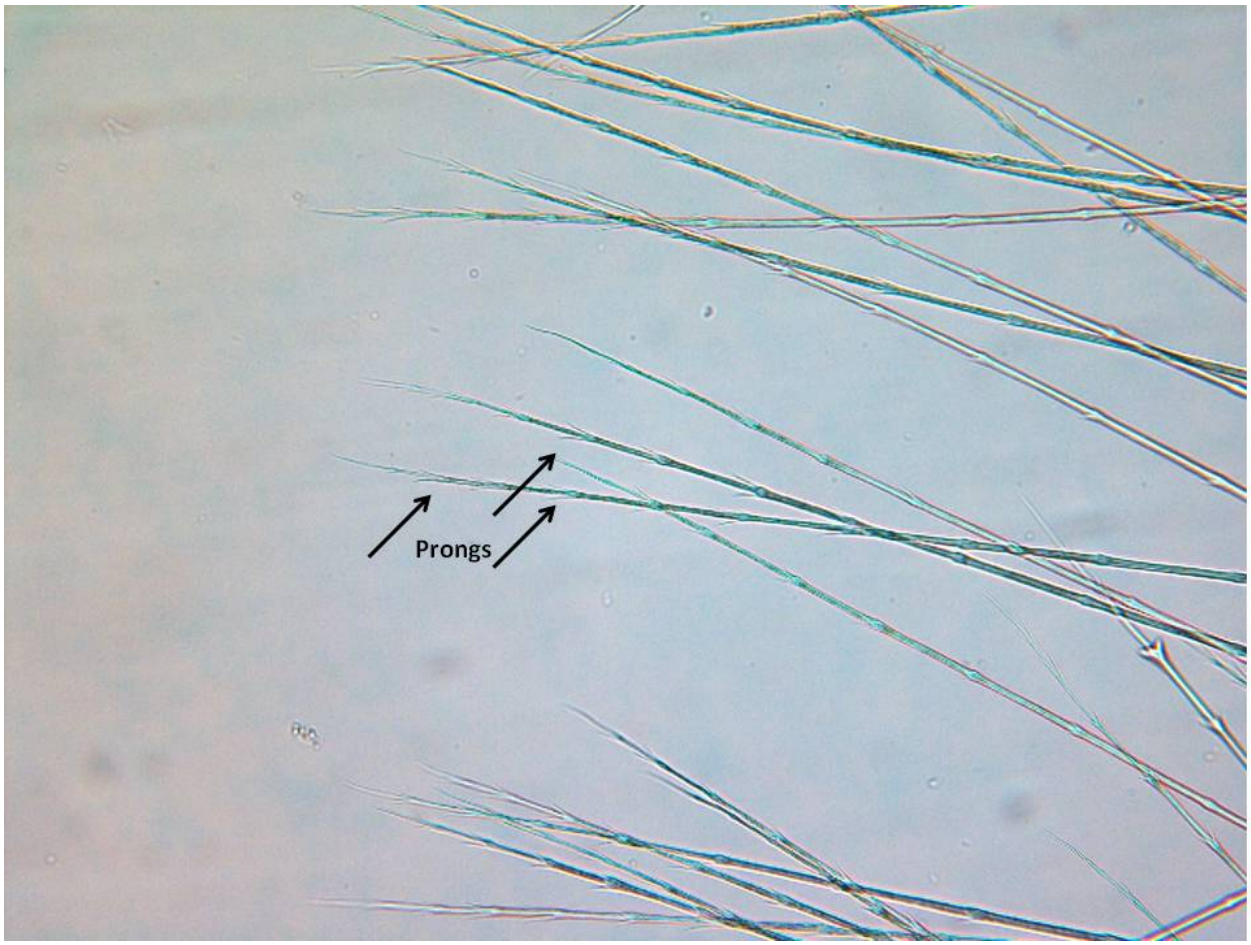

Figure 26. Prongs usually are visible at the tips of the barbs and barbules in waterfowl (Anseriformes) even when the diagnostic triangular-shaped nodes are lacking (200X).

Falconiformes (Birds of Prey)

The Order Falconiformes includes Eagles, Hawks, Osprey and Falcons. The microscopic features of two Families are described here (Accipitridae: Hawks; Falconidae: Falcons). Feathers from these Families of birds are often found in Wildlife Law Enforcement cases but also have been used in cult ceremonies, and sometimes in black-market trade. Eagle feathers are protected by additional laws with unlawful possession violations being subject to severe penalties, including lengthy prison terms and substantial fines. Owls are also sometimes considered "birds of prey" but because they are classified in a separate Order, those feather features are described later in this guide.

FAMILY : Accipitridae

The Family Accipitridae (Hawks, Eagles, etc.) is the largest and most heterogeneous family among the birds of prey with 233 species worldwide; 28 occur in North America. Due to similar microscopic characteristics, Hawks, Eagles and Vultures can be very difficult to separate based only on microscopic structures.

Barb length (100X): Long to very long.
Barbule length (100X): Long to very long (Figure 27).

Figure 27. Red-tailed Hawk (Buteo jamaicensis) has long barbules that extend beyond the field of microscopy view at 100X.

Node shape: Spines are typically uniformly distributed at nodes along the barbules (Figure 28). Spines are usually more visible proximally on barbules. Generally the nodes are only slightly expanded and are usually void of pigment (Figure 28). The barbules have a long "grass-like" appearance.
Node distribution: Nodes are spined and more easily observed proximally, becoming less apparent distally on barbules.

Pigment pattern: Internodal pigment is usually stippled and more predominate proximally on barbs and barbules. The nodes typically lack pigment. Pigment is usually present and stippled to varying degrees in the internode portion of the barbule but sometimes entire barbs and barbules lack any pigmentation. Focusing the microscope field of view at various depths of field sometimes a

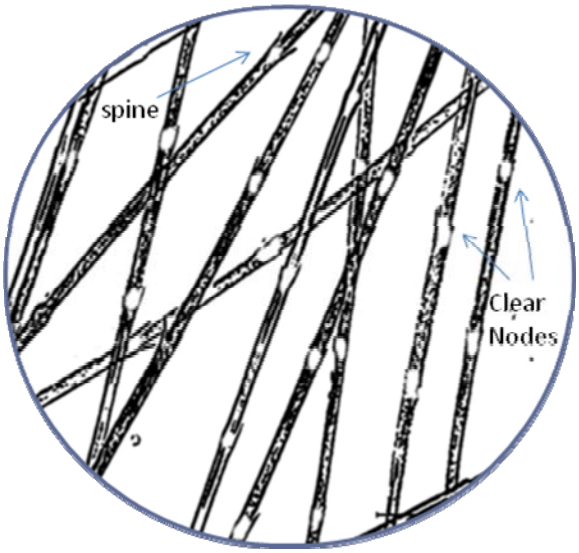

Figure 28. Schematic showing pigment stippled internodally and unpigmented areas at node junction that is typical of hawks and vultures.

ids in viewing the light internodal pigmentation. Not all barbs or barbules in this group of birds have pigment, so use caution when examining this feature. It is best to examine multiple barbs and barbules when assigning birds to the Family Accipitridae.
Diagnostic features: Birds of prey typically have long barbules, some degree of internodal pigment but lack pigment in the nodes.

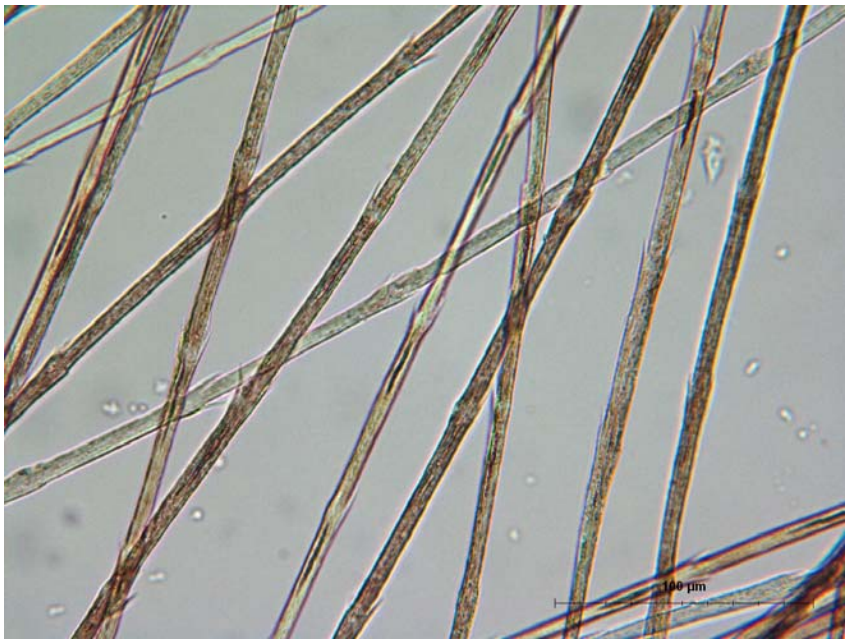

Figure 29. Turkey Vulture (Cathartes aura) is similar to hawks in feather microstructure but have somewhat shorter barbules and may lack the dark, stippled intermodal pigmentation.

Similar species: Birds of prey (except falcons) can appear to be very similar to each other microscopically. Hawks and Turkey Vultures (Figure 29) are especially very similar, but the Black Vulture usually has much more pigmentation in internodes and visibly shorter barbules. Osprey are unpigmented throughout most barbs and barbules. Use extreme caution when trying to identify this group based on microscopic feather structures alone and do not attempt it without corroborating evidence such as whole feathers for specimen comparisons.

Family Falconidae:

The Family Falconidae (falcons) is comprised of 64 species worldwide; 11 occur in North America and range in size from the small American Kestrel (*Falco sparverius*) to the large Gryfalcon (*Falco rusticolus*).

Barb length (100X): Long to very long.
Barbule length (100X): Long to very long; "wispy" in appearance (Figure 30).

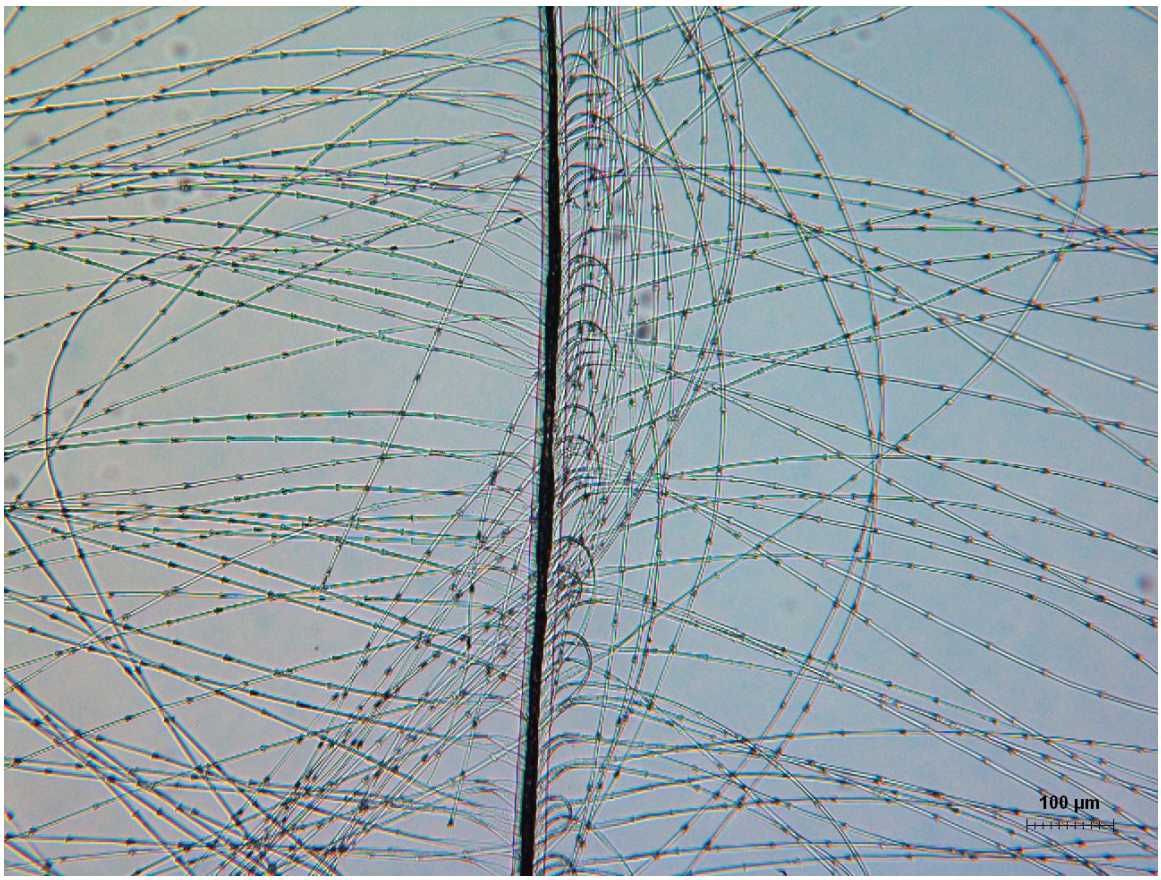

Figure 30. Falcons (Peregrine Falcon – Falco peregrines) are distinguished from hawks by having rounded pigmented nodes that are uniformly distributed on the long, wispy barbules.

Node shape: Expanded nodes are obvious all along the length of barbules and are diagnostically "plump" or rounded in shape. The appearance of the node is large and round which is in stark contrast to the narrow internodal width (Figure 31) on these barbules.

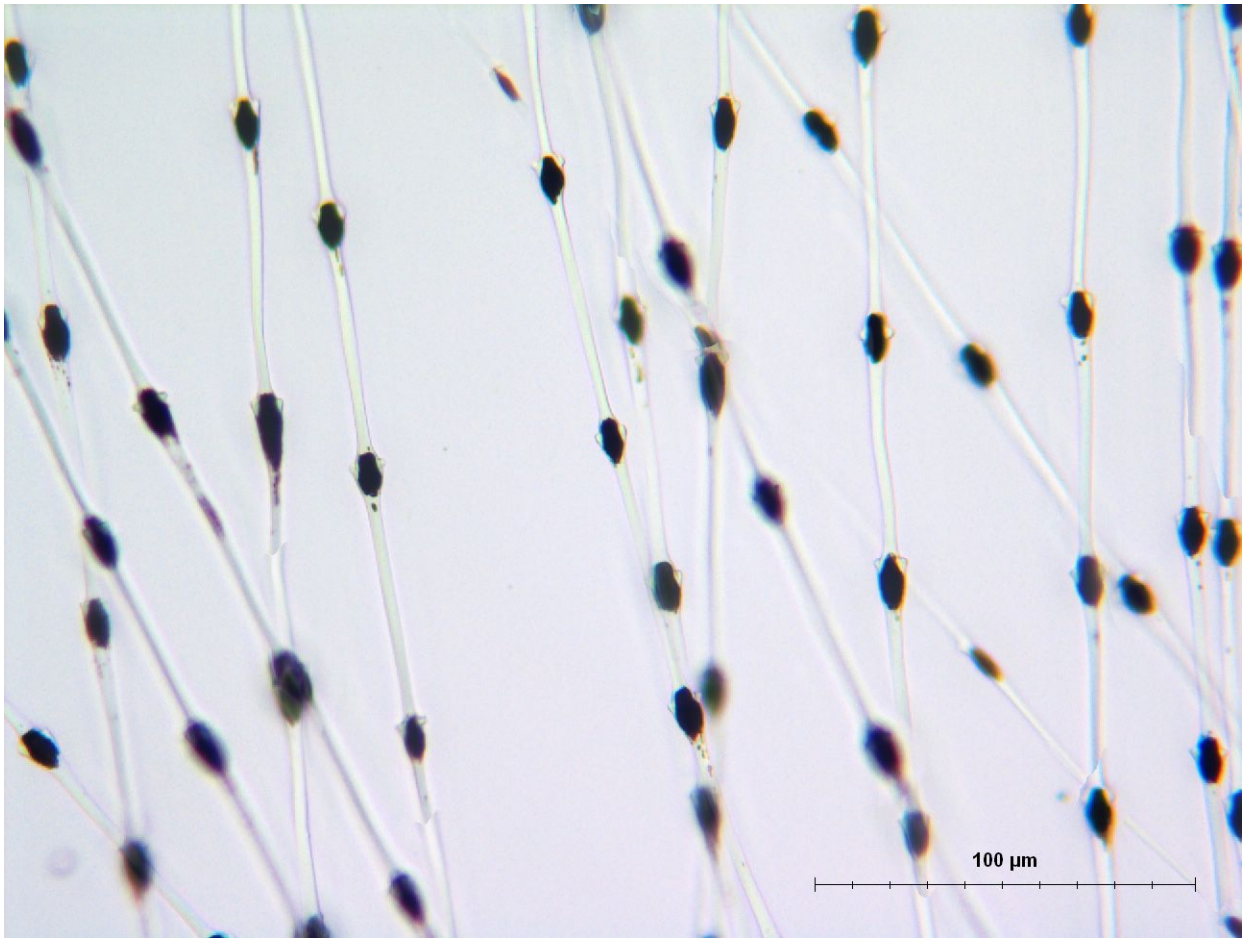

Figure 31. View of diagnostic pigmented nodes and narrow intermodal width typical of falcons (American Kestrel – Falco sparverius).

Node distribution: The nodes are consistently spaced at regular intervals all along barbules. The appearance of the node is somewhat 'knobby' due to the narrow diameter of the internode. The consistent presence of these knobby, pigmented nodes, throughout the barbule separates the Falcons from the other birds of prey in this Order. Hawks, Vultures and Eagles lack pigmented nodes and only have internodal pigmentation.

Pigment pattern: The pigment is concentrated at nodes and is round or oblong in shape on proximal nodes. Distal nodes have pigment that may extend below the node. Pigmented nodes are uniformly distributed throughout the length of barbules but decrease in width distally.

Diagnostic features: Round-shaped pigment concentrated at nodes on long "wispy" barbules. Pigmented nodes are typical of Falcons but in some cases there may be only slight pigment or even unpigmented nodes if the down is sampled from flight feathers.
Similar Species: At first glance, some songbirds (Passeriformes) may have a similar appearance because the microstructure also has the round pigment concentrated at the nodes. Passeriformes, however, have villi on the base cells and do not normally have long "wispy" barbules. Owls may seem similar at first glance but the proximal nodes of Owls are more flared and distinctly cupped. The distal nodes of owls are also elongate rather than round. Parrots may also appear similar to Falcons but have extremely long barbules with the pigment, which if present, is only concentrated at the node (never stippled below the node). Barbules of Parrots are usually so long that they appear tangled when viewed microscopically and the node shape in Parrots is usually flared throughout most of the barbule.

Galliformes (Fowl-like Birds)

The Galliformes include six families, the most familiar being Phasianidae: Pheasant, Quail, Chicken and Partridges. Feathers from these birds are often used in bedding, clothing, fly-tying, and are sometimes dyed or modified and attached to dream-catchers or other arts and crafts items. The Wild Turkey (Meleagrididae) and chicken do not differ from the domesticated varieties as far as feather microstructure is concerned.

Barb length (100X): Long.
Barbule length (100X): Long.
Node shape: The proximal nodes of the chicken and turkey sometimes are slightly expanded with small spines, but the most diagnostic characteristics of this group are the ring-shaped structures that surround the more distal nodes (Figures 32 & 33). These "rings" are usually located distally on the long barbules. Sometimes the 'rings' slip free at the node junction and slide along barbules (Figure 34 - arrow).
Node distribution: Typically, chickens and turkeys have two types of nodal structures; slightly expanded proximal nodes with short spines (Figure 35) and distinctly "ringed" distal nodes. Nodes are distributed regularly and evenly and are numerous throughout barbules. Not all samples will have the diagnostic ring-shaped nodes.
Pigment pattern: The pigment is typically darkest and more concentrated at the node or just below the node but pigment can also be darkly stippled in the internode and throughout the barbules.
Diagnostic features: The 'ring-shaped' structures that surround nodes are the most diagnostic feature of this Order, although not all Families or samples exhibit this characteristic.
Similar species: The only other Order of birds that have 'ringed' structures are the Tinamiformes (Tinamous). Although Tinamous physically resemble fowl, they are not

closely related. Because Tinamous do not occur in the United States and are unknown in criminal cases, the microstructures are not illustrated in this guide.

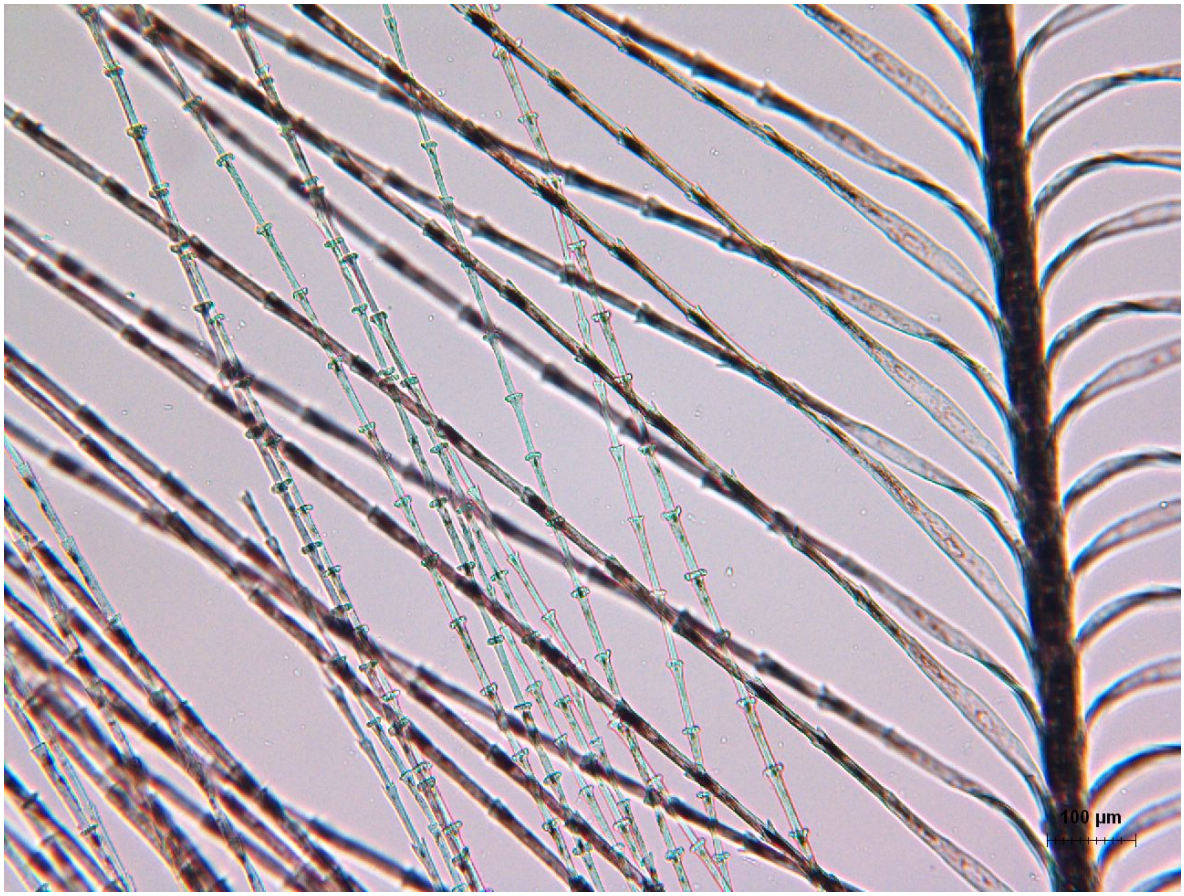

Figure 32. Ring-necked Pheasant (Phasianus colchicus). Most Galliformes that are involved in forensic cases have long barbules with expanded nodes proximally and ringed structures that surround the nodes distally (200X).

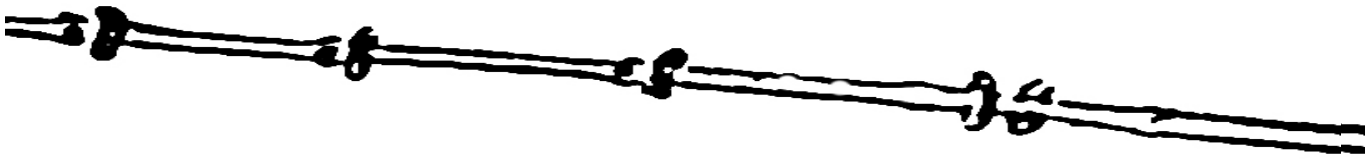

Figure 33. Schematic showing ringed nodes.

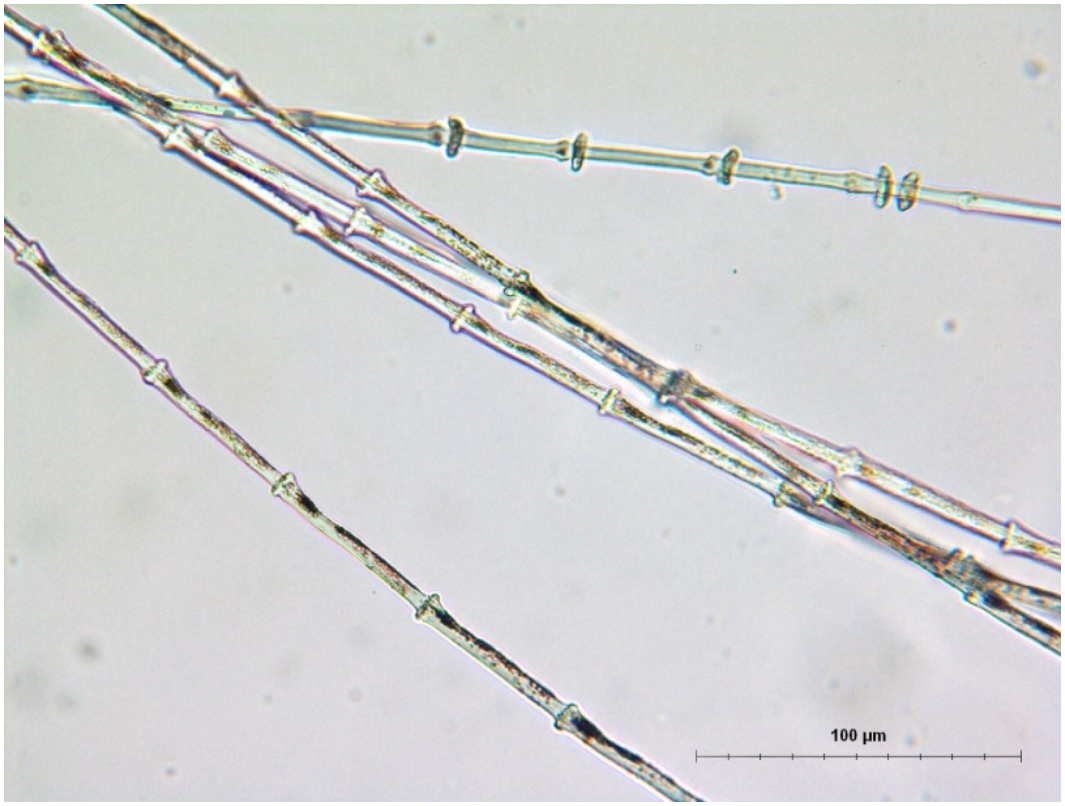

Figure 34. "Ring" structures sometimes slip free of the node and slide along the barbules (see arrow).

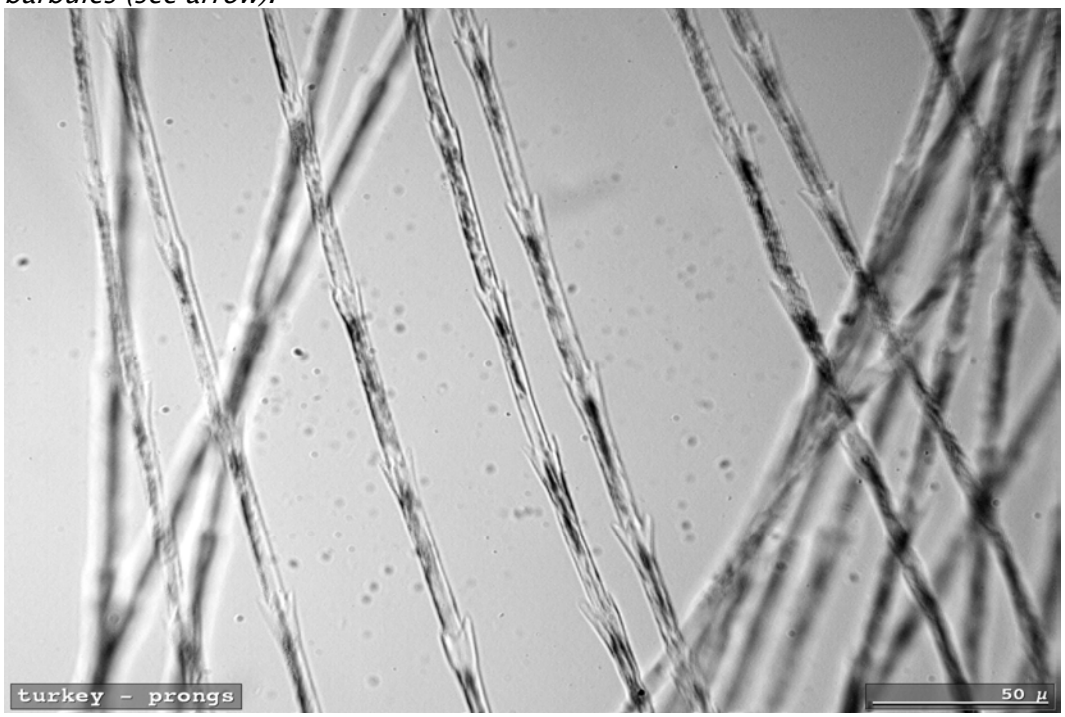

Figure 35. Spined nodes are also typical of some Galliformes (Turkey – Meleagris gallopavo) and are located on the proximal portion of the barbules. The 'ringed' structures may not occur in all Families within this Order.

Columbiformes (Pigeons and Doves)

There are 308 species in Columbidae worldwide; 18 different species occur in North America. Rock Pigeons (*Columba livia*) often roost in warehouses, barns, and bridges and are adapted to humans so their feathers may be encountered in the environments of different types of crime scenes.

Barb length (100X): Long to very long.
Barbule length (100X): Long to very long.
Node shape: The node shape of Doves and Pigeons are extremely flared on most proximal barbules and have a distinct shape that resembles a crocus flower. The node shape, therefore, is described as 'crocus-shaped' (Figures 36 & 37).

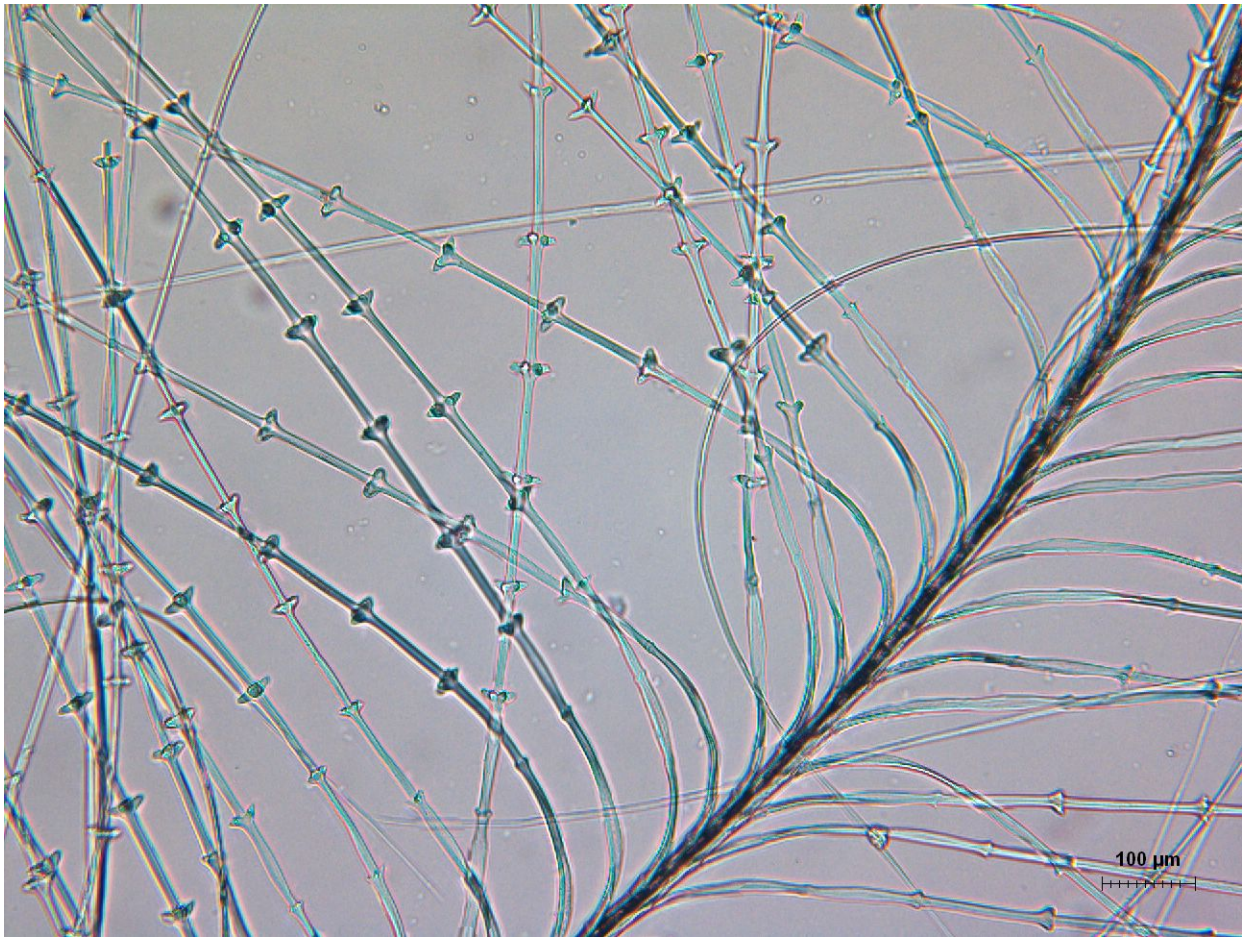

Figure 36. Long barbules with flared 'crocus-shaped' nodes (200X) and light stippled pigment in Rock Dove (Columba livia).

Node distribution: Nodes are numerous and evenly distributed along the length of barbules with the distinctly flared nodes becoming less apparent distally. The most diagnostic 'crocus-shaped' nodes are located proximally on barbs and barbules.

Sometimes asymmetry is also noted proximally, and is illustrated in Figure 38 in the Common Wood Pigeon (*Columba palumbus)*.

Pigment pattern: Pigment is usually not obvious, but if present, it is lightly stippled in the internode and not an immediate diagnostic feature.

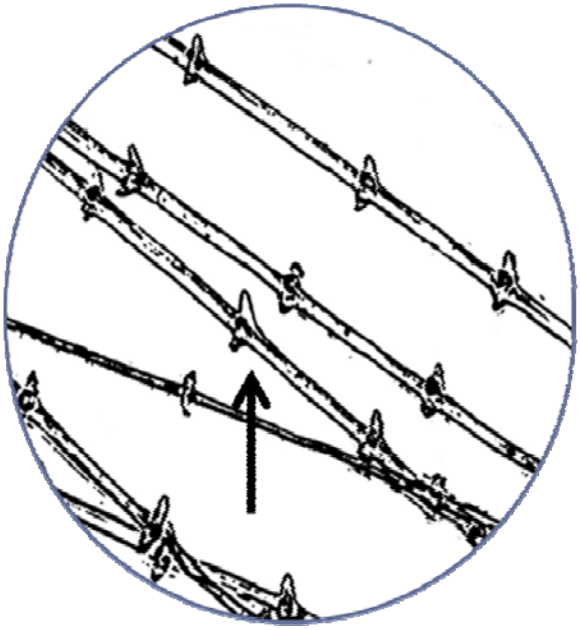

Figure 37. Schematic of Pigeons and Doves (Columbiformes) have 'crocus-shaped' flared nodes that are prominent on the proximal portion of barbules.

Diagnostic Features: The most diagnostic microscopic characteristic of Doves is the very flared, 'crocus-shaped' nodes that are most prominent proximally on the barbs and barbules. The node shape, together with the long barbs and barbules, are diagnostic of Doves (Figure 36). A unique feature in some Columbiformes is that the feathers are loosely attached to the skin. This allows

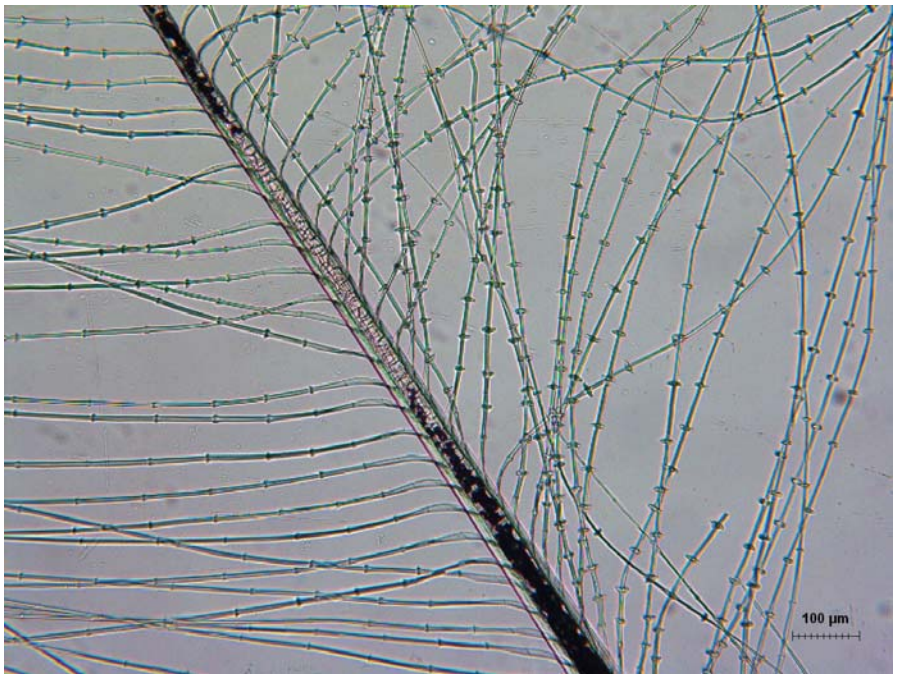

Figure 38. Photomicrograph showing asymmetry in vanules of Common Wood Pigeon (Columba palumbus) that is typical of some species in this family. Nodes on the right side (distal) in this image are much more expanded and numerous than those on the left side.

them to easily escape predation, but it also makes for a diagnostic feature for whole feather identification. The inferior umbilicus, or the most proximal point of the calamus on the feather rachis, narrows to an extremely fine point where the feather is attached to the skin and is easily recognizable as a "pin point" on the whole feather.

Similar species: Some Parrots have similar microscopic structure to Doves but Parrots usually do not have the distinct 'crocus' shaped nodes. While the basal nodes of Parrot down are very expanded, they do not have the same diagnostic shape as the Doves. Parrots typically have longer barbules, and sometimes have pigmented nodes.

Psittaciformes (Parrots)

There are 364 species worldwide within this Order. Although there are currently no native Parrots in the United States, 8 species have established populations from escaped caged birds and mainly occur in the southern parts of California, Texas and Florida. The sale of these colorful birds as pets and trade in the black-market increases the chance of finding these feathers in homes, and potentially at crime scenes.

Barb length (100X): Long to very long.

Barbule length (100X): Long to very long.

Node shape: Nodes are expanded much in the same manner and shape as Doves but many species of Parrots have pigmentation at the nodes (Figures 39 & 40). While the basal nodes of Parrot down are very expanded they do not have a diagnostic 'crocus' shape like Doves but rather are more laterally flared at the transparent area around the node instead of curved upward like a flower petal.

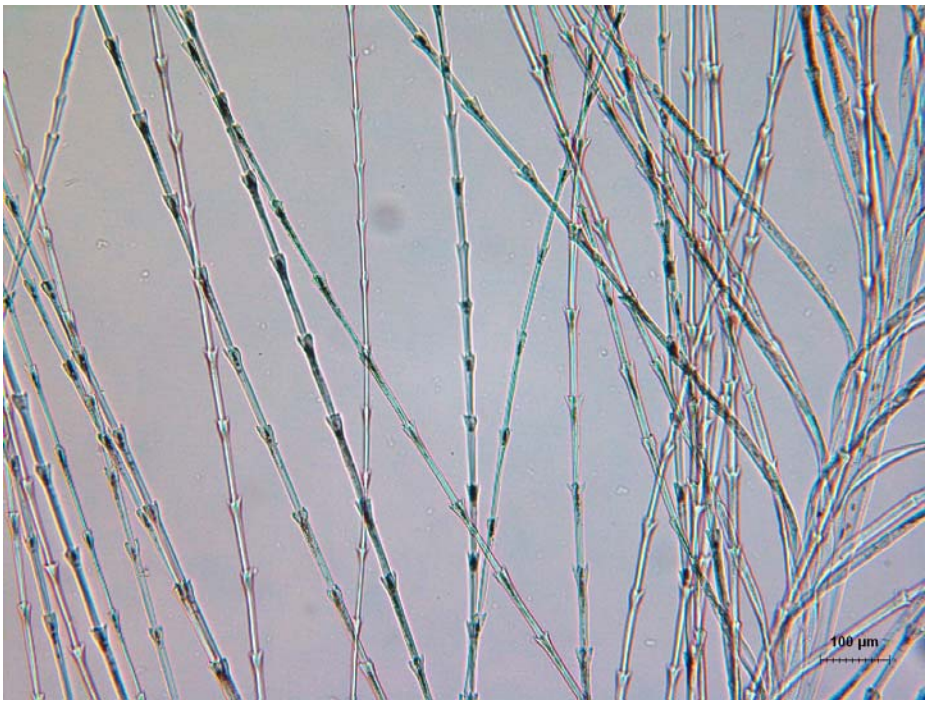

Figure 39. Parrots (Psittaciformes) typically have pigment that is rounded and concentrated at the nodes or extends slightly into the internode 200X (Scarlet Macaw – Ara macao).

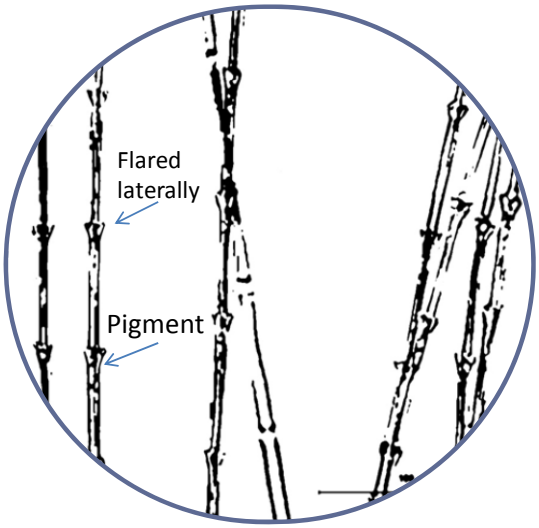

Figure 40. Schematic of structures typical of Parrots (Psittaciformes). Nodes are laterally flared and pigment is usually concentrated at the nodes. The internode is wide on the proximal portion of the barbule.

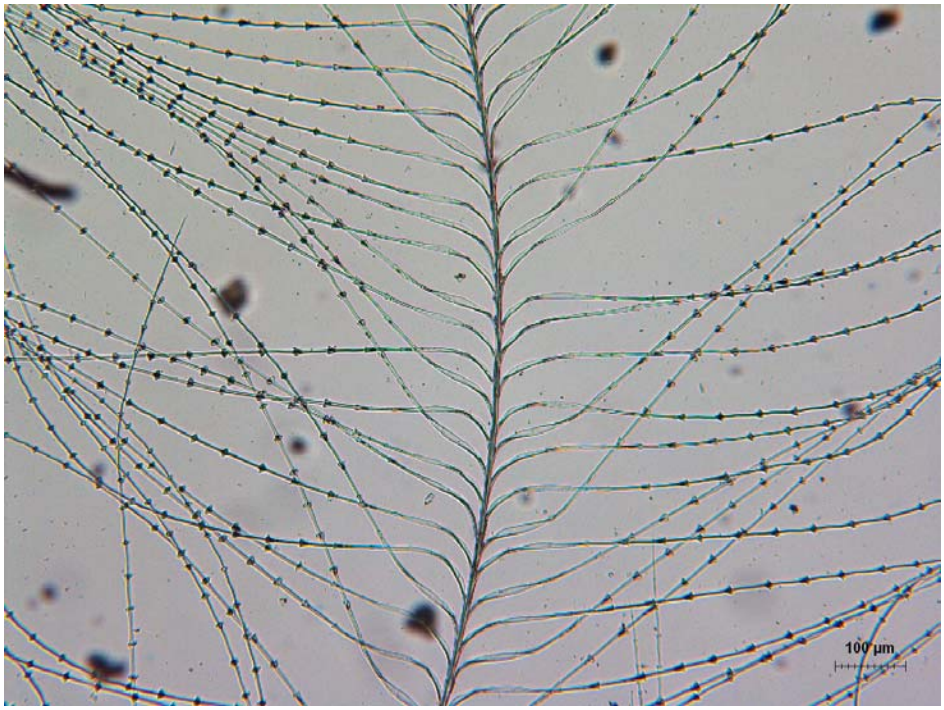

Figure 41. Masked Lovebird (Agapornis personata) 100X. The laterally flared nodes of parrots are diagnostic but some downy barbs may lack pigment at nodes while other barbs from the same individual bird may have pigment.

Node distribution: Nodes are very numerous and evenly distributed along the barbules. Nodes remain greatly expanded even at distal points on barbules.

Pigment pattern: Pigment, if present, is located at the node and is rounded or beaded when viewed at high power (Figure 39). Pigment is usually not stippled internodally as in Doves, and is concentrated at the node or barely extends below the node.
Diagnostic features: The very long barbules, the widely flared nodes that are observed all along the barbules length, and the pigmented nodes are characteristic of Parrots (Figure 41). Also, Parrots do not have villi on base cells. Not all samples will have pigmentation.
Similar species: Doves and Falcons. Doves usually lack pigment concentrated at the nodes and usually have more diagnostic 'crocus-shaped' nodes proximally on the barbules that become less apparent distally while Parrots have expanded nodes that occur consistently all along barbules. Falcons also look similar to Parrots but have a much finer internode width and typically have fewer expanded distal nodes. The proximal internode width of barbules is relatively wider in Parrots than in Doves and Falcons

Strigiformes (Owls, Barn-owls)

Although classified in a separate order, Owls are sometimes considered "birds of prey" due to their predatory behavior. There are 180 different species worldwide; 21 occur in North America. Feathers from Owls may be found in abandoned buildings where they nest, outdoor scenes, rituals, and are involved in wildlife law enforcement cases.
Barb length (100X): Long to very long.
Barbule length (100X): Long to very long; wispy distally.
Node shape: The proximal nodes of barbules are widely flared and expanded and appear cupped in shape (Figure 42). The wide nodes become less apparent medially on barbules and fade to simple or non-expanded nodes with elongated pigment at the distal portion of barbules. (Figures 43 and 44).
Node distribution: Most Owls have one to three proximal nodes that are widely expanded and cupped upward. The nodes taper distally to become completely indistinct from the long wispy barbule tips. Typically, pigment is present at all nodes but no expanded area is present around the medial to distal pigmented nodes (Figure 45).
Pigment pattern: Pigmentation is regular and uniform at nodes throughout the length of barbules. Nodes are not flared distally. Pigment is typically confined to the node but becomes very elongate as the node shape narrows distally (Figure 45).
Diagnostic features: Microscopically, Owl feathers have one to three proximal triangular nodes but quickly taper to unexpanded nodes that are not distinct from the barbule distally. Owls typically have pigment at nodes all along barbules.
The whole feather characteristics of these mainly nocturnal birds are distinctly soft and fluffy with long, 'hair-like' barbules on the pennaceous feathers that aid in silent flight.

Owls typically have this 'hair-like' texture on most whole feathers (Figures 46 & 47) and a diagnostic 'comb-like' edge on the outer primary (Figure 48).

Similar species: Owls are unique in their microstructure and are not similar to other species.

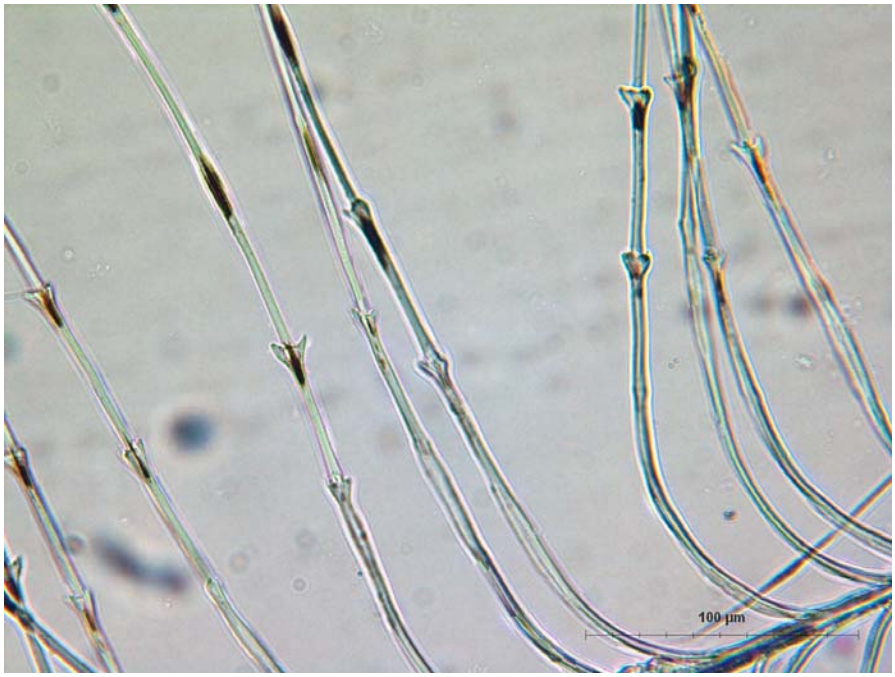

Figure 42. Short-eared Owl (Asio flammeus) showing proximal nodes that are cupped upward and have pigment that extends to the internode (400X).

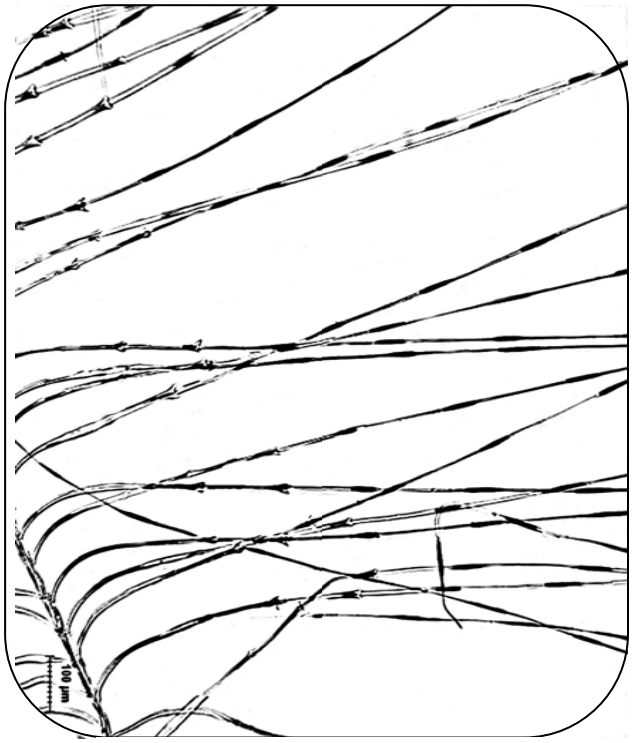

Figure 43. Schematic of node shape and distribution on barbules (100X).

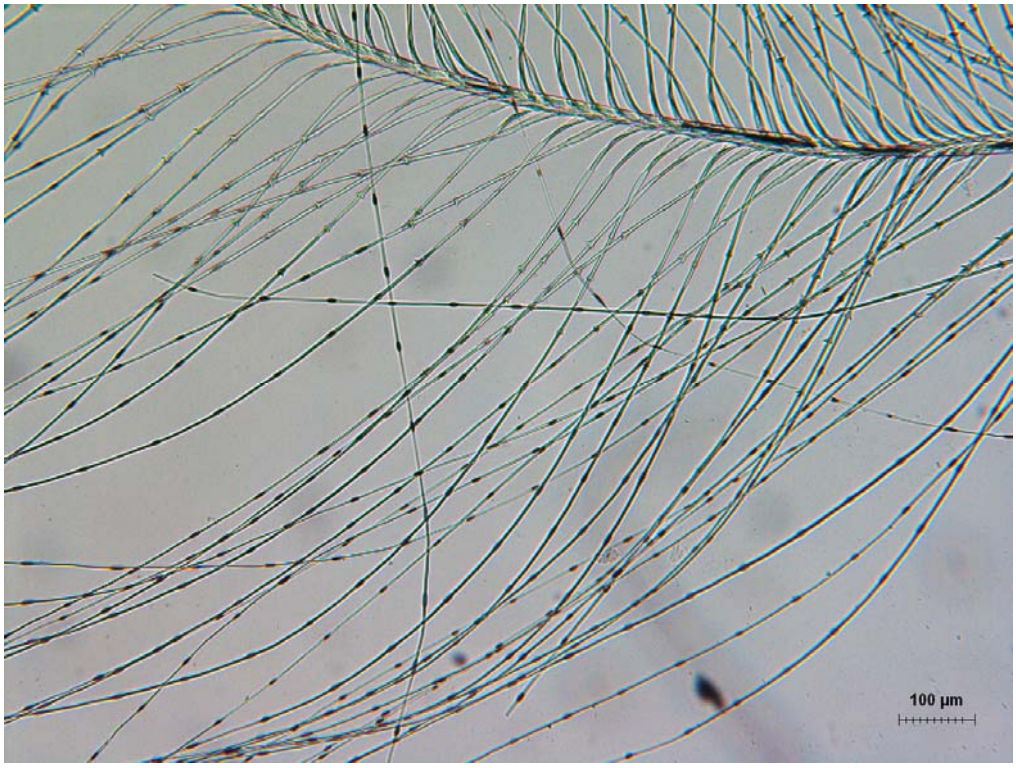

Figure 44. Barred Owl (Strix varia) showing long barbules with pigmented nodes.

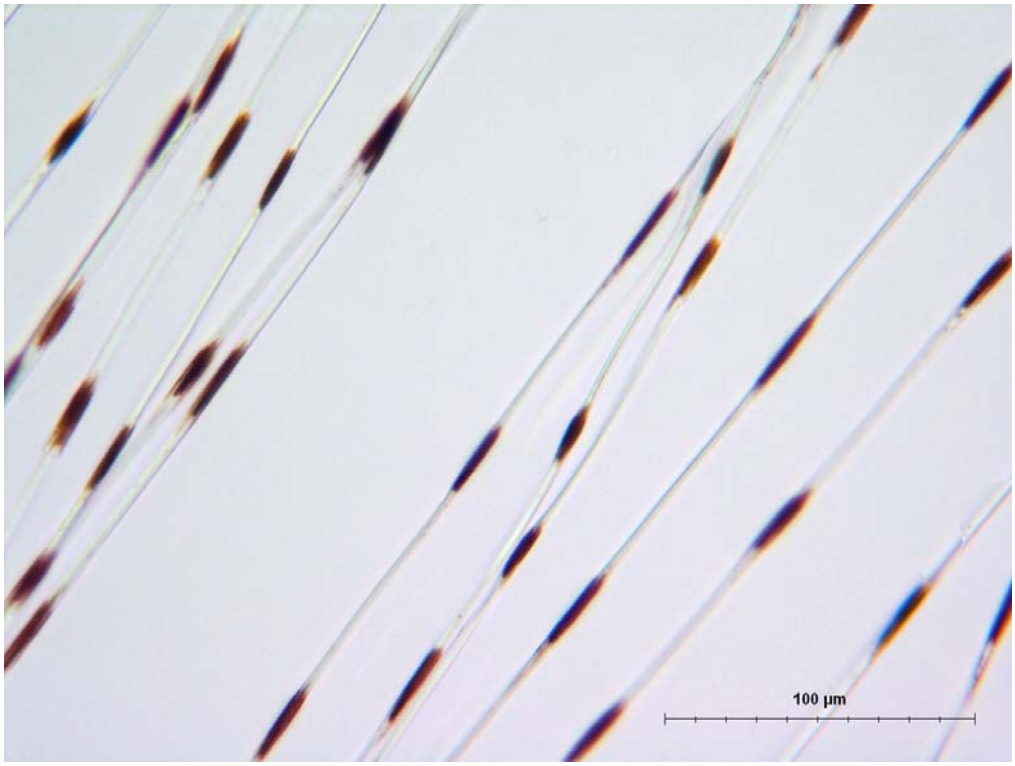

Figure 45. Barred Owl (Strix varia) showing the simple distal nodes and elongate pigment of owls (400X).

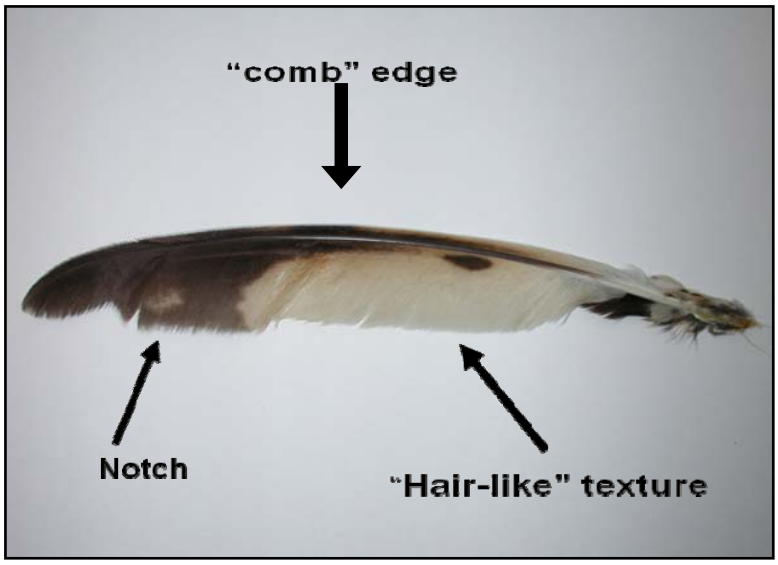

Figure 46. Strigiformes (owl) contour feathers have the diagnostic 'hair-like' texture on the inner vane of most flight feathers (Figure 47) and the 'comb' edge on the outer vane of the outer primary (Figure 48).

Figure 47. 'Hair-like' texture of owl feathers.

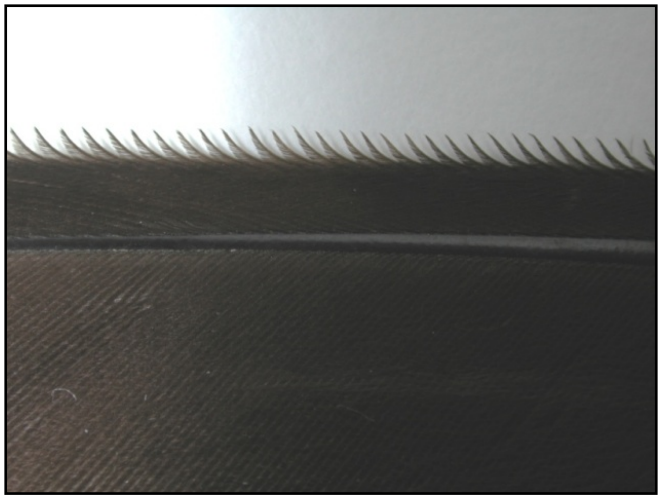

Figure 48. 'Comb-like' edge on outer vane of outer primary that is typical of owl feathers.

Passeriformes (Songbirds)

The birds in the Order Passeriformes include all of the songbirds and are commonly referred to as "passerines". This is the largest Order of birds and includes some 57 Families with more than 5,000 species worldwide. Passerines, or songbirds, are usually small birds, but the Raven is one of the largest members in this Order. Songbirds are not usually involved in human forensic crimes but because they are such a large group of birds, knowing some of the microscopic identification features will help eliminate these species from further consideration, especially in outdoor scenes. Because this group of birds is so large, only the basic microscopic characters are presented here. The key feature to passerine identification is the presence of villi. For a more detailed explanation of villi, see the special feather features section page 10.

Barb length (100X): Short to long.

Barbule length (100X): Very short to long.

Node shape: Nodes are typically slightly expanded with flared or rounded transparent projections at most nodes. Nodes are normally not spined, pronged, or formed into unique shapes. Pointed or knobbed villi are typically present on base cells.

Node distribution: Nodes usually occur all along barbules in uniform distribution with pigment mainly concentrated at the nodes throughout the barb and barbules (Figure 49).

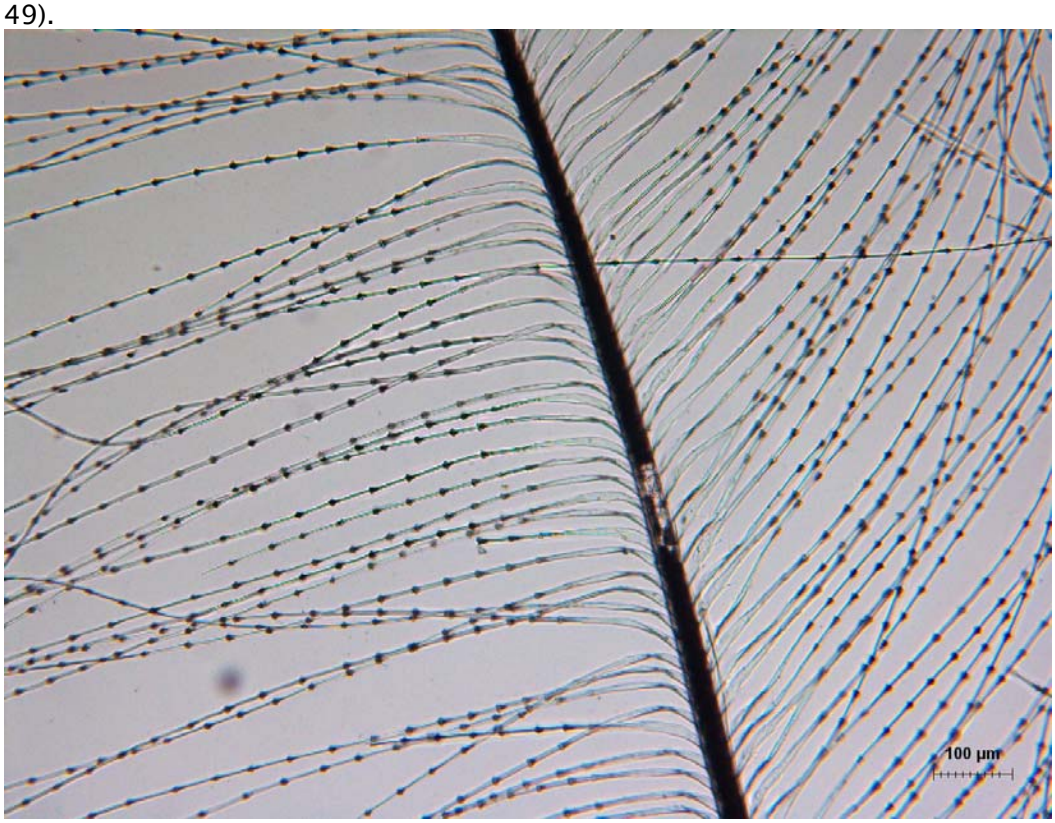

Figure 49. Photomicrograph of American Crow (Corvus brachyrhynchos) showing the typical features of passerine microstructures; many pigmented nodes distributed along the barbules (100X).

Pigment pattern: The pigmentation pattern of most passerines is uniformly distributed along barbules at the nodes and is normally confined to nodes or located near the node.

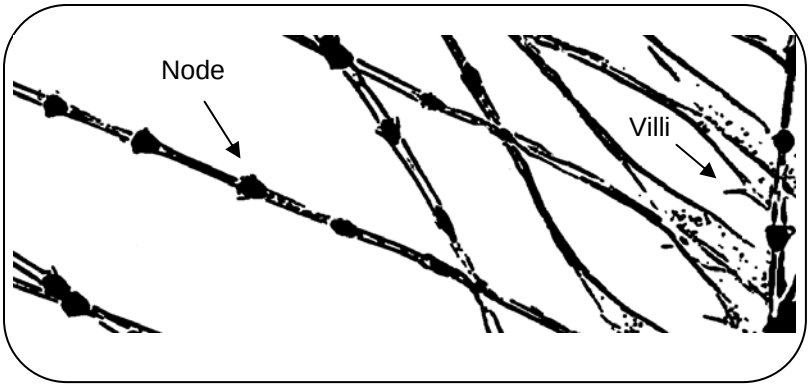

Figure 50. Schematic showing diagnostic villi and node structures typical of Passeriformes (songbirds

Diagnostic features: Villi are the characteristic feature of passerines and a few other groups of birds. Villi are located on the base cell region of proximal barbules (Figures 50 & 51) and are described in more detail earlier in this guide. The villi of passerines are typically knobbed (Figure 48), but sometimes may appear pointed. The first character to look for in a microscopic exam is the presence of villi. If villi are observed, then the feather is from a Passerine, Woodpecker, Hummingbird or possibly a Shorebird.

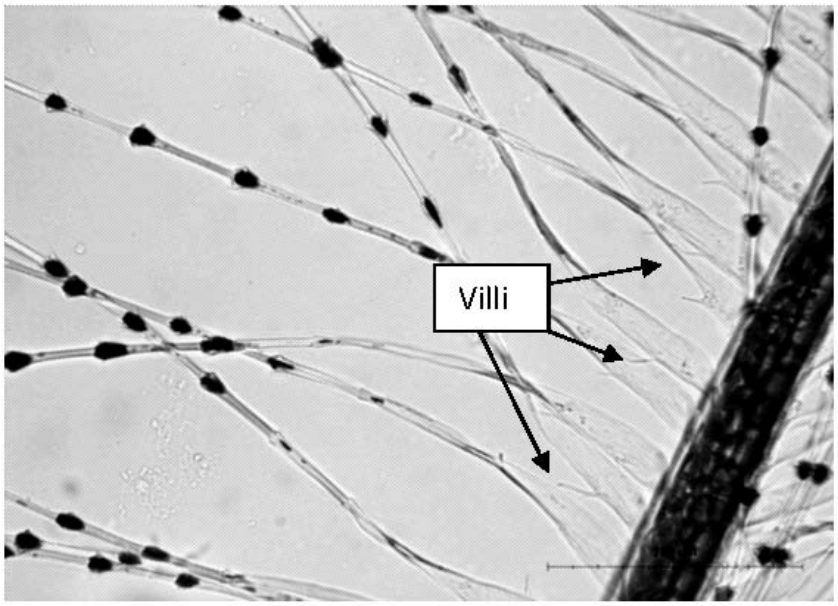

Figure 51. Western Kingbird (Tyrannus verticalis) showing the many knobbed villi typical of passerines (songbirds) 400X.

Similar species: Many species of birds appear to be similar to passerines microscopically because the nodes are typically pigmented uniformly along barbules as in Falcons, Shorebirds, and some other non-passerine groups. The presence of many knobbed and/or pointed villi on base cells set Passerines apart (Figure 51). Not all barbs and barbules will have villi and not all samples may have this characteristic; use caution when searching for this feature.

Conclusions

Three basic conclusions can be reached from a microscopic examination of feathers.

1. Confirmation that feathers or feather portions are present in the submitted debris; however, no diagnostic features are present that could lead to a more specific identification.

2. Identification of the Order, Family or species of bird.

3. Identification of the Order, Family or species of bird and comparison with a known source of the feathers resulting in an association or exclusion of an item or bird.

The feathers from the questioned (Q) source exhibit the same microscopic characteristics as the feathers comprising the feather portion of the known (K) sample and can be associated to the known or other sources containing feathers from the identified bird. This conclusion states that the questioned feather can be associated with a source bird; however, a caution must be made that the microscopic features of feathers are not unique to a particular bird to the exclusion of others within the same Order or Family.

Report

Some examples of report statements include:

Example 1:

The feathers found in/on the questioned source are consistent in microscopic structure to feathers found in Domestic Chicken feathers of the Order Galliformes. It should be noted that feathers cannot be identified to a particular bird to the exclusion of others within the same species, Family or Order using only microscopic structures. However, if sufficient feather fragments or whole feathers are available the sample may be positively matched to a museum specimen for exact identification.

Evidence: Q1 Down jacket (Item from victim), Q2 clothing from suspect

Duck and Chicken feathers were found on the clothing of the suspect that exhibit the same microscopic characteristics and structures to the feathers comprising the Q1 Down jacket. Accordingly, these feathers are consistent with having come from the Q1 Jacket; however, it should be noted that feathers cannot be identified to a particular bird to the exclusion of others within the same species.

Note – you may have to limit the conclusions to state the feathers came from the same Family, or Order instead of species depending on the features present and what you are able to determine from the feather microstructure.

Example 2:

Feathers were found in/on the questioned items; however, these feathers do not contain sufficient characteristics for a determination of species, Family or Order.

Evidence: Q1 shirt from suspect, Q12 down filled blanket

The feathers found on the Q1 shirt are consistent with Pigeons of the Order Columbiformes. The feathers comprising the Q12 down filled blanket are consistent with Geese of the Order Anseriformes. These feathers are not consistent and, accordingly, the Q1 feathers could not have come from the Q12 blanket.

In writing a report, the limitations of the examination must be addressed.

Testimony

When testifying regarding an identification or association of a feather or feather fragment, the expert witness must demonstrate their qualifications by detailing their training and use of an extensive reference collection. The witness should educate the jury on feather structures and what portions are diagnostic prior to discussing their conclusions. The limitations of the science need to be addressed as well because many jury members associate an "identification" with coming from a single source. In feather analysis, an identification is related to the bird type but the association is between the evidence and a known sample.

Significance

The significance of a forensic analysis of feathers will always be case dependent. An association of a feather to a source may indicate contact; however, the rates of transfer and persistence have not yet been fully analyzed for feathers. The reliability of an analysis of the microstructure must take into account the education and training of the examiner in feather identification. The weight placed on a bird identification or association should also consider that variability is found within some Orders or groups of birds.

The ability to identify and compare the microscopic characteristics of feathers is a skill gained by extensive training in microscopy and analysis of numerous groups of birds. Feather identification and matching tests must be conducted to demonstrate the

ability to correctly associate feathers with a particular source. Beyond establishing competency and proficiency in this analysis, feather identifications should be confirmed by another qualified examiner prior to a report being issued.

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Websites for Feather Identification

U.S. Fish and Wildlife Service Forensics Lab, Ashland, OR – Excellent website for wildlife forensics. Feather ID notes and Feather Atlas with digital feather images.

http://www.lab.fws.gov/index.php

Slater Museum of Natural History, University of Puget Sound, WA – digital images of bird wings.

http://digitalcollections.ups.edu/slater/

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Websites for Bird Protection Acts

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http://www.fws.gov/migratorybirds/RegulationsPolicies/mbta/mbtintro.html

Keywords: Feather, Identification, Plumulaceous, Downy, Barbs, Microscopy

Glossary of Terms

The following terms are defined as they are used in this guide.

Afterfeather – secondary structure of contour feathers which originates on the ventral side of feathers at the superior umbilicus of calamus. Present on feathers of most birds but may be absent or vestigial.

Barb – primary lateral branch of feather rachis that collectively form the vanes of feathers. Barbs are further divided into barbules. Barbs can be of pennaceous or plumulaceous (downy) type.

Barb length– for this guide we define the length of the barb as short or long. Long barbs extend well beyond the field of view when examined microscopically at 100 X.

Barbule – lateral branch off of the rachilla of a barb; smallest division of feather. Collectively forms vanules of a barb. Each barbule can be divided into a base and a pennulum. Downy barbules have diagnostic micro-characters that aid in the identification of some groups of birds.

Barbule length– for this guide we define the length of the barbule as short, medium, or long. Long barbules extend far beyond the field of microscopic view when examined at 100X; medium extend to the edge of the field of view; and short are well within the field of view.

Base cell – the proximal portion of the barbule that attaches to the rachilla. Usually delineated by a cell division just before the pennulum and are usually flattened or strap-like in appearance.

Calamus – the proximal portion of the feather shaft that lacks barbs. Attaches feather to the skin at the inferior umbilicus and is divided from the rachis at the superior umbilicus.

Contour Feathers – the surface layer of feathers which give a bird its characteristic form including the body, wings and tail.

Downy – see Plumulaceous

Flight Feathers – the wing (remiges) and tail (retrices) feathers of a bird that provide lift, thrust, and maneuverability for flight.

Hooklets – hooked tips of distal pennaceous barbules that interlock with the plate of adjacent pennaceous barbules.

Internode – the portion of a plumulaceous pennulum cell between two nodes. This portion of the barbule usually lacks any distinct morphological characters, may have pigment, and can vary in length and width.

Node – the portion of the plumulaceous pennulum (barbule) were cells join. Barbules are made up of telescoping cells that are connected at nodes. The node is typically the distal portion of each cell that expresses morphological shape, structure, and sometimes contains pigmentation.

Pennaceous – the distal barbs of a feather that consist of somewhat flattend, stiff barbules with interlocking hooklet structures forming coherent vanes. The region of the feathers visually seen on a bird (color/pattern) that also provides the protective outer covering of the body, and strength of flight feathers.

Pennulum – the main portion of a barbule that consists of cells (see Figure. 7). In plumulaceous barbules, the pennulum has the diagnostic node and internode characters with progressively tapering cells. In pennaceous barbules, the pennulum has interlocking hooklet structures. Pennulum is a term that is interchangeable with barbule but the term pennulum usually refers to barbule minus the base cell.

Plumulaceous – (analogous to "downy") the proximal barbs of a feather that lack interlocking hooklet structures creating a fluffy "downy" texture. Plumulaceous barbs are closer to the bird's body and the general function is thought to be for insulation. The plumulaceous down of body contour feathers is the best region for examining and analyzing feather characteristics for microscopic identification.

Primaries – the outer (distal) flight feathers of the wing. Most birds have 9-10 primaries.

Rachilla – the central shaft of a downy feather barb where the barbules attach.

Rachis – the central feather shaft that has barbs attached to it.

Remiges – the flight feathers of the wing (includes primaries, secondaries, and tertial feathers).

Retrices – the flight feathers of the tail. Most birds have 10-12 retrices.

Secondaries – the inner (proximal) flight feathers of the wing. The number of secondaries varies with species (usually range from 9-25).

Semiplumes – feathers intermediate in form and structure that have a developed central rachis, but lack well developed pennaceous regions. Found beneath and between contour feathers.

Shaft – the central, stiff structure of a feather that consists of the proximal calamus and the distal rachis.

Tertials – the innermost flight feathers of the wing next to the body. Birds usually have 3-4.

True Down – down feathers beneath and between contour feathers that provide added insulation; The lack of a well developed central rachis gives these feathers a "pom-pom" appearance.

Vane – the region of a feather on each side of the rachis.

Vanule – the region of a barb on each side of the rachilla.

Villi – small, transparent projections located on the base cells of plumulaceous barbules in some groups of birds. (singular - Villus).