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COMPARATIVE STUDY OF YELLOW-BILLED BABBLER (*TURDOIDES AFFINIS)* FEATHERS REVEALS UNIFORMITY IN THEIR MICROSTRUCTURES AMONG INDIVIDUALS

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KEYWORDS

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Plumulaceous

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ABSTRACT

Though a few in numbers, investigations on feather microstructures from the early 20th century till date, have contributed immensely to various fields such as phylogeny, palaeontology, archaeology, wildlife forensic, biomechanics and so on. However, existing studies on feather micro-structures of birds endemic to the India/Indian sub-continent are few in number and limited in their scope. Also, no study has ever been conducted to compare feather microstructures of different individuals of a species from India. To address this issue, a comparative feather microstructure study of three individuals of *Turdoides affinis*, a passerine endemic to the Indian sub-continent was done. Select microstructure parameters for five different types of feathers were studied in detail. The molecular sexing method was used to elucidate the sex of *T. affinis* individuals for gender based differences if any. Results of the study identified that two of *T. affinis* individuals were female whereas one of them was male. Morphometrically, tail contour was the longest (9.63±0.76 cm) and bristle were the shortest (1.00±0.07 cm) feather. Semiplume had the longest barb length (1.73±0.04 cm) and shortest barbs (0.16±0.01 cm) were present in bristles. Subpennaceous barbs and knob-shaped villi, characteristic of members of the *Passeriformes* family, was also observed in all three individuals. This study records no significant difference in feather characteristics amongst the three *T. affinis* individuals irrespective of the differences in their sex and size. Systematically documented feather micro-characteristics of *T. affinis* in this study could be used as a species identification tool and would provide baseline data for the feather catalogue of Indian bird species being compiled at SACON.

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1 Introduction

Studying feather microstructures can prove to be a very useful tool for avian species identification (Chandler, 1916; Robertson et al., 1984; Dove & Koch, 2011). In studies related to different domains such as bird hit with aircraft, museum specimens and wildlife forensics, the studies on feather microstructures have been broadly used for species identification (Brom, 1991; Dove, 1997; Dove & Koch, 2011). Previous studies of feather microstructures especially the plumulaceous (downy) barbs, has been used as a tool for identifying the respective Order or Family that the species might belong to (Chandler, 1916; Robertson et al., 1984; Dove, 1997; Dove, 2000; Lee et al., 2016).

Pioneer work on microstructures of feathers was conducted by Nitzsh (1867) where he first described details of different microstructures present in different barbs. By focusing on microstructures of pennaceous barbs of contour feathers, Wray (1887) established a model for feather study. Chandler (1916) is renowned for his highly systematic study of feather microstructures in numerous species of birds. Brom (1986) conducted a study on feathers samples from aircraft collision where he described differences in feather microstructures amongst different species of birds colliding with aircrafts. Birds have also been phylogenetically analyzed based on microstructures of feathers (Dove, 2000; Dove & Agreda, 2007). For forensic identification of avian species using feather fragments, Dove &Koch (2011) and Lee et al. (2016) have contributed immensely. The importance of the systematic study on feather microstructure has been accepted by various workers and different applications of feather study are well proven in different fields (Chandler, 1916; Brom, 1991; Dove & Koch, 2011; Lee et al., 2016). Furthermore, microstructures of feathers from fossilized samples have been investigated upon for their evolutionary significances (McKellar et al., 2011; Carney et al., 2012; Xing et al., 2016). Yet, there exists a huge lacuna in the field of feather microstructure study, especially from India. As such, there is no systematic study available on feather microstructures from an endemic Indian species. Hence, the information regarding feather micro characteristics in any bird species will be an important contribution. Also, no previous study has ever been conducted to compare the feather microstructure of different individuals of a species from India. Such lack of information has prohibited us from comparing inter and intraspecies feather microstructures and getting a baseline database for species identification.

To address this issue, we conducted a comparative feather microstructure study in three individuals of *T. affinis;* a passerine bird, endemic to the Indian sub-continent. By adopting the molecular sexing technique, we have identified the sex of the specimens to elaborate gender based differences in feather microstructures if any. We have compared different select microstructure parameters for five different types of feathers, which were sampled from five different body locations of the bird. Various feather microstructures for both pennaceous and plumulaceous barbs were documented and compared to find any intra-species microstructural difference. As such the feather catalogue of *T. affinis* created through this study also can be used for species identification. Providing empirical evidences towards the feather microstructure of different individuals of *T. affinis,* this study is a small contribution towards the vast field of plumology which has application in species identification.

2 Materials and methods

2.1 Collection details of sample specimens

T. affinis is a passerine (family Leiothrichidae) bird endemic to southern India and Sri Lanka. This species is also classified as "Least Concern" by IUCN. With due permission from the Tamil Nadu forest department (Ref.No.WL5 (A)/2219/2018; Permit No. 14/2018) road-kill survey for opportunistic sample collection were conducted in between the months of January-June, 2018. During road-kill surveys three individuals of *T. affinis* viz. Individual-1 (Ind 1) (N11°5'1.67'', E76°49'15.61"), Individual-2 (Ind 2) (N11°5'35.28", E76°47'48.60") and Individual-3 (Ind 3) (N11°5'29.28", E76°48'10.08") were collected from Thadagam-Anaikatty road of Coimbatore district (Figure 1).

2.2 Molecular sexing

The tissue sample from the mentioned road-kills were collected in DESS buffer (Seutin et al., 1991) and transported under favorable conditions to National Avian Forensic Laboratory (NAFL) at SACON, Coimbatore, Tamil Nadu. About 10 mg of the muscle tissue was digested in lysis buffer (10 mMTris, 10 mM EDTA, 10% SDS and 40µg Proteinase K) and subsequently used for DNA extraction using the Phenol-Chloroform-Isoamyl Alcohol method with minor modifications (Sambrook et al., 1989). PCR reaction (Supplementary Table 1, 2) was set-up with the extracted DNA as a template and using 2550F-2718R primers adopted from Fridolfsson & Ellegren (1999).

2.3 Sampling of feathers

Feathers were divided into two broad categories as a contour (primary flight contour, secondary flight contour, tail contour, body contour) and non-contour (semiplume, down, powder down, bristle, and filoplumes) feathers (Gill, 2007; Lovette & Fitzpatrick, 2016). By using surgical forceps (number 00) feathers were plucked carefully from the cadavers. Flight contour feathers i.e. primary and secondary contour feathers were sampled from both wings. Similarly, tail contours were sampled from the tail region of specimens. Body contour feathers were sampled from the dorsal and ventral sides of each carcass. Each

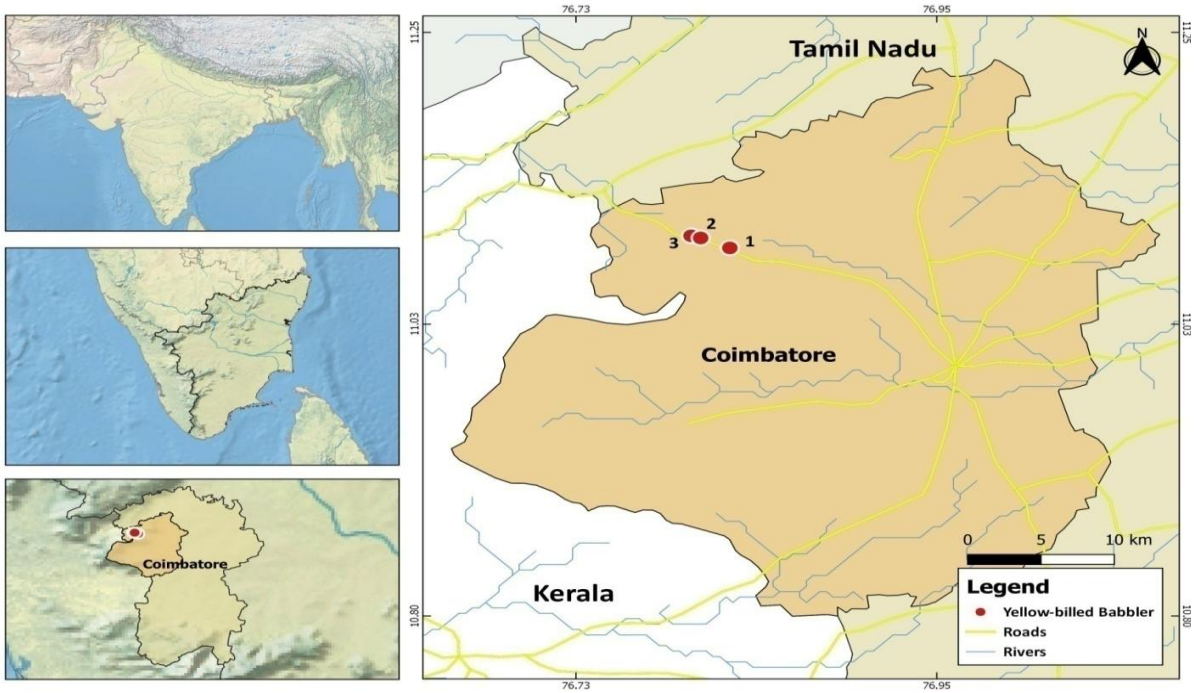

Figure1 Geo-locations of collected road-kill individuals of *T. affinis*

Table 1 Rachis length of different feather types of three individuals of *T. affinis*

Feather Type	Mean± S.E. (cm)	Range (cm)
Primary Contour	7.67±0.36	7.30-8.25
Secondary Contour	5.55±0.09	5.40-5.60
Body Contour	3.43±0.43	2.75-3.90
Tail Contour	9.63±0.76	8.40-10.30
Down	2.99±1.03	2.08-4.66
Semiplume	2.93±0.74	1.90-4.00
Bristle	1.00±0.07	0.90-1.10

Table 2 Average calamus length of three individuals of *T. affinis*

Types of feather	IND 1	IND 2	IND 3	Range (cm)	Mean±S.E. (cm)
Primary Contour	1.89	1.68	1.73	1.68-1.89	1.77±0.08
Secondary Contour	0.60	0.50	0.50	0.50-0.60	0.53±0.04
Body Contour	0.45	0.30	0.40	0.30-0.45	0.38±0.05
Tail Contour	1.00	1.10	1.00	1.00-1.10	1.03±0.04
Down Feather	0.20	0.24	0.22	0.20-0.24	0.22±0.01
Powder Down	0.16	0.14	0.14	0.14-0.16	0.15±0.01
Semiplume	0.14	0.20	0.18	0.14-0.20	0.17±0.02
Bristle	0.20	0.20	0.20	0.20-0.20	0.20±0.00

semiplume, down and powder down feathers were sampled from five different body locations viz. right and left wings, tail, dorsal and ventral sides of specimens. Five bristle feathers were sampled from near the eyes and chin of each specimen. Due to the degraded condition of cadavers' filoplume feathers could not be retrieved from the collected specimens of *T. affinis.*

2.4 Sampling of feather barbs

Sampled feathers were washed using 70% ethanol solution and air dried (Lee et al., 2016). Following Dove (1997), select feathers of *T. affinis* were divided into three distinct regions as proximal, intermediate and distal. By using needle and forceps (number 00); five barbs were carefully plucked from each one of these three regions for mounting on slides. From one feather, total of fifteen numbers of barbs were sampled from both sides of the vanes i.e. inner and outer vane. Total of 15 feather barbs were directly sampled from powder down feathers due to the unavailability of rachis on it. Five whole bristle feathers were sampled and mounted directly on single slides due to their tiny structures.

2.5 Slide preparation

Both dry and wet mount methods were performed for slide preparation of *T. affinis*. Slides and coverslips were cleaned using 70% ethanol. For dry mount, sampled single barbs were allowed to settle down on the slide using one-two drops of xylene. Xylene let feather barbs spread up on the slide and to stick on it by acting as an adhesive substance after drying (Dove & Koch, 2011). Dried barbs were covered with previously cleaned coverslips. To prevent dust and moisture edges of coverslips were sealed by using transparent nail varnish. Two-three drops of DPX have been placed on the xylene dried barbs for the wet mount. Coverslips were placed carefully on the drops, taking care of air bubbles. Finally, all prepared slides were labeled by using NAFL (National Avian forensic laboratory) slide coding protocol. Properly labeled slides were kept openly overnight for drying.

2.6 Macroscopic characteristics

By following Lee et al. (2016) careful observations were made for macro-characteristics as well as colour, pattern and texture of all feathers. Select feathers were photographed by placing them on a plain A4 sheet paper alongside with ruler scale. By using Image J software morphometry were taken from these photographs. Length of rachis, vane and calamus were measured for all select feathers except powder down (due to soft rachis). By using Canon Mark 7D camera prepared slides were photographed for the measurements of barb length for different types of feathers.

2.7 Microscopic characteristics

Prepared slides from select feather barbs were observed for select microscopic features viz. Hooklets, Villi, Nodes, Shape of Nodes, Shape of Internodes, Prongs, Size of prongs on barbules, Location of prongs at nodal structures, Pigmentation on ramus and Ventral Teeth. LABOMED Lx500 light microscope was used for observation and documentation of different feather microstructures from prepared slides under 100X and 400X resolutions. All select microscopic features of select feathers were documented by using Mia Image aR software. Using Image J software, the length of the mounted barbs was measured from photographs of prepared slides.

3 Results

In this study, microstructure characteristics of feathers of Yellow-billed Babbler (*T. affinis*) from different feather barbs were observed. Here, different parameters of feather microstructure of sampled feathers, as well as feather macro-characteristics among select feather samples of three *T. affinis* individuals, have been compared.

3.1 Molecular sexing

The PCR based assay adopted from Fridolfsson & Ellegren(1999) elucidated that individual 1 and 2 are female, whereas individual 3 was identified as male (Figure 2). The PCR amplification and agarose gel electrophoresis of the highly conserved primer pair of 2550F-2718R flanking the introns in the CHD1 gene of birds, display one (CHD1W) or two fragments (CHD1W and CHD1Z) in females while males only show one fragment (CHD1Z) different in size from the female-specific CHD1W fragment. As such upon amplification by the primers, individual 1 and 2 displayed two DNA bands (CHD1W and CHD1Z) and Individual 3 displayed a single band (CHD1Z). We successfully compared the feather microstructure of both sexes in this study.

3.2 Macro-characteristics

Macro-characteristics of feathers were studied for three parameters viz. colour, texture and pattern of the selected feathers (Supplementary Table 3). Colour of the select feathers varied from pale brown to creamy. Contour feathers were mostly pale brown in coloration whereas colour of non-contour feathers varied from creamy, pale brown or a shade of colour in-between. The texture of various contour feathers and bristle (a modified contour) investigated in this study was rigid, whereas the texture of all other feather types was soft and fluffy. The rigidness in contour feathers was due to the presence of pennaceous barbs whereas the fluffiness of other feather types was due to the presence of plumulaceous barbs. The rigid texture of contour

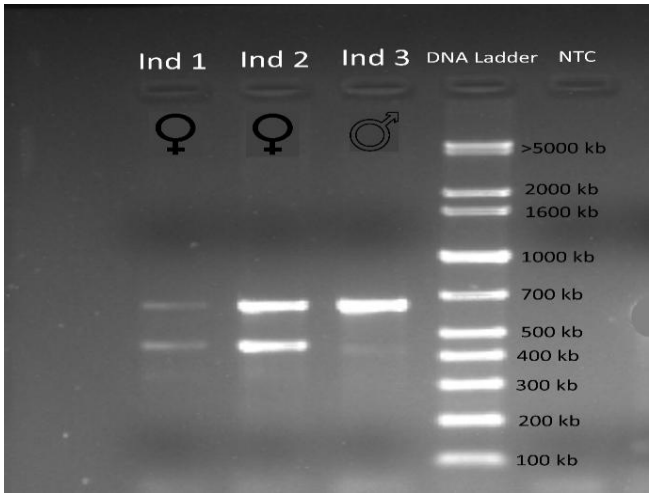

Figure 2 PCR results of the three individuals using primer pair 2550F-2718R shows two different banding pattern for male and female. Two bands (CHD1W and CHD1Z) represents female (Ind 1, 2) and a single band (CHD1Z) as male (Ind 3). Size standard for DNA is 1 kb DNA Ladder (Qiagen Inc., Germany). NTC signifies No Template Control

Table 3 Average length of vane of three individuals of *T. affinis*

Types of feather	IND 1	IND 2	IND 3	Range (cm)	Mean±S.E. (cm)
Primary Contour	5.41	5.77	6.52	5.41-6.52	5.90±0.40
Secondary Contour	4.80	5.15	5.10	4.80-5.15	5.02±0.13
Body Contour	2.30	3.35	3.50	2.30-3.50	3.05±0.46
Tail Contour	9.30	7.30	9.20	7.30-9.30	8.60±0.80
Down	1.88	4.22	2.00	1.88-4.22	2.70±0.93
Semiplume	1.76	3.80	2.72	1.76-3.80	2.76±0.72
Bristle	0.70	0.90	0.80	0.70-0.90	0.80±0.07

Table 4 Average barb length of three individuals of *T. affinis*

Types of feather	IND 1	IND 2	IND 3	Range (cm)	Mean±S.E. (cm)
Primary Contour	1.04	1.46	1.45	1.04-1.46	1.32±0.17
Secondary Contour	0.95	1.06	0.98	0.96-1.06	1.00±0.04
Body Contour	0.68	0.78	0.12	0.12-0.78	0.53±0.25
Tail Contour	1.16	1.03	1.22	1.03-1.22	1.14±0.07
Down	0.86	1.28	1.04	0.86-1.28	1.06±0.15
Powder Down	1.27	0.86	1.05	0.86-1.27	1.06±0.15
Semiplume	1.77	1.67	1.74	1.67-1.77	1.73±0.04
Bristle	0.18	0.16	0.14	0.14-0.18	0.16±0.01

feathers helped them in functions of flight, hard covering as well as aerodynamic maneuverability. The rigid texture of bristles helps in their specialized function, i.e. protection of the birds eyes. The soft and fluffy texture of down, semiplume and powder down feather types enabled them to insulate, waterproof and provide overall covering on the body surface. Overall, a difference in texture between contour and non-contour feather types was observed which may be attributed to their difference in functions.

3.3 Morphometric measurements of feathers

We observed rachis length varied amongst the different feather types. Tail contour feathers possess the longest rachis with 9.63±0.76 cm, followed by primary contour, secondary contour, body contour, semiplume, down, and bristle being the shortest at1.00±0.07 cm (Table1) (Figure 3, Figure 4).*T. affinis* as a bush dwelling passerine undertakes short jumpy flights while foraging rather than long high altitude flights, which is evident from the long tail feathers and comparatively shorter flight contours. Calamus was longest in primary contour (1.77±0.08 cm), followed by tail contour, secondary contour, and body contour. In this study we found, though rachis may be long in tail contour, primary flight feathers were more deep rooted into the skin (evident from the longest calamus length). Down, semiplume and bristle have the same mean calamus length of 0.2±0.0 cm with powder down having the shortest mean calamus length at0.15±0.01cm (Table 2; Figure 5, Figure 6). Among all studied feathers, barb lengths were measured as longest in semiplume feathers (1.73±0.04) whereas shortest barbs were present in bristle feathers (0.16±0.01) (Table 4; Figure 9, Figure 10). Short calamus length and very long barb length were essential to carry out the functional obligations of down, semiplume and powder down feathers. The highest mean feather vane length was observed in tail contour (8.60±0.80 cm) and shortest in bristle feathers (0.80±0.07 cm) (Table 3; Figure 7, Figure 8).

3.4 Micro-characteristics

The slides were examined for various feather microstructures utilizing different parameters viz. hooklets, villi, nodes, the shape of nodes and internodes, prongs, pigmentation on the ramus and ventral teeth (Table 5). Body contour feathers sampled from the dorsal and ventral side, possess sub-pennaceous barbs i.e. both nodes and hooklets are present on single barbs of the intermediate region of the select feathers (Figure 13, Figure 14). Sub-pennaceous barbs in feathers can be used as a species identification marker for various taxa. The identification of sub-pennaceous barbs in body contour feathers points towards the presence of both plumulaceous and pennaceous barbules together in a barb. Hooklets were prominently observed on the flight contour (primary contour, secondary contour, and tail contour), body contour, semiplume, and bristles with noticeably absence in down and powder down feathers (Figure 11). Hooklets in pennaceous barbs are what provides the basis for interlocking barbs, and as such attributes to the rigid shape and structural stability during flights. The plumulaceous barbs of *T. affinis* across feather types possessed multiple knobbed shaped villi on their basal cells (Figure 11, 12). *T. affinis*, feathers were densely pigmented with triangular shaped nodes on the plumulaceous barbs (Figure 11, Figure 12). Interestingly, body contour feathers displayed two different types of nodal structures on plumulaceous barbs, viz. triangular shaped with densely pigmented nodes and triangular shaped variably pigmented nodes (Table 5; Figure 11, Figure 12). Prongs though small in size are present (on the distal region of the nodes) amongst all the investigated feathers (Figure 11) Further, pigmentation on ramus varied from light to darker shade. Flight contour and body contour feathers have variably pigmented ramus while other investigated feathers contained densely pigmented ramus. Ventral teeth were observed in the basal cell region of pennaceous flight contour, body contour, semiplume, and bristle (Figure 11).

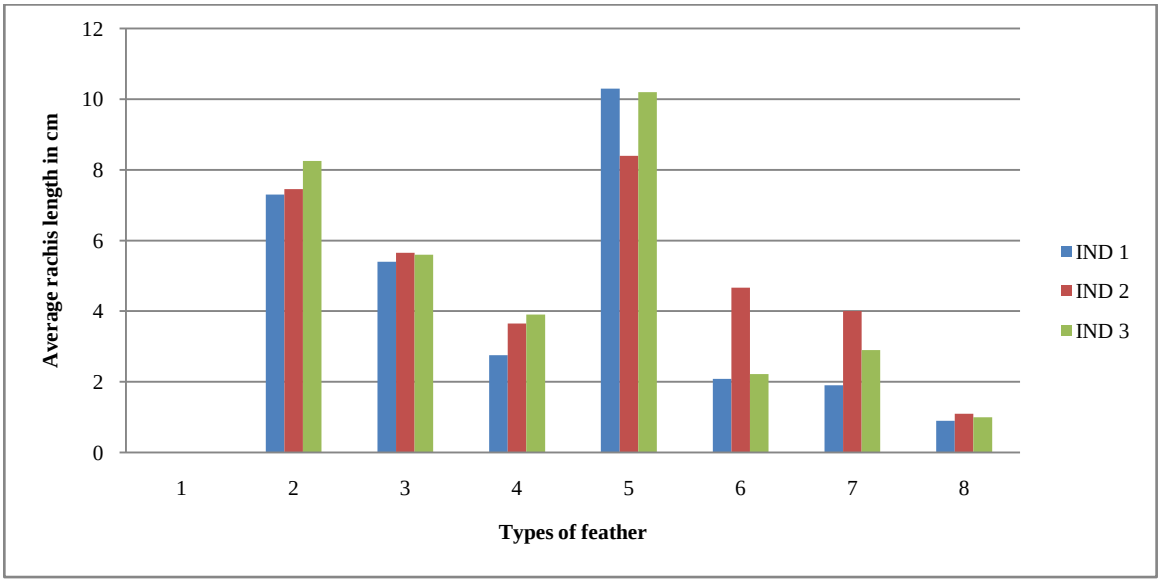

Figure 3 Comparison of average rachis length of the three individuals of *T. affinis*

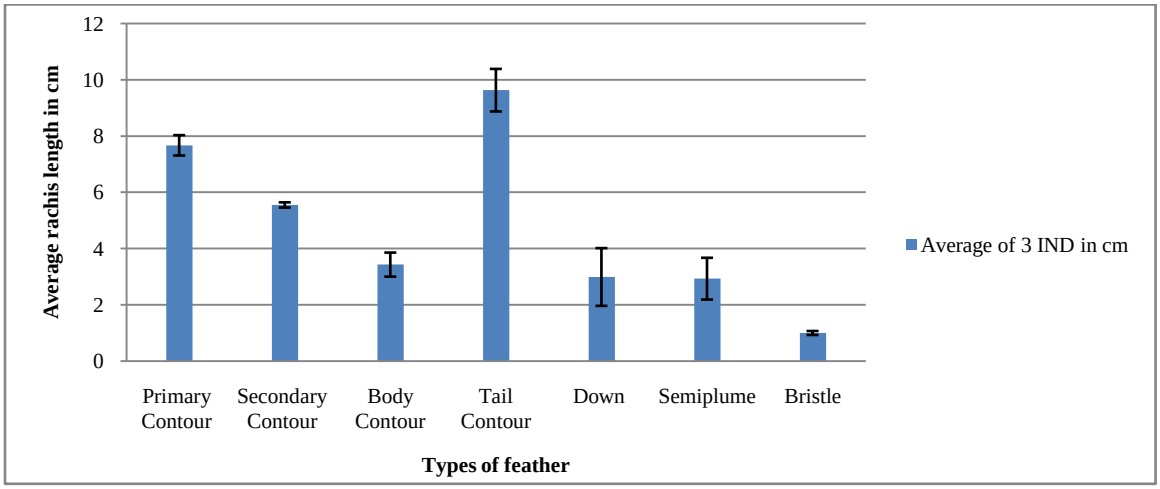

Figure 4 Mean rachis length of three individuals of *T. affinis*

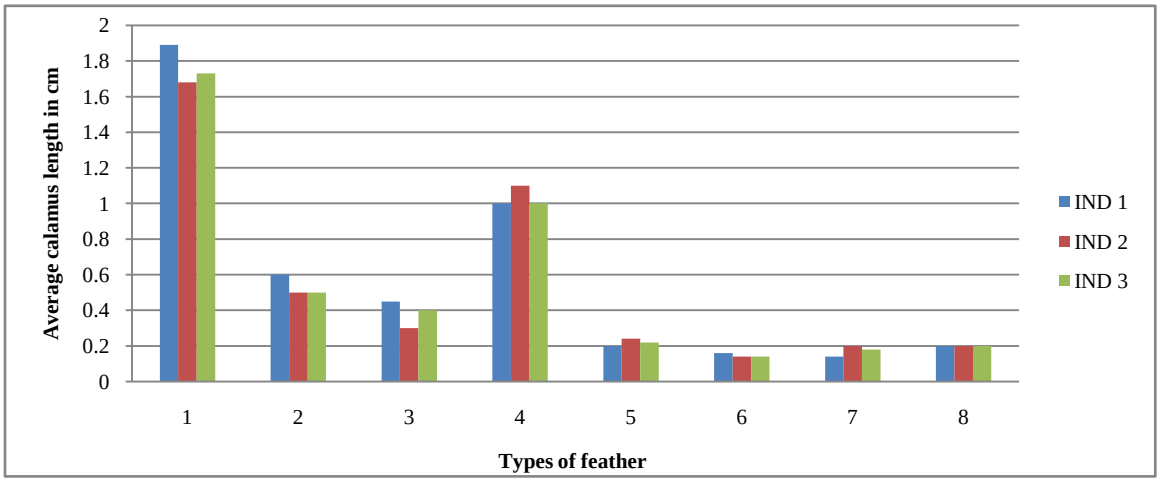

Figure 5 Comparison of average calamus length of the three individuals of *T. affinis*

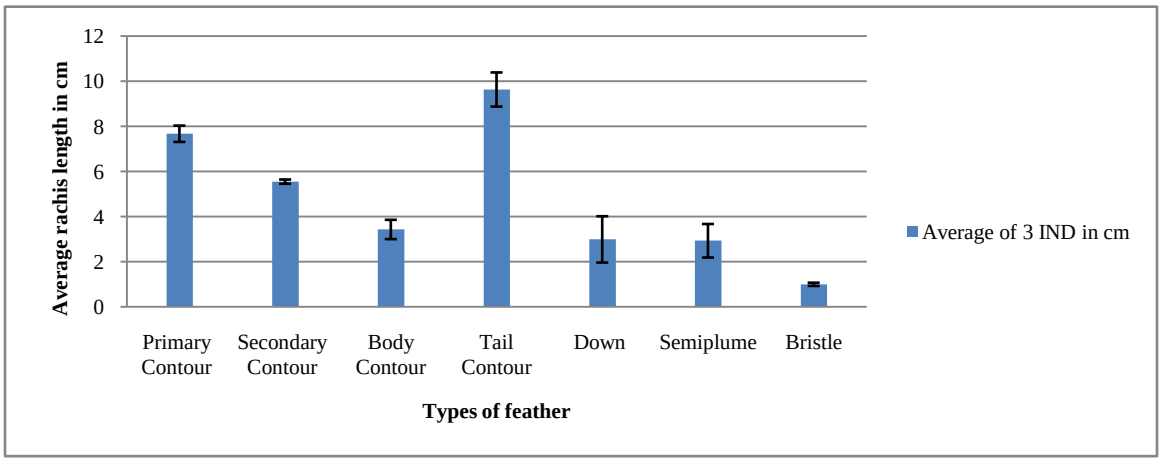

Figure 6 Mean calamus length of three individuals of *T. affinis*

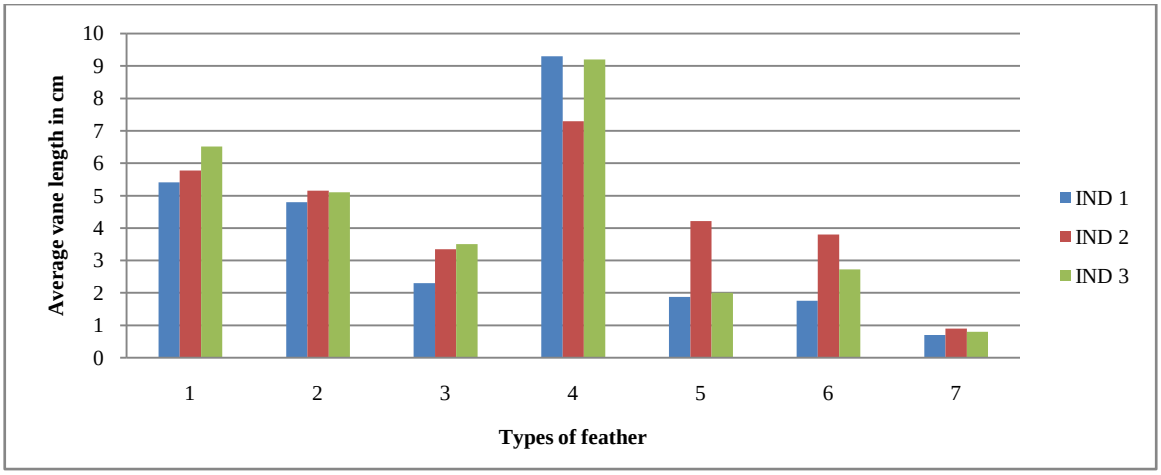

Figure 7 Comparison of average vane length of the three individuals of *T. affinis*

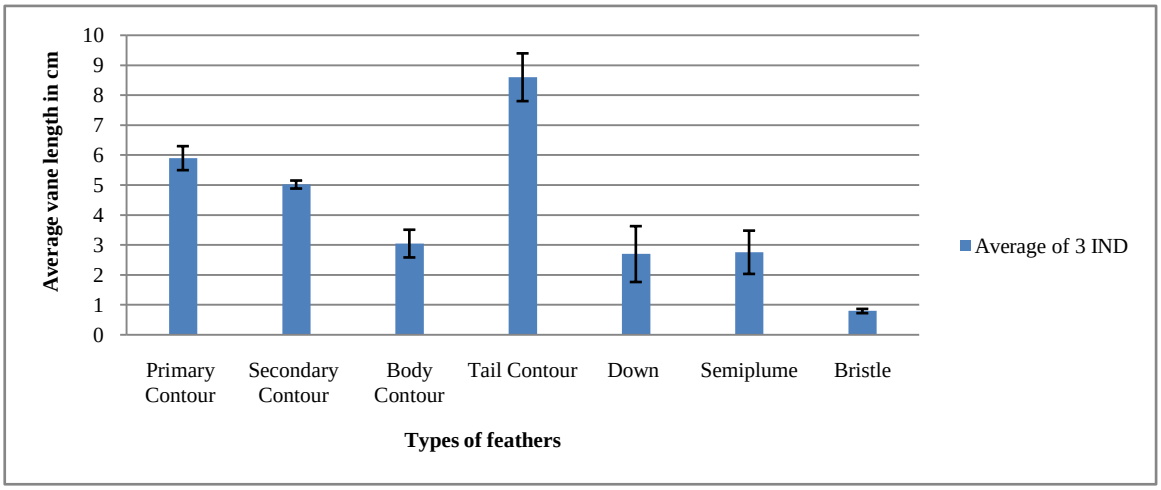

Figure 8 Mean vane length of three individuals of *T. affinis*

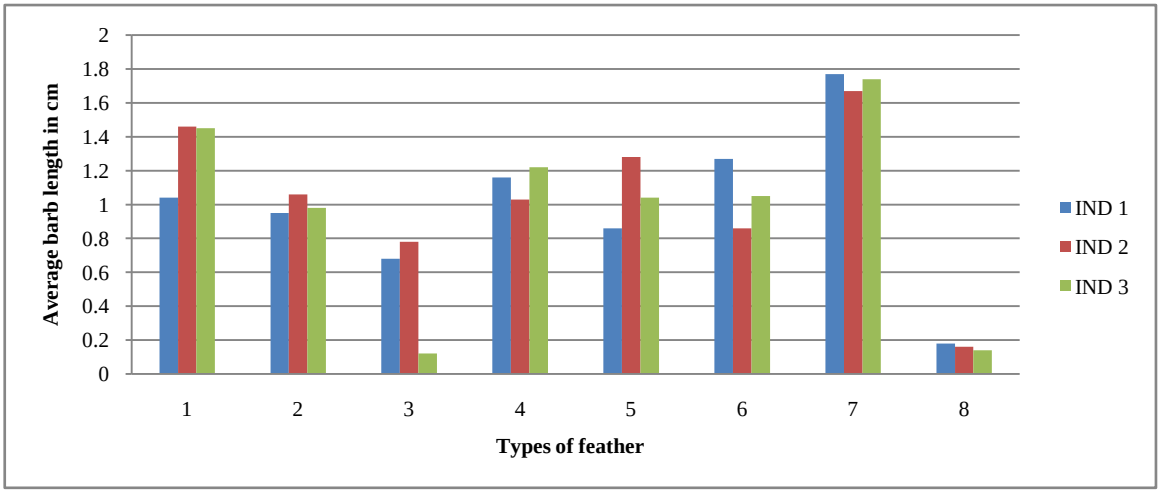

Figure 9 Comparison of average barb length of the three individuals of *T. affinis*

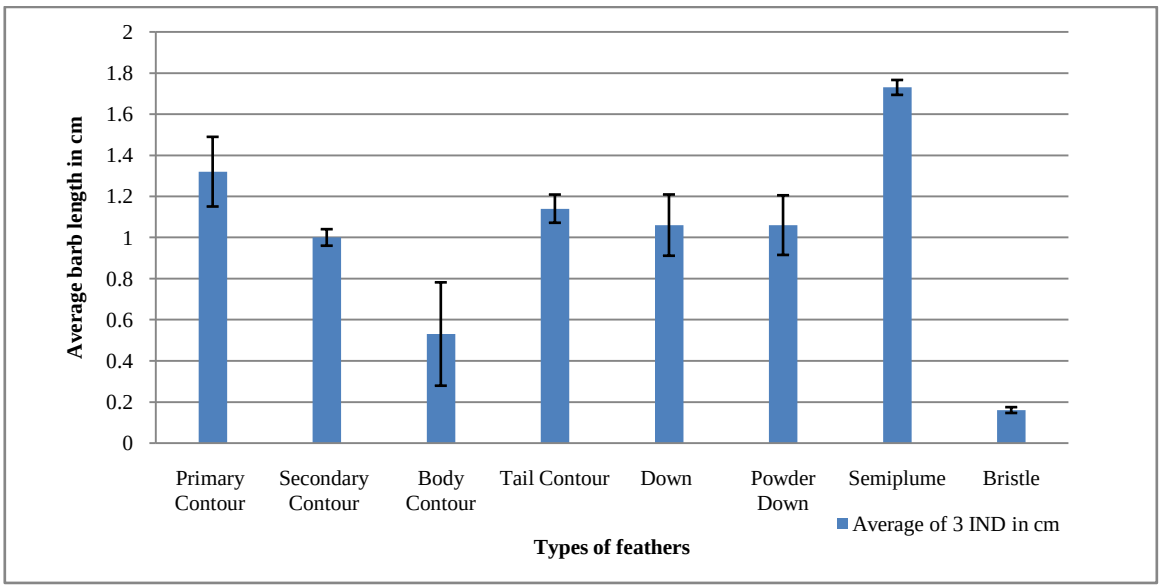

Figure 10 Mean barb length of three individuals of *T. affinis*

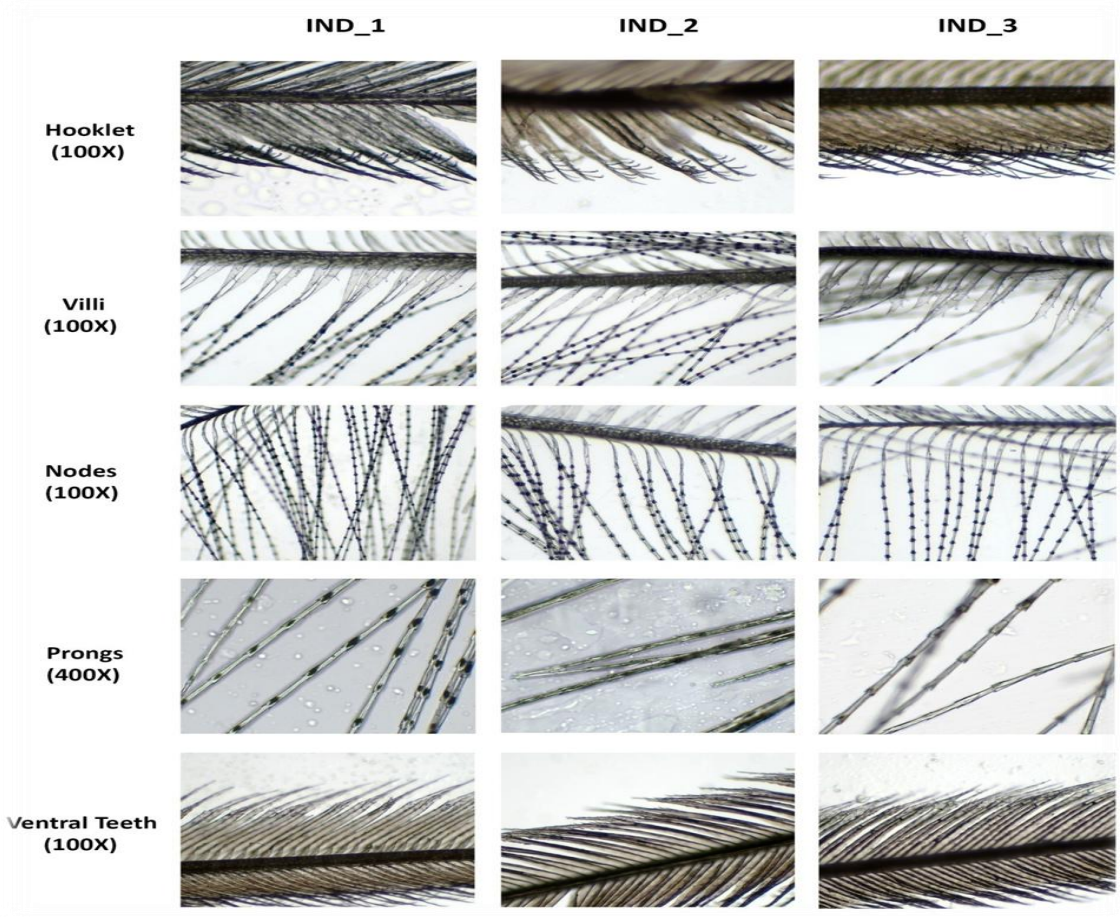

Figure 11 Microscopic feather characteristics of three different individuals on 100X and 400X resolutions.

Table 5 Microscopic observations for different parameters of pennaceous and plumulaceous feather barbs for all three individuals of *T. affinis*

Characteristics		Feather Types					
		Flight Feather	Body Contour	Semiplume Feather	Down Feather	Powder Down Feather	Bristle
Parameters for 3 individuals	Hooklets	1	1	1	0	0	1
	Villi	0	1	1	1	0	0
	Node	0	1	1	1	1	0
	Shape of Nodes	NA	3,2	3	3	3	NA
	Shape of Internodes	NA	STR	STR	STR	STR	NA
	Prongs	1	1	1	1	1	1
	Size of Prongs at Barbules	S	S	S	S	S	S
	Location of Prongs at Nodal Structure	D	D	D	D	D	D
	Pigmentation on the Ramus	4	5	5	5	4	5
	Ventral Teeth	1	1	1	0	0	1

Legends: 0 -Absent, 1 - Present, D - Distal, P - Proximal, 2 - Triangular Variably Pigmented Node Shape, 3 - Densely Pigmented Triangular Node Shape, 4 - Variable, 5 - Dark, NA - Not Applicable, STR - Straight, L- Large, S - Small

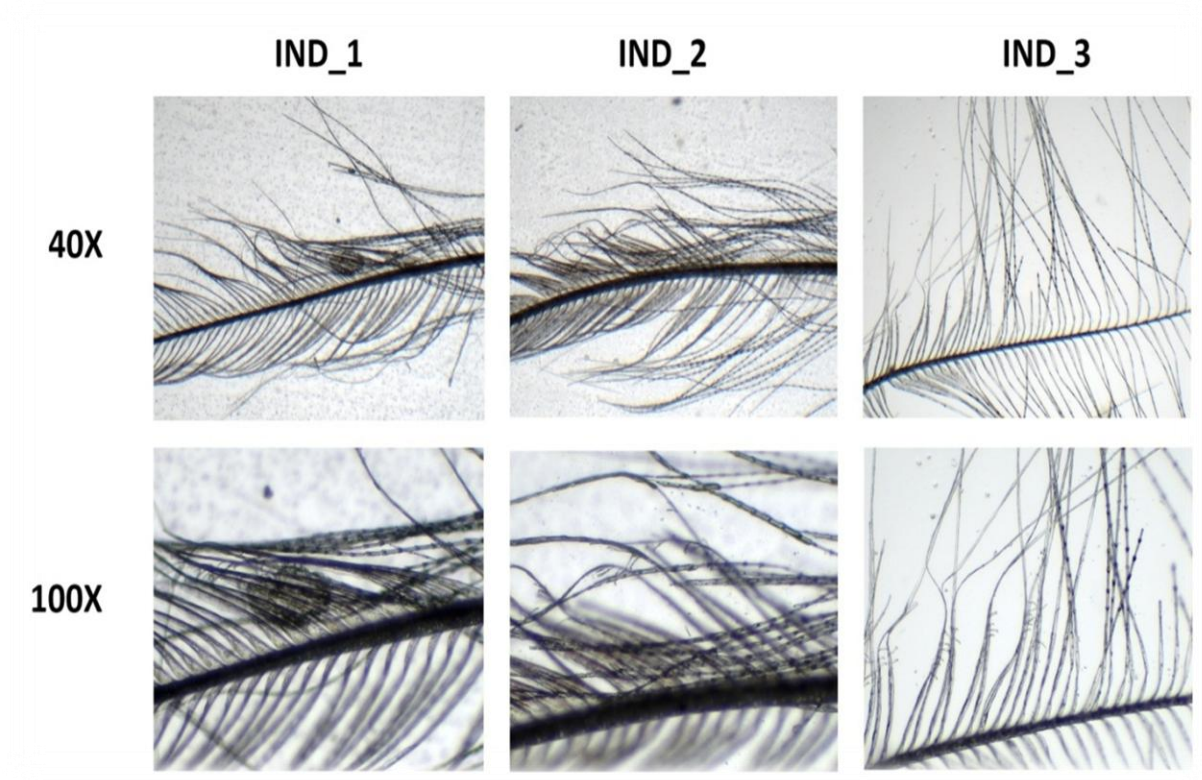

Figure 13 Hooklets and nodes present on body contour feathers from ventral side on 40X and 100X

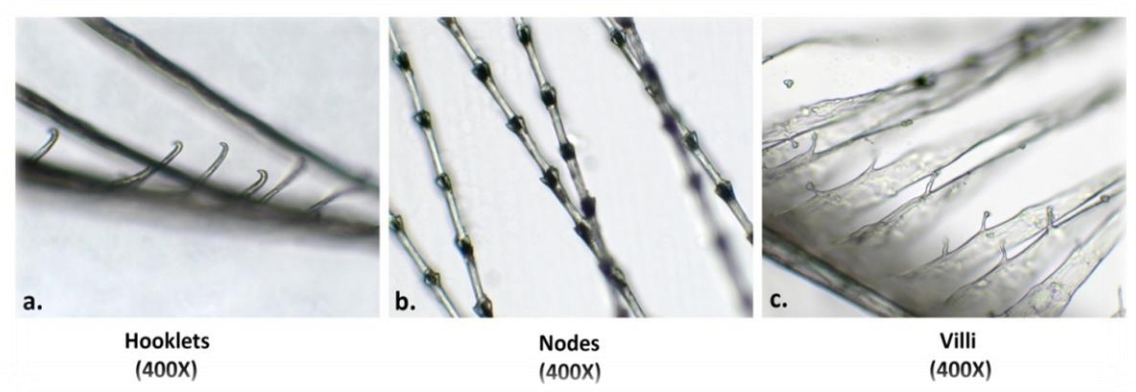

Figure 12 Hooklets (a), Nodes (b) and Villi (c) on 400X.

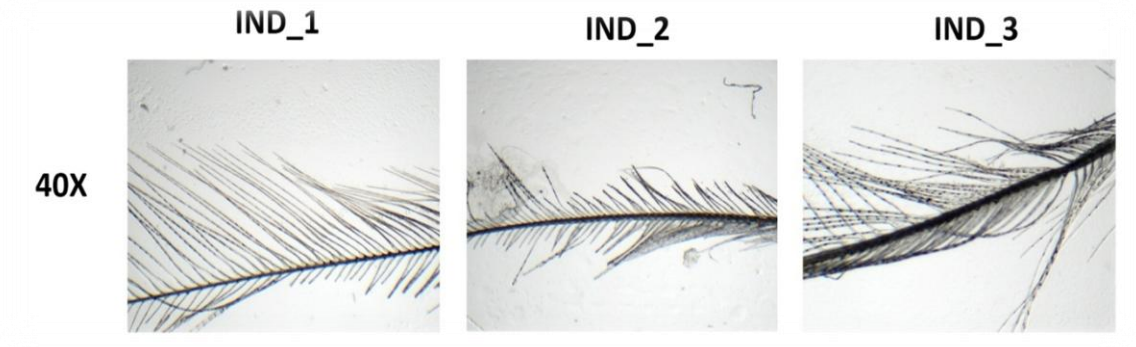

Figure 14 Hooklets and nodes present on body contour feathers from dorsal side on 40X

4. Discussion and conclusions

Investigations of different feather macro and micro characteristics in context of inter species variations have been reported by earlier workers (Brom, 1991; Dove, 2000; Dove & Peurach, 2002; Lee et al., 2016). The focused aim of this present study was to develop feather catalogue for the passerine species i.e. *T. affinis* based on their macro and microstructure of select types of feathers and also to quantify the intra-species difference if any.

Feathers possessing plumulaceous barbs were soft and fluffy in texture whereas the rigid texture of feathers (contour feathers) was due to the presence of pennaceous barbs. From the investigations on textures of various feather types, it is evident that feather texture corresponds directly to its functions. For instance, the rigid texture of contour feathers is due to the interlocking of barbs by hooklets, which is of utmost essential for maintaining structural stability and withstanding air pressure during flights. Similarly, the soft and fluffy texture due to the loose, fibrous nature of down, semiplume and powder down feathers is a necessity for providing insulation to the bird. The comparatively long length of tail rachis rather than flight feathers indicates that *T. affinis* as a species is mostly ground dwelling. Evident from the life history traits, *T. affinis* are considered as bush birds where they undertake very short flights between bushes or rocks while foraging. Hence, species with such behavior will require high maneuverability (aided by long tail feathers) rather than long flight feathers (which aids in high altitude flights). From the short calamus length and longer barb length of down, semiplume, and powder down feathers it is evident that these feathers contain loosely packed soft plumulaceous barb structure which can move independently from one another. Structurally such arrangement creates a blanket like covering all over the body of a bird, for insulation and covering purposes.

Variations in feather microstructure amongst species have already been reported by various authors (Lee et al., 2016). These variations amongst multiple species when compared may provide a key to taxa identification in birds (Lee et al., 2016). In this study of three individuals of *T. affinis*, we documented the presence of sub-pennaceous barbs in the intermediate region of body contour feathers and the presence of knobbed shaped villi. These select micro-characteristics recorded in this study (e.g. presence

Supplementary Table 1 Sequence of primers used for molecular sexing in this study adapted from Fridolfsson & Ellegren (1999)

S.No	Primers	Sequence
1	2550F	5'- GTTACTGATTCGTCTACGAGA -3'
2	2718R	5'- ATTGAAATGATCCAGTGCTTG -3'

Supplementary Table 3 Summarized macro-characteristics of the individuals of *T. affinis* for their select feathers

S. No	Feather type	Location	Colour	Texture	Pattern
1	Primary contour feather	Right wing	Pale brown	Rigid	No pattern
2	Primary contour feather	Left wing	Pale brown	Rigid	No pattern
3	Secondary contour feather	Right wing	Pale brown	Rigid	No pattern
4	Secondary contour feather	Left wing	Pale brown	Rigid	No pattern
5	Body contour	Dorsal	Pale brown	Rigid	No pattern
6	Body contour	Ventral	Pale brown	Rigid	No pattern
7	Tail contour	Tail	Pale brown	Rigid	No pattern
8	Down feather	Right wing	Creamy	Soft and fluffy	No pattern
9	Down feather	Left wing	Creamy	Soft and fluffy	No pattern
10	Down feather	Dorsal	Creamy	Soft and fluffy	No pattern
11	Down feather	Ventral	Creamy	Soft and fluffy	No pattern
12	Down feather	Tail	Creamy	Soft and fluffy	No pattern
13	Semiplume	Dorsal	Pale brown	Soft and fluffy	No pattern
14	Semiplume	Dorsal	Pale brown	Soft and Fluffy	No pattern
15	Semiplume	Ventral	Pale brown	Soft and fluffy	No pattern
16	Semiplume	Ventral	Pale brown	Soft and fluffy	No pattern
17	Semiplume	Tail	Pale brown	Soft and fluffy	No pattern
18	Powder down	Right wing	Pale brown	Soft and fluffy	No pattern
19	Powder down	Left wing	Pale brown	Soft and fluffy	No pattern
20	Powder down	Dorsal	Pale brown	Soft and fluffy	No pattern
21	Powder down	Ventral	Pale brown	Soft and fluffy	No pattern
22	Powder down	Tail	Pale brown	Soft and fluffy	No pattern
23	Bristle	Near eye	Creamy	Rigid	No pattern
24	Bristle	Near eye	Creamy	Rigid	No pattern
25	Bristle	Near beak	Creamy	Rigid	No pattern
26	Bristle	Near beak	Creamy	Rigid	No pattern
27	Bristle	Chin	Brownish	Rigid	No pattern

Supplementary Table 2 All PCR reactions were performed in a 10 µl volumes using 5 µl of 2X TaqMastermix, 0.5 µl of primers, 2 µl water and 40 ng of template DNA on an Eppendorf Thermocycler in the following temperature conditions

Parameter	Stage1		Stage 2		Stage 3	
	Incubate		35 cycles		Final	
Temperature	95°C	95°C	45.4°C	72°C	72°C	4°C
Time (mm:ss)	05:00	00:30	00:45	0:50	10:00	∞

of sub-pennaceous region and shape of villi) may provide a key for taxa identification of *T. affinis* as these features have been generally observed across the family Passeriformes (Dove & Koch, 2011). Although to validate the same more investigation on feathers from various other species are required.

Overall, the microstructures of feathers of all the 3 individuals of *T. affinis* are identical and no intra-species differences in microstructures were found. Despite their sexual differences, we observed all three individuals of *T. affinis* displayed similar feather microstructures. Through this study, it can be confirmed that feather microstructures identical in all members of the species. Although, there exists morphometric variation between the individuals it may be attributed to the difference in age and size of the three individuals compared in this study. As the quantitative characteristics of feathers structure tends to changed based on the age of an individual the qualitative feather characteristics (i.e. the microstructural features) are appointed as the best approach for feather study (Dove,1997), and through this study, we also confirm the same.

This study records a nonsignificant difference in feather characteristics amongst the three *T. affinis* individuals. Irrespective of the differences in their sex and size of feathers, the structural characteristic remains the same as in all three individuals of *T.affinis* that were investigated in this study. Thus, micro-characteristics of *T. affinis* feathers presented in this study can be used as reference characteristics for species identification. The data generated through this study forms the baseline for the feather catalogue of Indian bird species being compiled at SACON. More comparative studies are needed in plumology of Indian avifauna which can help in species identifications as well as wildlife crime investigations.

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Conflict of interest

Authors declares no conflict of interest

Author contribution

Conceptualization: Ram Pratap Singh, Data generation and compilation: Swapna Devi Ray, Prateek Dey, Nozrul Islam.

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