29.0 Identification of Galliformes using the Microstructure of Feathers: Preliminary Findings

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Introduction

The correct identification of a species is extremely important in wildlife offence cases such as poaching and illegal trade in wildlife. One of the important contribution of wildlife biology is identification of species using a variety of techniques, thereby helping law enforcement agencies and control of illegal trade. Birds are poached for 'meat', 'feathers' and for the illegal pet trade. Identification of birds by studying the fine structure of feathers is not a common practice in India (Grub, 1989; Mathew, *et al.* 2003). Some studies, however, are available in connection with identification of bird hits on aircrafts (Satheesan, 1994; Grub, 1989; Khola, 1988; Sodhi, 2002; Mathew *et al*. 2003).

Identification of bird feathers particularly those obtained after skinning a bird or from other products that are decorated with feathers, has been a difficult task. Due to lack of standardized methods and expertise in this subject in India (Grub, 1989) as well as in most parts of the world (Prast and Shamoun, 1997; Ciecelak and Dul, 2007), identification of a species feathers remains complicated. In this connection, the Wildlife Institute of India (WII) has initiated a study aimed to standardize the methodology for identification of birds by studying the fine structure of feather especially for the successful enforcement of Wildlife (Protection) Act, 1972. Preliminary findings of this study, particularly with respect to galliformes are discussed in this paper.

Structure of a body feather

Generally five types of feathers cover the body of a bird: contour, semiplumes, down, bristles and filoplumes. Of these, *contour feathers* are further classified into two categories depending upon their location on the body, typical *body feathers*, and *remiges-rectrices*. The structure of *body feathers* (Fig. 1) is easily reviewed by examining the most familiar type of feathers, those that covering over most of the body (Prast and Shamoun, 1997). The base of the feather shaft is called calamus which is short, tubular and implanted in a socket known as feather follicle. Beyond the calamus is a long, tapered section, the rachis, which bears branches, the barbs, on its sides. The division between the calamus and the rachis is marked by the lowermost barbs and by a bit on the underside of the shaft, the superior umbilicus. At the bottom of the calamus is an opening, the inferior umbilicus, through which the pulp enters a feather while it was growing. The rachis is composed of an outer thin, solid layer, the cortex, and an inner, thick, spongy core, the medulla or pith. The barbs on each side of the rachis constitute a sheet known as a vane. A barb consists of an axis, the ramus, and many closely spaced branches, the barbules, or radii. A barbule is essentially a stalk of single cells that are serially differentiated to some degree. It has a base of compressed, fused cells and a distal segment, the pennulum, of cylindrical, jointed cells. In a downy barbule (plumulaceous), the base is short and straplike, and the pennulum is long and simple, resembling a stalk of bamboo. The distal ends of the pennulum cells are variously swollen or furnished with tiny prongs. Pennaceous (distal) barbules are often highly differentiated and diverse outgrowths, collectively termed barbicels. Proximal barbules, those facing the proximal end of a feather, have a pronounced flange along the dorsal edge of the base, and barbicels that are small, simple, or absent. A barbule has different types of nodes, basal nodes, which are distributed towards the base, distal nodes are the areas towards the tip of the barbule, middle portion of the pennulum is called middle nodes and the terminal node is the distal-most node on the barbule (Prast and Shamoun, 1997).

Methods

Various zoos and wildlife field biologists were requested to collect feathers from different localities spread throughout the countries. All the collected feathers were labeled with information such as name, sex of the species, place, date of collection, type of feather along with name of the collector. After receiving the samples from the field all the labeled information was recorded in the laboratory file. If the feather sample was in good condition then it was considered for further identification. Typical *body feathers or down feathers* were used to study the fine structure of the feathers. *Body feathers* were chosen because of its ubiquitous distribution

over the body of a bird and they have two distinct regions called pennaceous and plumulaceous. First, entire feathers were scanned for its colour patterns. Both pennaceous and plumulaceous colours and its barb distribution patterns were noted. These features often provide sufficient basis for definitive identification by experienced ornithologists. Afterwards, a barb from plumulaceous region was picked off without damaging any part of the barb and mounted on the slide by using DBX mountant. After drying the slides for 12 hours at room temperature, they were observed under high-power optical and light transmission binocular microscope attached with video screen (Sony, Trinitron). Image of the barbules was viewed and read on the screen. With the help of cytometer (0.01 mm accuracy), length of barbule, length of internodes and length of basal nodes were measured. Number of nodes in the entire pennulum was counted and its distribution patterns also recorded. Structure of node, intensity of pigmentation, structure of prongs and terminal nodes were observed. After studying the fine structure of barbules, a part of barb was photographed by using the Black Box of Nikon camera, which was also attached to the same microscope. All the slides were carefully preserved for future reference.

In order to differentiate the bird groups by using all the morphometric and meristic characters of barb, discriminant function analysis was used with all those variables for which the data were recorded. Measuring the length of a barbule was much easier than counting the number of nodes in a barbule. If there is a correlation between the length of barbules and number of nodes then it may advisable to avoid time on counting the number of nodes present in barbule. Correlation test was performed to find out the relationship between the barbule length and number of nodes, the length of internodes and number nodes.

Finally, a reference sheet was prepared for each species with the help of existing literature references. Each reference sheet gives information regarding the classification of species, local name, general body appearance, distribution range, population status, legal status and microstructure of feathers, which includes colour, length of barbule, number of nodes, length of internode, nodal structure, structure of terminal node, prong structure, node distribution, node density, length of basal node and pigmentation. Apart from this, a picture of the species, scanned picture of the feathers and a photograph of a part of the barbule was also included in the reference sheets (see example Reference sheet of Temminck's Tragopon).

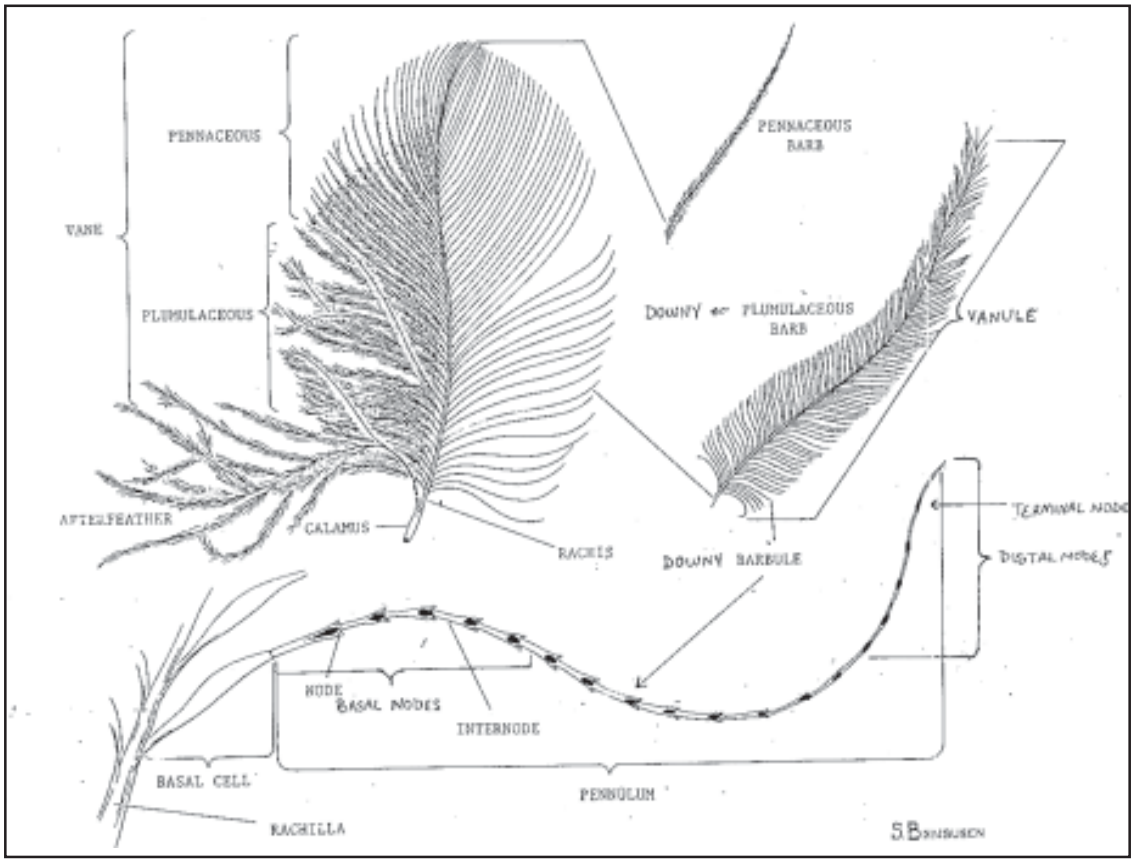

Figure 1. Structure of a contour feather (Source: Prast and Shamoun, 1997)

Results

A total of 64 feather samples were received from various zoos and some wilderness areas of India. Of these, 30 samples belonging to 24 species representing five orders were found suitable for the study. Of the 30 samples, 10 samples belonged to galliformes, which included eight wild species and two domestic fowls (which had two different body colour). In total, nine characters of a barbule were used to discriminate the five groups of birds. Of the nine characters, total number of nodes in a barbule, density of nodes and structure of prongs were extracted by the test as being highly useful to discriminate the five groups of birds *viz*. Galliformes, Pelecaniformes, Falconiformes, Coraciiformes and Psittaciformes. Pelecaniformes could easily be differentiated from other groups because of its very low (or even absence) pigmentation and its number of nodes. Psittaciformes and Coraciiformes are very close, however, these groups were distinct due to the differences in the sizes of the basal nodes. Hornbills have longer basal nodes compared to the parakeets. Apart from basal node, structure of nodes was also useful to differentiate these two groups, hornbills has narrow nodes. However, parakeets had heart-shaped or triangular shaped nodes. Compared to parakeets, hornbills had less pigmentation on the barbule. Because of identical prong structure and number of nodes, these two groups appeared to be close. Group centroids of all five groups of birds occupied different locations in a plot, which were separated along the extracted characters of barbule.

Galliformes (Phasianidae)

A total of 10 birds belong to eight species of wild birds and two domestic fowls which had two different body colour were studied (Table 1). All the species were discriminated (DCA) using the feather characters such as the total number of nodes in a barbule, density of nodes and structure of prong. Red Junglefowl (RJF) and Western Tragopan (WT) were close, however, RJF was slightly different from WT because of nodal shape. RJF has prominent triangular shaped nodes and WT also has triangular shaped nodes but very narrow. Pigmentation was also more in WT barbule than in RJF. Ring-necked Pheasant, Grey Junglefowl and grey coloured Domestic fowl were very close, however, these species were different due to different shaped nodes and length of prongs (Table 1). Interestingly, grey coloured domestic fowl and red coloured domestic fowl were varied widely, however, these two birds have identical node structure. Three species of tragopan were studied and found that all the species varied due to different structures of node, terminal node and prong.

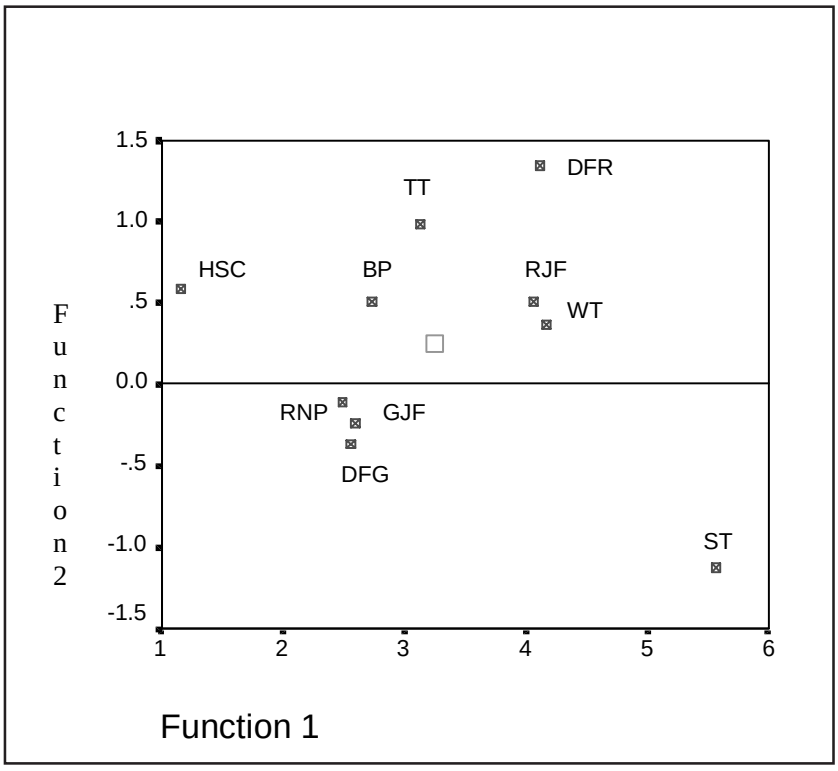

Figure Plot of species centroids on the first two axis derived from a discriminant function analysis to classify species by fine structure of feather (Function 1: number of nodes and structure of prongs, function 2: node density). TT- Temminck's Tragopan, DFR - Domestic fowl (red in colour), HSC - Himalayan Snow cock, BP - Burmese Peafowl, RJF – Red Junglefowl, WT – Western Tragopan, RNP – Ring-necked Pheasant, GJF – Grey Junglefowl, DFG – Domestic fowl (Green in colour), ST – Satyr Tragopan.

Discussion

Bird species could be identified at three different levels depending upon the type of samples which can be received from the field station or from the enforcement authorities. If the sample is a whole bird then it could be identified without any difficulty by referring to the Part-I of the 'Reference Sheets' (see Reference Sheet of Temminck's Tragopan) or the available bird field guides. If only feathers are received, it also could be identified without much difficulty except in case of some groups of birds such as warblers, babblers, raptors and female birds of most of the groups, by referring the Part-II of the Reference Sheet. Third level of identification is required if a part of the feather (plumulaceous portion) is received, or which could not be identified using the first two parts of the Reference Sheet and would be identified with the Part-III that describes the microstructure of the barbule.

Combination of both morphometric and morphological characters of barbules are essential for the identification of birds at group level. The total number of nodes in a barbule, density of nodes and structure of prong appears to be unique to a group. We believe that these characters are useful for the group identification. Sometimes, species could also be identified by studying these characters of a feather. Pelecaniformes can be easily differentiated from the other groups (studied) because of either low or absence of pigmentation on barbule. Once the bird group is identified, for then the species level identification, a combination of both morphometric and morphological characters such as structure of nodes, terminal node and the length of basal node is required. In the case of Falconiformes species could be identified by the using the same characters, which were used for the group identification. However, in the case of Pelecaniformes, morphological characters of barbule alone may not be useful to identify the species since almost all the species have similar morphological features. Due to difficulties in getting the samples of more species belongs to Psittaciformes and Coraciiformes (Bucerotidae), it may not be appropriate to comment on these groups based on low sample size.

We strongly believe that if birds were identified at group level then it would not be difficult in identifying the same at species level since we have preserved the Reference Slides of all the species studied. Our preliminary experiment clearly shows that different birds belonging to the same species may have different meristic characters but will certainly not have different morphological characters such as structure of nodes, prongs, terminal nodes and the intensity of pigmentation on the barbule.

Conclusion

Our study demonstrates that identifying upto order level such as Galliformes, Pelecaniformes, Falconiformes, Coraciiformes and Psittaciformes using the fine structural characters of feather is possible. The reference sheets and slides prepared through this study will aid in this process. However, going upto species level remains a limitation. Parameters such as number of nodes, shape of the nodes, and pigmentation distribution will be helpful regarding identification at species level. We had some difficulties while describing the structure of nodes due to vague shapes, in this case we could refer the previously mounted slides for clarification as well as confirmation. Since all the slides of studied species are preserved, identifying them won't be a problem. This study has demonstrated the usefulness of the present approach for primarily five bird orders. However, with the availability of more samples from other groups in trade, the scope of this study could be expanded.

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Table 1 : Microstructural characters of Down Deather of selected Galliformes.

Name	Length of barbule (mm)	Nodes (Nos.)	Basal node (mm)	Internode (mm)	Density of nodes/mm of barbule	Node structure	Pigmentation	Terminal node	Size of Prongs
Domestic fowl (grey in colour)	2.66	43.57	0.13	0.06	16.86	Triangular	Low	Flared	Large
Domestic fowl (red in colour)	3.86	61.71	0.16	0.06	16.42	Triangular	Neck region of nodes has thick pigmentation	Flared	Small
Grey Junglefowl	2.09	35.57	0.15	0.06	17.87	Triangular	High	Flared	Medium
Red Junglefowl	2.61	46.00	0.12	0.05	18.04	Ring like	Low	Flared	Small
Ring Necked Pheasant	1.90	31.29	0.16	0.06	17.38	Slightly triangular shaped	High	Flared	Small
Himalayan Snowcock	4.02	59.57	0.14	0.07	15.12	Ring like	Medium	Un-flared	Nil or very small
Satyr Tragopan	4.26	63.14	0.10	0.07	14.95	Ring	Low	Usually not flared	Nil or very small
Temminck's Tragopan	3.83	59.29	0.21	0.06	16.11	Triangular	Medium	Flared	Medium
Western Tragopan	3.35	51.00	0.21	0.06	15.91	Triangular like	Medium	Usually not flared	Small
Green Peafowl	1.81	33.86	0.14	0.05	19.67	V shaped	High	Usually not flared	Small

Reference Sheet

Temminck's Tragopan *Tragopan temminckii*

Classification:

Order: Galliformes
Family: Phasianidae

Local name(s): *Bop* (Tibet); *Oua oua ky* (China)

Part I (Fig 1) Part II (Fig 2) Part III (Fig 3)

David Mead

General appearance: Male has grey spotting on red underparts, and red colouration to upperparts. Female has prominent white spotting on underparts (Fig 1).

Distribution: Resident East Himalayas in Arunachal Pradesh.

Status: Rare

Legal status: The Indian Wildlife (Protection) Act, 1972: Schedule I – Part III

Feather Characteristics:

Colour : Pennaceous portion is smaller than plumulaceous vane. Pennaceous region with or without larger, greyish drop-shaped spotting, surrounded by red colour. Plumulaceous barbs are blackish in colour (Fig 2).

Length of barbule: Small/Medium/Long, average length of barbule is 3.83 (±0.21) mm. Fig 3.

Terminal node: Terminal node is flared and has cilia like three prongs at the tip.

Prongs structure: Short and cilia like prongs at the slightly enlarged nodes.

Distribution of nodes: Uniformly distributed throughout the barbule

Node density: 16.11 nodes mm^{-1}, an average number of nodes per barbule is 59.28 ± 2.97)

Length of basal node : 0.21 mm

Pigmentation: More pigmentation stippled in the node regions but it gradually diminished towards the tail.