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COMMUNICATION

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COMMUNICATION

Identification of a unique barb from the dorsal body contour feathers of the Indian Pitta *Pitta brachyura* (Aves: Passeriformes: Pittidae)

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Abstract: Earlier research on feather morphology emphasized comprehensively on the body contour feather than various other types of feathers. Therefore, we conducted a systematic study on all feather types of the Indian Pitta *Pitta brachyura*, a passerine bird native to the Indian subcontinent. Feather barbs from wing contour, tail contour, body contour, semiplume, down, powder down, and bristle feathers were retrieved from the bird and observed under a light microscope. Primary flight feathers from the right and left wing were longest (85.17 mm and 87.32 mm, respectively), whereas bristle feathers were the shortest (5.31 mm). The mean barb length was observed to be the highest (11.37±0.47 mm) in the wing feather followed by body contour (8.31±0.39 mm), semiplume (8.27±0.22 mm), tail feather (7.85±0.50 mm), down (6.45±0.21 mm), powder down (6.04±0.23 mm), and bristle (2.70±0.07 mm). Pearson correlation was found positive for barb length and feather length of down feathers (r= 0.996, p ≤0.05). We observed a novel type of barb the first time from dorsal body contour feather having plumulaceous barbules at the base followed by pennaceous barbules. This unique barbule arrangement is termed 'sub-plumulaceous' as it is distinct and analogous to known 'sub-pennaceous' type arrangement found absent in passerines.

Keywords: Feather, microscopy, Pitta brachyura, sub-pennaceous.

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Ethics statement: This research was conducted in compliance with the government guidelines.

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Author contributions: R.P.S collected the sample; conceived the idea; and supervised the research. R.P.S and P.P generated the funds for the study. P.D, S.D.R and S.K.S generated the data. R.P.S and P.D wrote the manuscript and analyzed the data. All the authors reviewed the manuscript.

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The Communication published in the 26 June 2021 issue of JoTT was withdrawn due to a faulty map. The same Communication with a corrected map is published here.

INTRODUCTION

Feathers are the most numerous, elaborate, and diverse derivatives of avian integument (Gill 1995). Collectively referred to as plumage, feathers are extraordinary evolutionary innovation evolved over a million years that perform a wide variety of functions in birds from insulation, protection, mate attraction, sound production to locomotion (Gill 1995; Lovette & Fitzpatrick 2016). All birds have different types of feather assorted in their plumage (Gill 1995). These feathers vary considerably in macroscopic (colouration, texture, pattern, shape, and size) and microscopic characteristics (minute morphological appendages) (Dove 1997a). The studies on types, characterisation and microstructures of feathers give us a deeper understanding of feather form and function (Lee et al. 2016). Although a handful of studies on feather examination have been reported over the years, still many questions regarding feather morphology have not been answered (Lee et al. 2016). Morphological examination of feather structures in the present day has acquired importance in diverse range of disciplines such as phylogeny (Dove 1997a ; Bensch et al. 2009), palaeontology (Messinger 1965; Dove et al. 2010), archaeology (Harwood 2011), avian ecology (Galván 2011; Fairhurst et al. 2013), wildlife forensics (Dove & Coddington 2015), biomechanics (Kulp et al. 2018), and material sciences (Lingham-Soliar 2017).

Typically a feather is made of a central rod like staff with numerous interlocking barbs attached to it on either side. The central shaft of each barb has minute branch like structures called barbules (distal/proximal) that extend on its either side (Images 1, 2). Barbules can be either pennaceous /plumulaceous and harbour various microstructures such as hooklets, nodes, internode, cilia, villi, prongs etc. Though individual groups of feathers may vary amongst themselves on various accounts of functionality, they share vast similarities in certain basic structural characters (Gill 1995). The literature regarding the nomenclature of feather structure and its micro characteristics till date lacks uniformity and is mostly based on convenience of the authors (Lovette & Fitzpatrick 2016). The authors would also like to shed light on the fact that many intermediate and exceptions might exist within the nomenclature used in this report. Adapting from various previous text books (Chandler 1916; Gill 1995; Lovette & Fitzpatrick 2016) and research papers we have resorted to widely applicable terminology based on most logical nomenclature perceived by the authors.

Over the years very few research reports have been published on feather identification and its structural characterisation (Lee et al. 2016). A few notable and exceptional reports do exist in the discipline of feather studies. In this particular aspect, a landmark work on feather characterisation and systematic studies was done by Chandler (1916). In his work Chandler (1916) adopted a universal nomenclature for various microstructures in a feather and classified feathers into plumules (unspecialized plumules, powder down, oil gland feathers, and nestling types), filoplumes, and contour (remiges, retrices, unspecialized contour feathers, ornamental plumes, ear coverts, facial bristles, and facial ruffs). In 1965, Messinger with the help of Hargrave successfully standardized a method to identify feather fragments from archaeological feather remains (Hargrave 1965; Messinger 1965). Day in 1966 studied the microstructures of plumulaceous barbs of contour feathers to identify various species of birds, using basic methodology as described by Chandler (Day 1966). Robertson (1984) studied plumulaceous barbs of contour feathers and prepared a detailed scheme for species identification from feather microstructure. He quantified the numerical variations in feather microstructure amongst species by measuring the length of barbules and number of nodes per barbules thus addressing the lack of numerical evidences in Chandler’s (1916) work. In recent times, Carla J. Dove (Dove 1997a, 2000) used plumulaceous barbs of body contour feathers to successfully demonstrate inter-species differences and develop various forensic techniques useful in identification of species. In 2015, Lee and colleagues used the microstructures in plumulaceous barbs of body contour feathers for taxonomic identification of Australian birds (Lee et al. 2016). The study was remarkable in the fact that it used simple methods inspired from Chandler (1916) to create a feather identification catalogue of various illegally traded birds in Australia.

Previous studies on feather morphology are inclined in the direction of species identification and phylogenetic differentiation (Robertson et al. 1984; Dove 1997a, 2000). Barring Chandler (1916), previous reports have cleverly avoided elaborating on micro-structural differentiation in different ‘groups’ of feathers, selecting mostly ‘body contour feathers’ from the breast region as the subject of study. Such studies elaborated more on species differentiation but created a considerable knowledge gap in the understanding of differential structural characteristics of various types of feathers present in an individual bird. Most of the previous studies on feather investigations have been conducted either in bird species of northern America or Australia (Dove & Coddington 2015; Lee et al. 2016). Very few minor reports have been published on birds of southern Asia (Songyan et al. 1995; Lee et al. 2010) and even fewer reports about feather morphology of bird species of Indian subcontinent has ever been published. With absolutely no in-depth reports of feather morphological studies of birds of southern Asia (birds of Indian subcontinent in particular) has led to a considerable knowledge gap in this particular aspect. Therefore, our group has taken an initiative to create a feather atlas for Indian birds, and this study is a part of the same feather atlas initiative.

The main objective of this study is to quantify macro and micro characteristics of various types of feathers from different anatomical locations of an individual bird species, the Indian Pitta *Pitta brachyura*. Secondly, we aim to create the very first comprehensive report on feather morphological examinations in any endemic species of bird of the Indian subcontinent. Third, we aim to standardize a protocol that can be used for systematic identification and morphological studies of various applied aspects of feather investigations. The implications of our study can inculcate a whole range of in-depth feather analysis as a tool for feather form and function elaboration or as a phylogenetic identification tool or can be used for applied wildlife forensic research.

METHODS

Target bird description

The Indian Pitta is a member of the Pittidae family of the order Passeriformes. Classified under IUCN category as 'Least concern', one such individual was found dead in the premises (11.059^{0}N, 76.814^{0}E) of our institute and was used as a specimen for this study. The dead specimen was collected with due permission from forest department (Ref.No.WL5 (A)/2219/2018; Permit No. 14/2018). Covering a large range, the Indian Pitta migrates to various parts of peninsular India during winter (Figure 1). Generally the Indian Pitta is extant up to an elevation of 1,700 m in the entire peninsular India inhabiting deciduous and evergreen forests (Lambert & Woodcock 1996).

Feather sampling

We classified feathers broadly into contour (wings, tail, and body contour) and non contour (semiplume, down, powder down, bristle, and filoplumes) category (Gill 1995). Wing feathers were further sub-divided into primary flight feathers and secondary flight feathers following Lovette & Fitzpatrick (2016). The specimen was searched meticulously to collect all the various types of feathers. One primary flight feather, one secondary flight feather each from left and right wing was sampled along with a single feather from the tail. Similarly, one body contour from the ventral side and another body contour

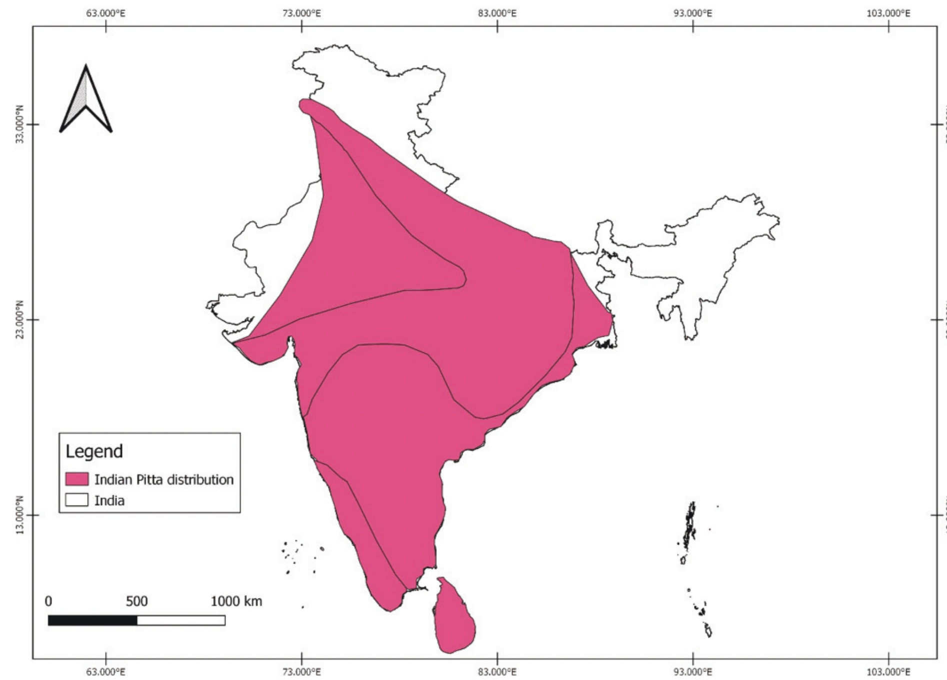

Figure 1. Species distribution map of Indian Pitta bird showing endemicity to the geographic range of Indian subcontinent. The map is adapted with permission from BirdLife International and Handbook of the Birds of the World (2017).

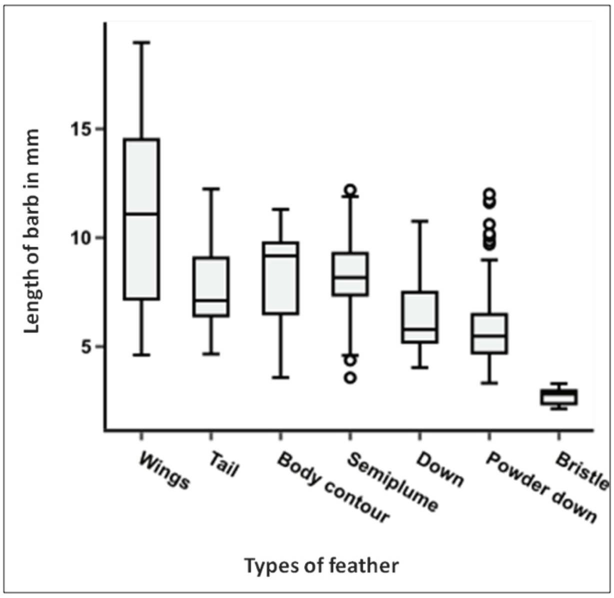

Figure 2. Numerical distribution of barb lengths in various types of feather.

from the dorsal side of the bird were sampled. In case of semiplume, two feathers each from ventral and dorsal side of the bird and one feather from the tail were sampled. One feather each from ventral portion, dorsal portion, right wing, left wing and tail were sampled for down feathers. In the same way, one feather each from ventral portion, dorsal portion, right wing, left wing, and tail were sampled for powder down feathers. Five bristle feathers were sampled from the chin and orbital region of the bird. After repeated searches through the cadaver of Indian Pitta, filoplume feathers couldn't be identified leading to omission of Filoplume observations in this study. The feathers were plucked carefully using surgical forceps (number 00) during sampling (Image 3).

Feather barb sampling

A methodical representation of the number of barbs sampled are mentioned in Table 1. After a feather was carefully plucked from the specimen, it was cleaned using 70 % ethanol solution (Lee et al. 2016). At first, each feather was placed carefully on a plain paper along with a scale, labelled properly and photographed. The length of the feather was noted and the feather was marked into three equal intervals termed 'proximal', 'intermediate', and 'distal' sections, respectively (Dove 1997b) (Image 4). For wings and tail feathers, vanes were classified as outer vane and inner vane following Chandler (1916) (Image 4). Three barbs from both vanes (inner and outer) of each section (proximal, intermediate, and distal) from a single feather from wings and tail were sampled (Image 4). A total of 18 barbs were sampled from each of the wing and tail feathers (5 feathers in total) (Table 1). For other types of feathers (body contour, semiplume, down, and powder down) five barbs from each of the three sections (proximal, intermediate, and distal) were plucked carefully following the same sampling procedure. Due to the minute size of the bristle feathers five entire bristle feathers were mounted onto separate glass slides. All the samplings of barbs were conducted carefully using surgical forceps (number 00) with minimal damage to the barbs.

Feather barb slide preparation

The sampled feather barbs/whole bristle feathers were placed onto a small drop of Xylene (Fisher Scientific, product No. 35405) on a microscope glass slide which were previously cleaned by using 70% ethanol. The drop of Xylene allowed the feather barbs to spread apart its barbules and after its evaporation kept the barbs attached onto the glass slide (Lee et al. 2016). In the meantime the slides were labelled properly using printed label stickers according to their slide codes to avoid confusion. Previously cleaned cover glasses were placed directly onto the completely dried feather barbs for dry mount (Lee et al. 2016). By using nail varnish (Nail Trend; Pearl White, India) the four sides of the cover glasses were sealed and allowed to dry for proper microscopic observations.

Macroscopic characteristics

Whole feathers were observed for macro-characteristics such as feather colour, pattern and texture following Lee et al. (2016). As mentioned above, the feathers were placed on a plain paper along with a scale, labelled properly and photographed. The slides mounted with barbs were also placed carefully on a plain paper along with a scale and photographed. Using ImageJ software distance (in the digital images) was standardized using the scale in each individual photograph (Schneider et al. 2012). Using the same ImageJ software length of feathers and length of each barb was calculated following the software as per instructions. Length of all the feather types was calculated, except powder down feathers because these feathers have extremely soft and rudimentary rachis,leading to no distinct orientation.

Microscopic characteristics

All the prepared slides were observed carefully for a number of selected microscopic features of feather barbules. These parameters include presence or absence of sub-pennaceous region, villi, nodes, prongs, hooklets

Table 1. Sampling details of types of feather, location of feathers sampled, number of barbs sampled, slides prepared, feather length in millimetre (mm) and mean barb length in millimetre (mm).

Types of feather	Location of feathers sampled	No. of barbs sampled	Number of slides prepared	Length of feather sampled (mm)	Length of barbs sampled (mm)	
					mean	±SE
Contour	Right Wing (Primary flight feather)	18	18	85.17	8.71	0.82
	Right Wing (Secondary flight feather)	18	18	76.39	14.55	0.68
	Left Wing (Primary flight feather)	18	18	87.32	8.52	0.68
	Left Wing (Secondary flight feather)	18	18	76.10	13.70	0.75
	Tail feather	18	18	37.55	7.85	0.50
	Body contour (Ventral)	15	15	28.24	7.11	0.63
	Body contour (Dorsal)	15	15	24.86	9.50	0.14
Semiplume	Ventral-1	15	15	33.66	7.88	0.35
	Ventral-2	15	15	32.55	7.30	0.26
	Near the tail	15	15	29.59	9.32	0.53
	Dorsal-1	15	15	36.59	9.04	0.49
	Dorsal-2	15	15	27.69	7.83	0.68
Downy	Dorsal	15	15	23.42	9.56	0.22
	Ventral	15	15	10.24	5.19	0.22
	Right wing	15	15	10.67	5.72	0.13
	Left wing	15	15	12.53	5.94	0.25
	Near tail	15	15	11.74	5.83	0.38
Powderdown	Right wing	15	15	*	4.99	0.19
	Left wing	15	15	*	5.52	0.31
	Ventral	15	15	*	9.48	0.40
	Dorsal	15	15	*	5.11	0.16
	Near tail	15	15	*	5.09	0.19
Bristle	Orbital region and chin	5	1	5.31	2.77	0.25
	Orbital region and chin	5	1	6.53	2.70	0.09
	Orbital region and chin	5	1	6.90	2.82	0.11
	Orbital region and chin	5	1	6.87	2.79	0.17
	Orbital region and chin	5	1	6.06	2.42	0.07
	Total of 27 different feathers sampled	Total of 370 Feather barbs sampled	Total of 350 slides prepared			

* Length of powder down feathers couldn't be calculated due to very miniscule rachis and no particular orientation of feather observed.

and ventral teeth; shape of nodes and internodes; presence of prongs, hooklets and ventral teeth on both side of barb; size of prongs and pigmentation of nodes, internodes and ramus (Image 5).

The slides were observed at 100X or 400 X magnifications under a light microscope (Weswox BXL, India) for select microstructures.

All the feather observation and recording were carried out by a single observer to minimize observer bias. Important microscopic morphological characters were photographed using binocular light microscope with an attached camera (Labomed Lx500, India) at 100X and 400X magnifications using Image aR software.

Statistical analysis

All the analyses were performed using MS-Excel (Microsoft, U.S.A) (with XLSTAT add-in software). We calculated the length of all the sampled feathers as well as the length of barbs sampled from these feathers. For descriptive analysis the feathers were grouped into six groups (wings, tail, body contour, semiplume, down, powder down, and bristle) (Table 2). For each group the mean barb lengths, standard error, range and coefficient of variation was calculated. Comparative analysis were made and presented as box and whiskered plots (Figure 2). Correlation between the mean barb length and feather length was calculated using Pearson correlation coefficient. The feathers were grouped into four groups (contour, semiplume, down, and bristle), powder down was excluded from this analysis as the length of powder down feathers couldn't be calculated. For each feather, length of the feather was paired with mean barb length (of all the sampled barbs) during correlation analysis.

RESULTS

We observed 370 feather barbs from 27 different feathers (Table 1). We found morphological features such as hooklets and ventral teeth that were exclusive to contour and semiplume feathers only (Table 5). Features such as villi, nodes, prongs and internodes were recorded in down, powder down, semiplume feathers as well as in body contour feathers also (Table 5).

We observed a unique uncharacterized barbule arrangement in body contour feather barbs (Image 13). The barb was composed of plumulaceous barbules at the base of the barb with pennaceous barbules immediately following it (Image 13). Such unique arrangement of barbules in barbs was named as "sub-plumulaceous region" and was observed exclusively in the intermediate section of body contour feathers from the dorsal portion of the bird (Image 13). We reported in this study for the first time that bristles display microscopic morphological characteristics similar to down or powder down feathers (Image 12) (Table 5). The barbs of bristle feathers were characterized by the presence of villi, nodes, prongs and absence of hooklets and ventral teeth same as in down and powder down feather types. Even the shape of nodes was exactly similar as recorded in down and powder down feather barbs (Image 12).

Macroscopic characteristics

Primary flight feathers from the right and left wing (Table 1) were measured longest (85.17 mm and 87.32 mm, respectively), whereas bristle feather was the shortest (5.31 mm) (Table 1). The mean barb length of the wing feathers (primary and secondary flight feathers of left and right wing of contour type) was observed to be the highest (11.37±0.47 mm) and shortest in bristle (2.70±0.07 mm) (Table 2). Correlation (Pearson correlation coefficient) calculated using pair-wise comparison indicated that correlation was positively high for only the pair of barb length and feather length of down feathers ($r = 0.996$, $p \leq 0.05$) other feather types (contour, semiplume, powder down, and bristle) had no significant correlation between barb length and feather length (Table 3). The findings for various attributes (colour of feather, pattern in vanes, texture of barbs, and texture of rachis) of different feathers groups are presented in Table 4.

Microscopic characteristics

The feathers were divided into groups (wings, tail, body contour, semiplume, down, powder down, and bristle) and microscopic structures were scored in a predominantly binary (0/1) or tertiary (3/4/5) scores (Table 5).

Wings and tail were composed of entirely pennaceous barbules on feather barbs, characterized by the presence of hooklets on the distal barbules, teeth on both distal and proximal barbules and variable pigment on the rachis (Table 5; Image 6, 7). Body contour feathers were composed of barbs containing purely pennaceous barbules, purely plumulaceous barbules and both plumulaceous and pennaceous barbules (Image 8; Table 5). Semiplume feathers were composed of barbs containing purely pennaceous barbules and barbs containing purely plumulaceous barbules characterized by the presence of villi, nodes, prongs, hooklets and teeth (Image 9; Table 5). Down feathers (Image 10; Table 5), and powder down (Image 11; Table 5) were composed of barbs containing plumulaceous barbules characterized by presence of villi, nodes, and prongs. Two types of nodal shape were noticed in down feathers opposed to only singular type in powder down. Bristle feathers were characterized by presence of villi, nodes, and prongs displaying characteristics nearer to non-contour feathers (Image 12; Table 5).

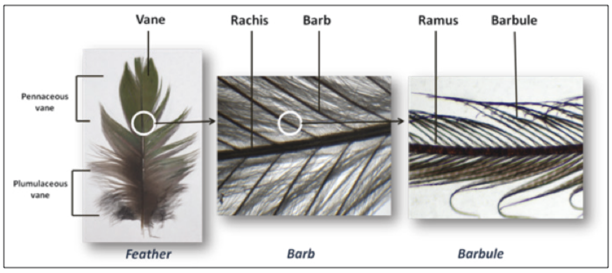

Image 1. Illustration of topography of a feather. © Prateek Dey & Swapna Devi Ray.

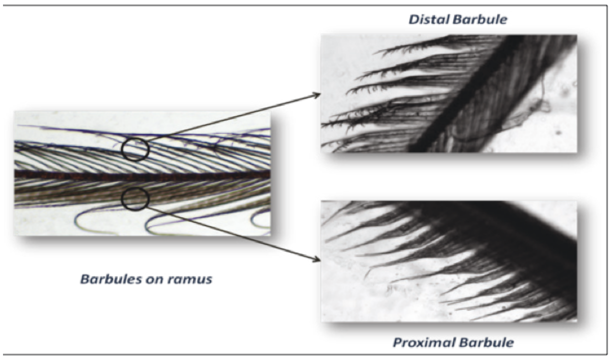

Image 2. Illustration of distal and proximal barbules on the barb of a pennaceous vane. © Prateek Dey & Swapna Devi Ray.

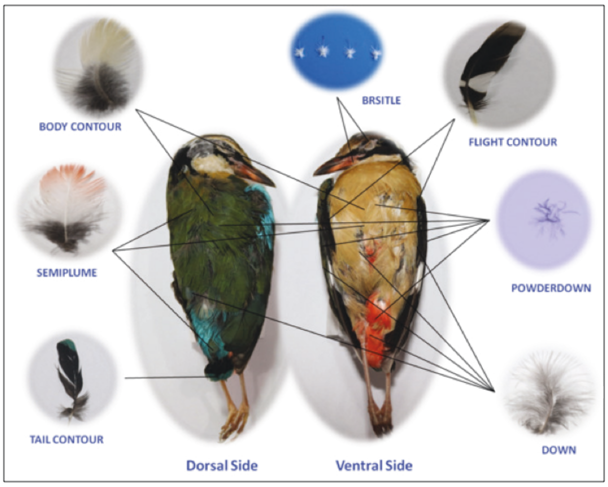

Image 3. Illustration of site of sampling of various feathers from different anatomic locations on the cadaver of Indian Pitta. © Prateek Dey & Swapna Devi Ray.

DISCUSSION

Morphological characteristics of various types of feathers were successfully studied in this report. Primarily we found, feathers grouped under same types but from different anatomical location have the

Table 2. Descriptive statistics of sampled barbs from various types of feathers.

Types of feather	No. of barbs (N)	Mean barb length (mm)	Range (mm)	Coefficient of variation (%)
Wings	72	11.37 ± 0.47	18.96–4.60	35.65
Tail	18	7.85 ± 0.50	12.23– 4.65	26.65
Body contour	30	8.31 ± 0.39	11.29–3.56	26.24
Semiplume	75	8.27 ± 0.22	12.19–3.56	23.32
Down	75	6.45 ± 0.21	10.75–4.03	28.34
Powderdown	75	6.04 ± 0.23	12.00–3.31	33.10
Bristle	25	2.70 ± 0.07	3.29–2.13	12.89

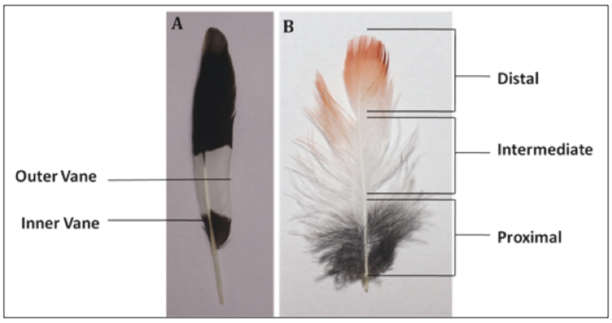

Image 4. A—Inner and outer vane in wing and tail feather | B—Sections considered on a feather for barb sampling (proximal, intermediate, and distal). © Prateek Dey & Swapna Devi Ray.

exact same microscopic characteristics. In this study we have provided such evidence after macro and micro level examination of 350 slides prepared from 370 feather barbs obtained from 27 feathers sampled from an individual of Indian Pitta. Another study in such a systematic manner was conducted by Chandler (1916) without any empirical data to it's annexure. About a century later in 2021, Ray and workers systematically documented feather micro-characteristics of yellow billed babbler (Ray et al. 2021). Similarly in this study we have successfully assessed and recorded the select feather characteristics of Indian Pitta into empirical information. In this study we have successfully assessed and recorded the selected parameters into empirical information.

The identification of a unique barb in the dorsal body contour feathers for the first time sheds light on possible subtle differences even in the same type of feather but from different anatomical location. Though such a barb is an exception as in all other cases we found that feathers grouped under same type have exactly similar structure. Such a unique structure might be an adaptation to its function. Having plumulaceous barbules and pennaceous

Table 3. Pearson correlation coefficient of mean barb length and feather length of various feather types. Confidence interval 95% ($p \leq 0.05$).

	Types of feather							
	Contour		Semiplume		Down		Bristle	
	r	p - value	r	p - value	r	p - value	r	p - value
Mean Barb length (mm) (α= 0.05, $p \leq 0.05$) r= Pearson correlation coefficient	0.447	0.315	0.820	0.020	0.996	.000	0.287	0.640

Table 4. Details of macroscopic characteristics observed in various feather types.

Type of feather	Colour	Texture of barbs	Pattern in vanes	Texture of Rachis
Wings	Mostly black with patch of white	Stiff	Bold	Stiff
Tail	Mostly black with tinge of green at one end	Stiff	Bold	Stiff
Body contour	Black with cream/green colour at one end	Stiff, fluffy	Bold	Stiff
Semiplume	Whitish black, orange	Stiff, fluffy	Bold	Stiff
Down	Mostly black with hints of grey	Soft, fluffy	Dull	Soft
Powder down	Grey with black tinge	Soft, fluffy	Dull	NA
Bristle	White with cream complexion	Stiff	Dull	Stiffened & strongly tapered towards one end

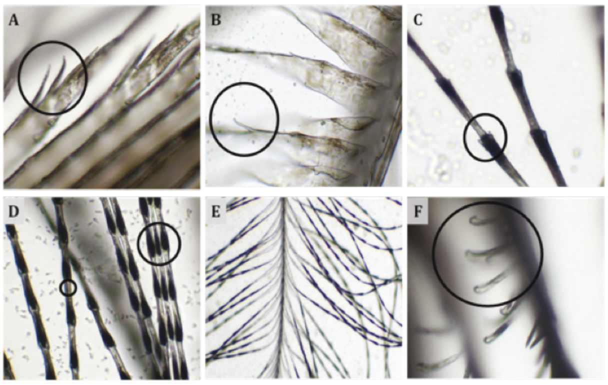

Image 5. Illustration of some of the microstructures present in a feather: A—Ventral teeth | B—Villi | C—Nodes with prongs | D—Nodes and Internodes | E—Plumulaceous barbules on a ramus | F—Hooklet. © Prateek Dey & Swapna Devi Ray.

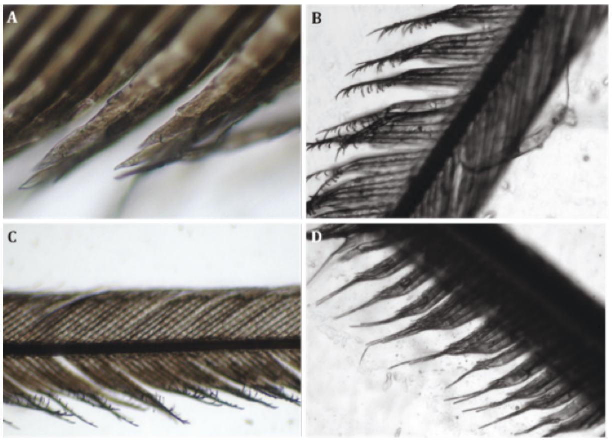

Image 6. Microscopic feather characteristics of wing feathers illustrated using light microscopy: A—Ventral teeth | B—Hooklets on distal barbule | C—Distal and Proximal barbule on ramus | D—Proximal barbule. © Prateek Dey & Swapna Devi Ray.

barbule in the same barb helps the feather in insulation as well as in flight. Such a specialized barb might be a necessity for the dorsal feathers that bear the blunt of air currents during a bird's flight. Previous studies displayed that passerines are generally characterized by the absence of sub-pennaceous region (Lee et al. 2016), possibly the presence of newly identified sub-plumulaceous region in dorsal body contour feather barbs is specific to these birds. Although more studies containing multiple passerine species from various families are needed to authenticate such a hypothesis nevertheless the above discovery is unique enough in its own right.

The bristle feathers are believed to be structurally modified contour feathers in the existing literature (Lovette & Fitzpatrick 2016). However microscopic analysis in this study placed them structurally closer to down and powder down feathers. The characteristics that separates bristle from contour feather is the absence of ventral teeth and hooklets, thus placing it closer to down and powder down types.

Correlation between barb length and feather length was significant for down feathers only. Such a correlation can be explained by the fact that length of down feather

Table 5. Details of microscopic characteristics observed in various feather types.
0—Absent | 1—Present | 3—Variable | 4—Node shape flared on one side and diminished towards other | 5—Rod shaped node | S/L—Small/Large | NA—Not applicable | D/P—Distal/Proximal barbule | STR/KNK—Straight/Kinked.

Types of feather	Micro-characteristics																	
	Sub-pennaceous region	Villi	Nodes	Node shape	Prongs	Presence of prongs on both side of barb		Prong size	Hooklets	Presence of hooklets on both side of barb		Teeth	Presence of teeth on both side of barb		Internode shape	Pigmentation		
	0/1	0/1	0/1		0/1	0/1	D/P	S/L	0/1	0/1	D/P	0/1	0/1	D/P	STR/KNK	Nodes	Inter-nodes	Ramu-s
Wings	0	0	0	NA	0	NA	NA	NA	1	0	D	1	1		NA	NA	NA	3
Tail	0	0	0	NA	0	NA	NA	NA	1	0	D	1	1		NA	NA	NA	3
Body contour	0	1	1	4	1	1		S	1	0	D	1	1		STR	1	3	3
Semiplume	0	1	1	4	1	1		S	1	0	D	1	1		STR	3	3	3
Down	0	1	1	4,5	1	1		S	0	NA	NA	0	NA	NA	STR	3	3	3
Powderdown	0	1	1	4	1	1		S	0	NA	NA	0	NA	NA	STR	3	3	3
Bristle	0	1	1	4	1	1		S	0	NA	NA	0	NA	NA	STR	3	3	3
Repeated barbs*	0	1	1	4	1	1		S	1	0	D	1	1		STR	1	3	3

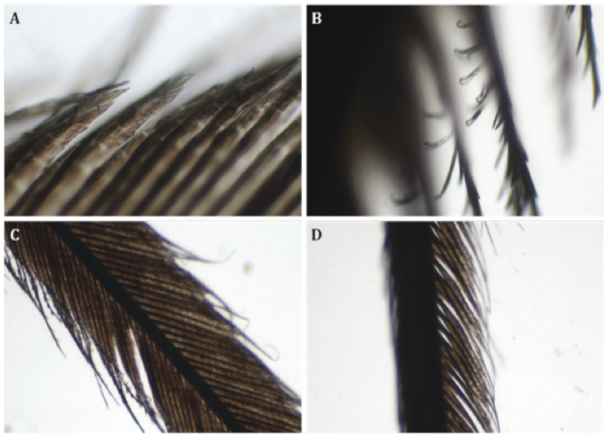

Image 7. Microscopic feather characteristics of tail feathers illustrated using light microscopy: A—Ventral teeth | B—Hooklets on distal barbule | C—Distal and Proximal barbule on ramus | D—Proximal barbule. © Prateek Dey & Swapna Devi Ray.

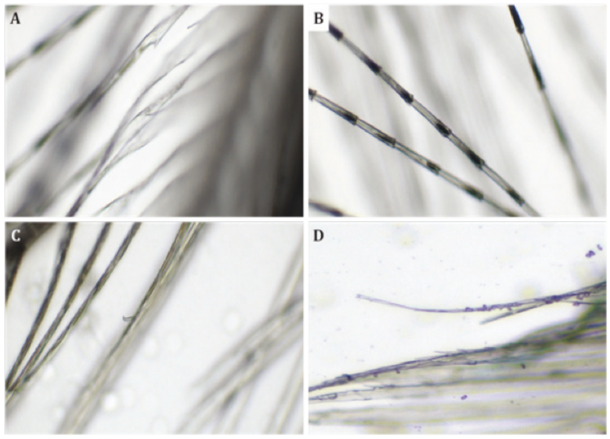

Image 8. Microscopic feather characteristics of body contour feathers illustrated using light microscopy: A—Villi | B—Nodes and prongs | C—Hooklet on distal barbule | D—Ventral teeth on proximal barbule. © Prateek Dey & Swapna Devi Ray.

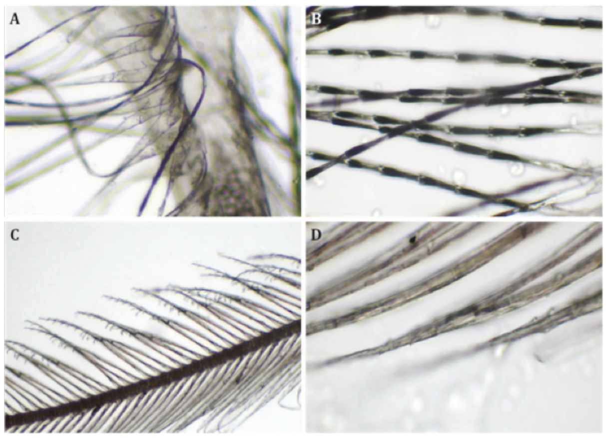

Image 9. Microscopic feather characteristics of semiplume feathers illustrated using light microscopy: A—Villi | B—Nodes and prongs | C—Hooklet on distal barbule | D—Ventral teeth on proximal barbule © Prateek Dey & Swapna Devi Ray.

barbs and length of down feathers are both essential to perform its function of insulation and thermoregulation. Whereas, barb length and feather length of other types of feather aren't correlated enough in their functionality. Through our study on the various feather types in Indian Pitta, we would like to suggest that based on barbules, feather barbs can be divided broadly into three types. These include: (i) barbs without any sub-pennaceous or sub-plumulaceous region (in case of Indian Pitta wing, tail, semiplume, down and powder down feathers), (ii) feather containing barbs with sub-pennaceous or sub-plumulaceous region (sub-plumulaceous region as present in body contour feathers from dorsal portion of Indian Pitta), and (iii) feather containing barbs which are specially modified for specific functions (bristle feathers). Such a morphology based classification of barbs, can possibly be beneficial for designing feather related studies in future.

Robertson (Robertson et al. 1984) pointed that Chandler (1916) and Day (Day 1966) had reported schemes for feather identification which lacked any corroborating evidence. Also, the works of Hargrave (1965) and Messinger (1965) were based on qualitative assessment of feather microstructures.

In 1984, Robertson et al. (1984) quantified and provided numerical data of node density and barbule length for consideration as species identification parameters; however their data demonstrated that variation in barbule length and node density within a species is considerably high and provides limited scope for inter taxa identification. Same was also established by Joannah Lee (Lee et al. 2016) through qualitative identification of feather micro characteristics. Through our result we also state that, assessment of feather microstructure qualitatively without any numerical data is capable of differentiation between various types of feather.

The studies of Robertson (1984) and Chandler (1916) state that pennaceous parts of contour feather vary hugely amongst the feathers of same individual; however, through our study we found that the pennaceous region of feathers of wings, tail, body contour, and semiplume have exactly the same microstructure, contradicting the findings of the previous studies. The similarity in micro structures is expected as the feathers performing similar functions are supposed to have exactly same microstructure. And as such the similar trend is observed in all other feather types.

Previous studies (Dove 1997b; Lee et al. 2016) have emphasized on the fact that an appropriate reference collection, well trained staff and standardized

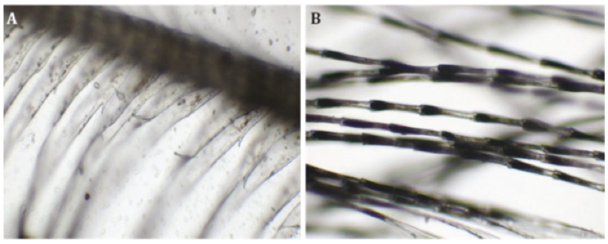

Image 10. Microscopic feather characteristics of down feathers illustrated using light microscopy: A—Villi | B—Nodes, prongs, and internodes. © Prateek Dey & Swapna Devi Ray.

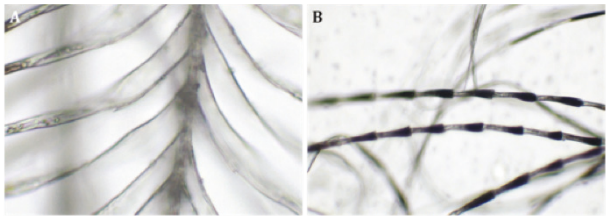

Image 11. Microscopic feather characteristics of powder down feathers illustrated using light microscopy: A—Villi | B—Nodes, prongs, and internodes. © Prateek Dey & Swapna Devi Ray.

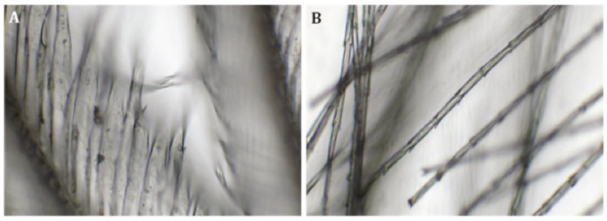

Image 12. Microscopic feather characteristics of bristle feathers illustrated using light microscopy: A—Villi | B—Nodes, prongs, and internodes. © Prateek Dey & Swapna Devi Ray.

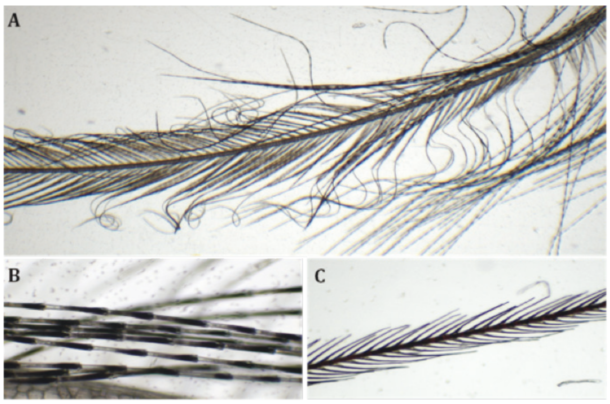

Image 13. Microscopic feather characteristics of unique feather barbs identified from dorsal body contour feather illustrated using light microscopy: A—Both pennaceous and plumulaceous barbules on the ramus of the barb | B—Nodes, Internodes, and prongs on the plumulaceous barbule | C—Distal and proximal barbule on the pennaceous barb. © Prateek Dey & Swapna Devi Ray.

techniques is necessary for such feather related studies. Through our work we have pioneered in such challenge for the first time in India and aim to create a feather identification repository armed with trained personnel to perform various feather investigations. Our study found that the technique of dry mount followed by our study (Robertson et al. 1984; Lee et al. 2016) is best suited for mounting feather barbs for observation under light microscope. The technique of mounting the barb in medium (Dove & Coddington 2015) might chaperone the delicate microstructures of villi and hooklets, leading to faulty recording of observations. Systematic studies on feather morphology helps us understand the form and functions of feathers better as well as provide us better understanding of inter-species differences in feather structures. The practical implications of our study can inculcate a whole range of in-depth feather analysis as a tool for feather form and function description or as a phylogenetic identification tool or as an aid in applied wildlife forensic research.

Data availability: Analyses reported in this article can be reproduced using the data provided by the author upon acceptance of the manuscript.

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