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Microdermetoglyphic morphometry of shed-off skin: a novel approach to solving snake identification complexity

Harij Uddin[1*], Md. Habib Ullah[2], Najmul Hasan[1], Md. Towfiq Hasan[1], Aniruddha Ghose[3], Mohammad Abdul Wahed Chowdhury[1, 4*], Ibrahim Khalil Al Haidar[1, 4*]

[1] Animal Immunization Lab, University of Chittagong, Chattogram 4331, Bangladesh
[2] Department of Science, Graduate School of Integrated Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka-Shi, 422-8529, Japan
[3] Department of Medicine, Chittagong Medical College, Chattogram 4203, Bangladesh.
[4] Department of Zoology, University of Chittagong, Chattogram 4331, Bangladesh
* Corresponding authors

INTRODUCTION & AIM

Snakes are cold-blooded, often perceived as creepy, and highly elusive animals that generally prefer to avoid detection by humans and other animals. Their secretive nature makes them difficult to observe in their natural environment during field surveys, which in turn hampers biodiversity studies. In contrast, shed snake skins are easy to detect, can remain in good condition for weeks, and may serve as a valuable source of biological information if studied properly.

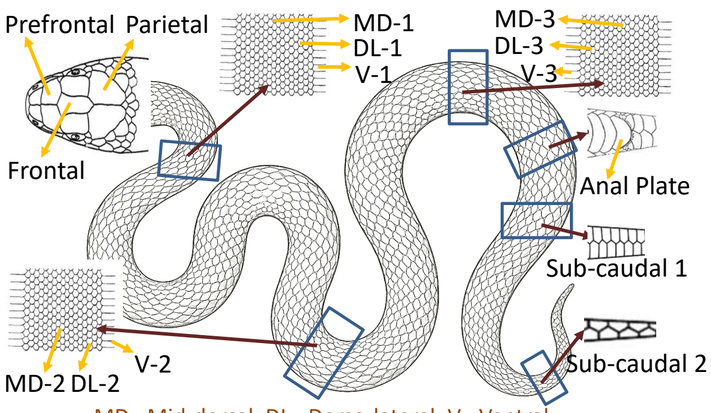
Snakes—particularly venomous species—are associated with substantial human mortality and morbidity in many parts of the world; therefore, rapid and accurate identification of snake is essential. Although several methods for snake identification currently exist, none are free from limitations. Traditional morphological approaches are often unreliable and pose serious envenomation risks during live handling, while molecular techniques, although highly precise, require costly infrastructure and specialised technical expertise, restricting their accessibility in many regions.

These challenges highlight the urgent need for a safer, reliable, and field-friendly alternative method for snake identification. In this study, we present a novel, non-invasive snake identification method based on the morphometry of microdermatoglyphic features of shed-off snake skin.

METHOD

Sample Collection, Scale selection, and Macro photography: We used shed-off skin of 14 adult cobra specimens; 7 *Naja naja* (*Nn*) and 7 *Naja kaouthia* (*Nk*), and these snakes were randomly collected from different locations of Bangladesh responding to the rescue call with due permission of the Bangladesh Forest Department (permission letter no.: 22.01.0000.101.23.2019.3173.). We studied 15 scales from each specimen (**Figure 1**). We prepared temporary slide of these 15 scales, and observed and photographed using calibrated light microscope (Labtron Digital Microscope, Model No. LBM-D13) and EUROMEX Microscope Camera (CMEX-10PRO). We examined and Photographed Inter-scale follicle and edge region of each scale (**Figure 2**).

Microdermetoglyphic morphometry: We measured six microdermatoglyphic characters from the photographs (**Figure 3**) using ImageFocusAlpha (v13229) software.



MD= Mid-dorsal; DL= Dorso-lateral; V= Ventral

Figure 1: Schematic representation of fifteen studied scales covering the entire body of a snake.

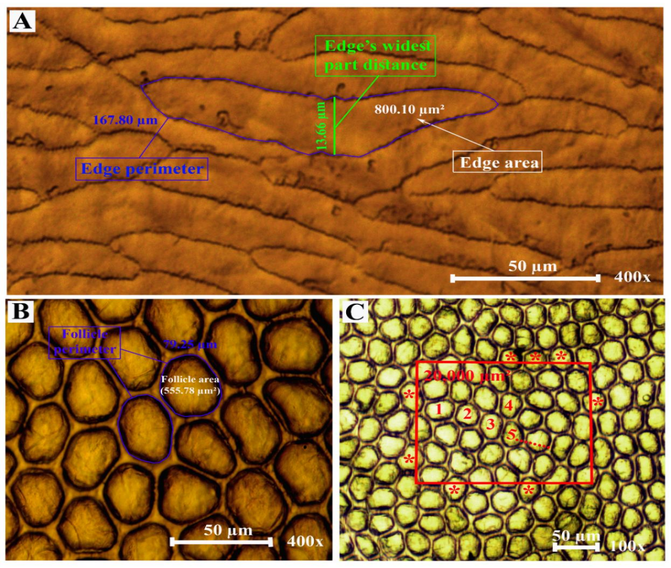


Figure 3: (A) Macro photograph of the edge area showing three microdermatoglyphic characters. Edge perimeter and edge area were measured by drawing a polygon outlining the edge structure, while the widest part distance was measured using a vertical or arbitrary line. (B) Macro photograph showing follicle perimeter and follicle area measured by outlining follicles with a polygon at 400× magnification. (C) Number of follicles per 20,000 µm² was calculated at 100× magnification by counting follicles within a randomly drawn 20,000 ± 50 µm² rectangle, excluding follicles with more than ~50% of their area outside the rectangle (*).

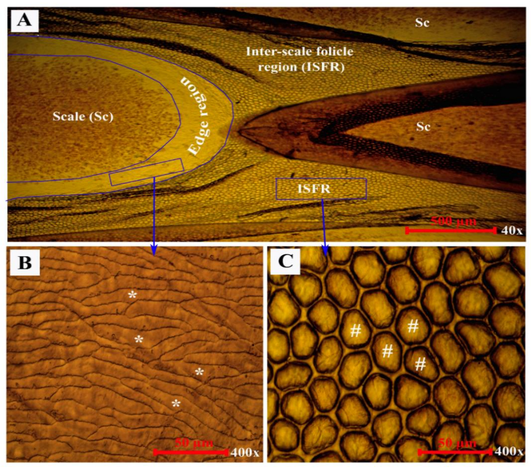


Figure 2: Microscopic view of a shed-off skin (A) showing two micro-locations, edge region and inter-scale follicle region at 40X magnification. Microscopic structures present in the edge region (B) termed as edges (* marked) and in the inter-scale follicle region (C) termed as inter-scale follicles (# marked) are shown at 400X magnification.

Normality test: We conducted the Shapiro-Wilk normality test to investigate whether the data follows normal distribution or not.

Paired t-test and Wilcoxon signed-rank test: We conducted paired t-tests for Gaussian data and Wilcoxon signed-rank tests for non-Gaussian data to assess whether microdermatoglyphic characters differed significantly between the two species, as well as between male and female individuals within each species.

Principal component analysis (PCA): We have conducted Principal Component Analysis (PCA) to reduce the dimension of the dataset and visualize the relationships between *Nk* and *Nn* species, and the relationship between the male and female snakes of *Nk* and *Nn.*

RESULTS & DISCUSSION

Paired t-tests and Wilcoxon signed-rank tests showed that studied microdermatoglyphic characters significantly differentiate *Naja naja* and *Naja kaouthia*, as well as the male and female individuals within each species. Among the six studied microdrmatoglyphic characters, edge widest part distance (edge_wpd) emerged as the most powerful discriminator, correctly separating 93.3% of scales between the two species ($p < 0.001$), followed by edge area (86.7%) and number of follicles per 20,000 µm² (46.7%). Furthermore, sex-based differences were significant ($p < 0.001$), with edge area distinguishing 60% of scales in *N. kaouthia* and 20% in *N. naja* (**Table 1**). In Principal Component Analysis (PCA), we have obtained species-wise (**Figure 4**) as well as gender-wise (**Figure 5**) clustering that shows the potential of these microdermatoglyphic characters in species as well as sex determination from a shed-off snake skin.

Table 1: The table shows character-wise percentages of significant comparisons ($p < 0.001$) between *Naja kaouthia* and *Naja naja* for the same character measured on the same scale, using paired t-tests for Gaussian data and Wilcoxon signed-rank tests for non-Gaussian data. Each comparison involved the same character (e.g. edge area) on the corresponding scale in both species, resulting in six characters compared across fifteen scales.

No.	Characters	Total comparisons	Significant comparisons		Non-significant comparisons	Significant comparisons (%)
			No.	Scales*		
1	Edge perimeter	15	2	DL2, V2	13	13.33
2	Edge area	15	13	PF, F, P, MD1, 2, 3; DL1, 2, 3; V1, AP, SC1, 2	2	86.67
3	Edge widest part distance	15	14	PF, F, P, MD1, 2, 3; DL1, 2, 3; V1, 2; AP, SC1, 2	1	93.33
4	Follicle perimeter	15	4	PF, F, AP, SC1	11	26.67
5	Follicle area	15	5	PF, F, DL3, AP, SC1	10	33.33
6	number of follicles per 20,000 µm²	15	7	PF, F, MD1, DL1, V1, 2; SC1	8	46.67

PF=Prefrontal; F=Frontal; P=Parietal; MD=Mid-dorsla; DL=Dorso-lateral; V=Ventral; AP=Anal plate; SC=Sub-caudal

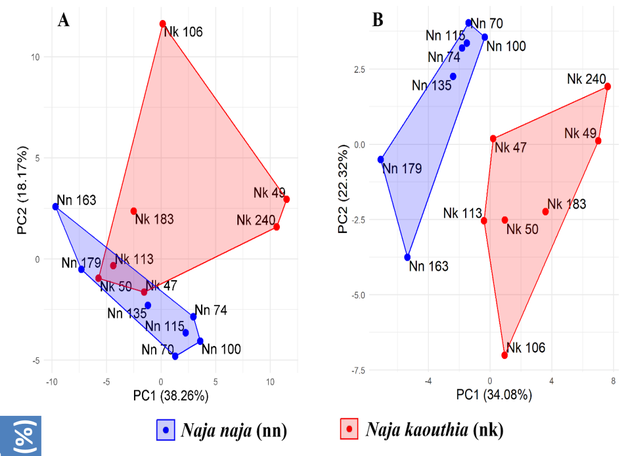


Figure 4: Principal Component Analysis (PCA) showing the ability of microdermatoglyphic characters to differentiate species. A) PCA using all characters separates *Nk* and *Nn* with slight overlap. B) PCA using the most significant characters (45 of 90) produces completely distinct species clusters.

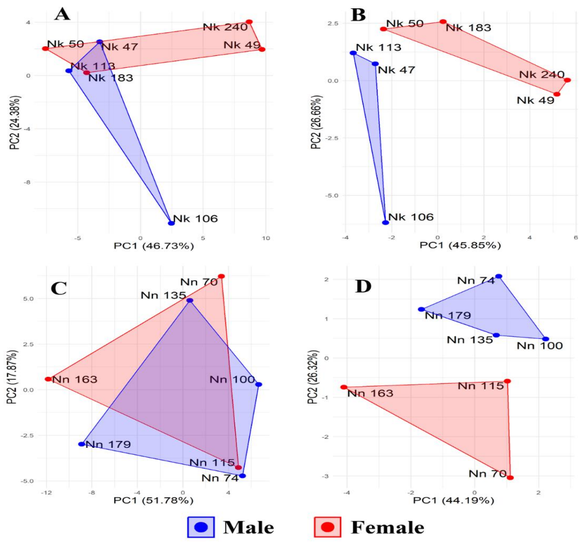


Figure 5: Efficiency of microdermatoglyphic characters in gender differentiation of *Naja kaouthia* (*Nk*) and *Naja naja* (*Nn*). Panels A and C show PCA results for male and female specimens of *Nk* and *Nn*, respectively, using all microdermatoglyphic characters. Panels B and D show PCA results for male and female specimens of *Nk* and *Nn*, respectively, using only statistically significant characters.

CONCLUSION

This study introduces the first demonstration of quantitative, rather than qualitative, shed-skin microdermatoglyphics as a taxonomic and ecological tool. By eliminating risks of live handling and bypassing molecular constraints, this approach offers a practical, non-invasive, and widely applicable method for snake identification. Beyond taxonomy, it holds promise for evolutionary studies, biodiversity monitoring, and informing conservation and public health strategies in snakebite-prone regions.

FUTURE WORK / REFERENCES

We further aim to apply this non-invasive, cost-effective, and safe approach to other groups of snakes and reptiles those shed their skin regularly. By applying the method across a wider taxonomic range, we aim to further test and strengthen its robustness and general applicability.