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APPLICATION OF TWO INTERESTING STATISTICAL TOOLS, USED IN DIFFERENTIATION OF CLOSELY RELATED SPECIES OF *Cornudiscoides* KULKARNI, 1969 (PLATYHELMINTHES: MONOGENOIDEA: DACTYLOGYRIDAE)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author NA designed the study. Authors JV and SR contributed in wet lab as well as fieldwork. Author AA performed the statistical analysis. All the authors contributed in preparing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A novel method is used to represent multivariate data as cartoon faces (Chernoff faces) for evaluation of inter-specific and intra-specific variations. Linear Discriminant Analysis has also been performed for classifying them. Five hundred specimens, belonging to nine species of the genus *Cornudiscoides* viz. *C. geminus C. agarwali*, *C. tukarami*, *C. bleekerai*, *C. mystusi*, *C. sclerovaginalis*, *C. longicirrus*, *C. aori*, *Cornudiscoides* n. sp. and one species of the genus *Bifurcohaptor* (*B. indicus*) were chosen and their morphometric data are subjected to statistical analysis. Ten sets of obtained Chernoff faces expressing differently in all the species, proving them distinct at a specific level. Being a different genus, *B. indicus* shows a significant difference.

Keywords: *Cornudiscoides* Kulkarni, 1969; *Bifurcohaptor* Jain, 1958; *Mystus* Scopoli, 1777; *Sperata* Hamilton, 1822; Chernoff faces; classification; R software.

1. INTRODUCTION

Faces are the graphical representation of multivariate data, using which one can find relevant information quickly, existing among them and these representations also have potential to enhance user's ability to study the phenomenon more accurately, thus easy to remember [1]. The technique that represents

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multivariate data into cartoon faces has been described by Chernoff [1]. This is a novel method, evolved through the chain of mathematical and geometrical calculations of relationship. With the help of these cartoon faces, different distinguishing features existing among them can be summarized. The image is constructed with multivariate data sets where each facial feature assigns a particular variable. The difference in frequencies of these variables results in a unique image.

The genus *Cornudiscoides* established by Kulkarni [2] at Hyderabad, reported from India, Sri Lanka, Malaysia and Pakistan [3,4,5,6]. To date, 16 species of *Cornudiscoides* have been described from India [7]. Of which, 13 species infesting 4 species of genus *Mystus* viz. *M. cavasius, M. vittatus, M. bleekeri* and *M. tengara* and three species have been described from *Sperata aor.*

Wide range in morphometric data of species description of monogenoidean population indicates the presence of morphological variation in nature [8]. Depending upon the environmental conditions like the season, locality and geographic distribution of host and age of parasites, the haptor and copulatory complex, important diagnostic characters, may exhibit variations in their morphology [9] proving that morphological studies alone are not completely reliable. Earlier researchers have used one-way analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), principal component analysis (PCA) and LDA to disclose inter-specific and intra-specific variation in monogenoidean parasites [10,11,8].

In the present study, we noticed small but a group of morphometric differences present in haptor of *Cornudiscoides* species like differences in size and shape of a dorsal anchor, ventral anchor, dorsal bar, ventral bar and hooks, the rising question that these variations are interspecific or intraspecific since many of the *Cornudiscoides* species shows similarity with each other.

The utility of Chernoff faces in distinguishing Indian species of the genus *Cornudiscoides* Kulkarni, 1969 parasitizing *Mystus* Scopoli,1777 (commonly known as 'Katanna') and *Sperata* Hamilton, 1822 (earlier included under the genus *Mystus*, commonly called 'Bada tagan'), restricted to southeast Asia [7] collected from river Gomti and various water bodies of U.P. can be checked here. Linear discriminant analysis (LDA) is another statistical method that can be used in quantitative measurements, to give the best classification model, explaining the distinction between different species groups.

However, so far no study has been conducted, using Chernoff faces for multivariate datasets of monogenoidean parasites. Here nine species of *Cornudiscoides* (whose number was enough) namely, *C. geminus* Gusev, 1976; *C. agarwali* Agrawal and Vishwakarma, 1996; *C. tukarami* Agrawal and Vishwakarma, 1996; *C. bleekerai* Agrawal and Vishwakarma, 1996; *C mystusi* (Rizvi, 1971) Dubey et al. 1992; *C. sclerovaginalis* Devak and Pandey 2007; *C. longicirrus* Agrawal et al. 2016; *C. aori* Agrawal et al. 2016 and *Cornudiscoides* n.sp. and one species of another genus *Bifurcohaptor* Jain, 1958 sp. i.e. *B. indicus* Jain, 1958, belonging to the same subfamily and parasitizing the *Mystus* is included in the present work.

2. MATERIALS AND METHODS

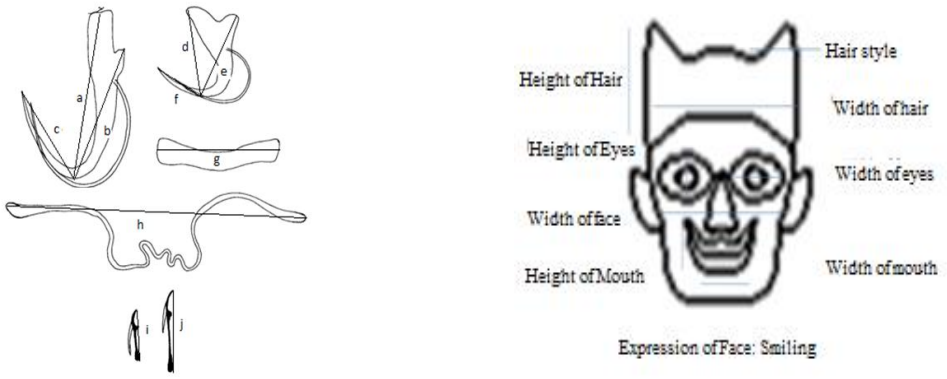
Fish hosts (commonly available freshwater fishes for which ethical clearance is not required) have been collected from different water bodies of Lucknow(26º51ºN 80º57º E), Barabanki (26.92ºN 81.20ºE), Gorakhpur (26.7588ºN 83.3697ºE) and Basti (27º 15ºN83º00ºE) (Table. 1), identified with the help of Fish base [12] and sacrificed. Gills were removed and transferred into glass Petri-dishes, containing water. Live worms were isolated with the aid of a binocular microscope. Gills of fishes were fixed in 3% formalin, diluted with lukewarm water [13]. A total of 500 specimens of ten species are thus collected and identified with the help of "An Encyclopaedia of Indian Monogenoidea" [3]. Temporary slides (glycerine mounts) [14,15] and permanent slides ware prepared according to Kritsky et al. [16] were prepared for the study of hard parts the monogenoids considering in the present study. The morphometric data is recorded from temporary and permanent specimens with the aid of Image Pro Express 0.6. Illustrations of parasites were according to Gusev [17].

In this study, ten parameters (variables); dorsal anchor inner length, dorsal anchor outer length, dorsal anchor recurved point, ventral anchor inner length, ventral anchor outer length, ventral anchor recurved point, dorsal bar length, ventral bar length, small hook length and large hook length were measured in micrometer (µm) (Fig. 1 and Table 2), with the help of Olympus BX 51 image analysis software. The Chernoff faces were drawn with the help of obtained these measurements over these 10 different variables. The LDA is used to develop a classification rule to classify the individuals of *Cornudiscoides* spp. correctly based on measurements of hard parts of haptor. The analysis was performed with the help of R-2.9.0 software. The different variables were used to define the different dimensions of the Chernoff faces

(Table 2). With the change in the values of these variables, the different dimensions of Chernoff faces also changed. For example with the increase in the DAOL the width of the face also increased. The Discriminant Analysis is a statistical tool used for the development of a classification rule, with the help some prior information (measurements on the different variable) to classify a new individual into one of the known populations based on its measurements on the same variables. Therefore, Discriminant analysis is used to develop a classification rule to classify the individuals of *Cornudiscoides* spp. correctly on the basis of measurements. The Chernoff faces were constructed and Discriminant Analysis is performed with the help of R-2.9.0 software. Total 500 faces corresponding to nine species of *Cornudiscoides* and one species of *Bifurcohaptor* (*B. indicus*) are presented in the sequential order depicted in Fig. 2.

Table 1. Host-parasites list, with locality and number of specimens of each species used in the present study

***Cornudiscoides* spp.**	**Host species**	**Localities**	**No. of individuals used in this study**
C. geminus	*Mystus vittatus* (Bloch, 1794)	Gonda	50
C. agarwali	*Mystus bleekeri* (Day, 1877)	Barabanki	50
C. sclerovaginalis	*Mystus cavasius* (Hamilton, 1822)	Barabanki, Lucknow	50
C. tukarami	*Mystus cavasius* (Hamilton, 1822)	Barabanki, Lucknow	50
C. n.sp.	*Mystus bleekeri* (Day, 1877)	Lucknow	50
C. bleekerai	*Mystus cavasius* (Hamilton, 1822)	Lucknow	50
C. mystusi	*Sperata aor* (Hamilton, 1822)	Lucknow	50
C. longicirrus	*Sperata aor* (Hamilton, 1822)	Lucknow	50
C. aori	*Sperata aor* (Hamilton, 1822)	Lucknow	50
Bifurcohaptor indicus	*Mystus vittatus* (Bloch, 1794)	Lucknow	50



A B

Fig. 1. (A) Measured parts of haptor used in the study: a- dorsal anchor inner length, b- dorsal anchor outer length, c- dorsal anchor, d- ventral anchor inner length, e- ventral anchor outer length, f- ventral anchor recurved point, g- dorsal bar length, h -ventral bar length, i – small hook length, j- large hook length. (B) Facial characters, constructed using variables

Table 2. Full name of measured variables, their abbreviations, and characters they represent

Serial number	Name of variables	Abbreviation used	Characters
1.	Dorsal anchor inner length	DAIL	Hair Style
2.	Dorsal anchor outer length	DAOL	Width of face
3.	Dorsal anchor recurved point	DARP	Shape of face
4.	Ventral anchor inner length	VAIL	Height of mouth
5.	Ventral anchor outer length	VAOL	Width of mouth
6.	Ventral anchor recurved point	VARP	Expression of Face
7.	Dorsal bar	DB	Height of eyes
8.	Ventral bar	VB	Width of eyes
9.	Hook small	HS	Height of hair
10.	Hook large	HL	Width of hair

3. RESULTS

By finding mean and standard deviations (S.D.) of different variables, in ten different species, it is observed *that B. indicus* can easily be separated from other species whereas, there exist some overlapping among these variables among the nine species of the genus *Cornudiscoides* under study, have some overlapping among these variables. The distinction among these species, by taking a look on the measurement over these variables, is not so easy (Table 3). To overcome this difficulty, for the five hundred parasites that were placed in 10 distinct groups (group I includes species of *C. mystusi*, group II includes species of *C. sclerovaginalis*, group III includes species of *C.* new species, group IV includes species of *C. bleekerai*, group V includes species of *C. agarwali*, group VI includes species of *C. longicirrus*, group VII includes species of *C. geminus*, group VIII includes species of *C. aori*, group IX includes species of *C. tukarami* and group X includes species of *B. indicus* Chernoff faces are constructed (Fig. 2). Each group is represented by 50 Chernoff faces, which showed variation among the groups (Fig. 2).

Individually, the group I shows most of the constant features like the shape of eyes, height and width of mouth but some variations can be observed in their head region, like the height of hair and style of hair. Worms of Groups II, IV and IX are also congeners and are found on *Mystus cavasius* (Hamilton, 1822). Groups II and IV have the structure of eyes and ears constant but the width of face and height of hair represent significant variation. Parasites of Group III and V infect to *Mystus bleekeri* (Day, 1877). In group III, facial expression, like smile, shows variation while height and style of hairs are constant. Members of group V show significant variations in the height of faces and smile. Group IX and X depicts constant features within their group members. The faces produced by 500 specimens, divided into 10 groups look somewhat similar (group III, IX, X) but some do not completely do justice as they show less similarity with other group members (Fig. 3). However, in individual group, some species depicted variation in their characters like the shape of eyes, style of hair and mouths among others. Interestingly, the reason behind these variations could not be determined. To explain this phenomenon of a discrepancy, the linear discriminant analysis was performed.

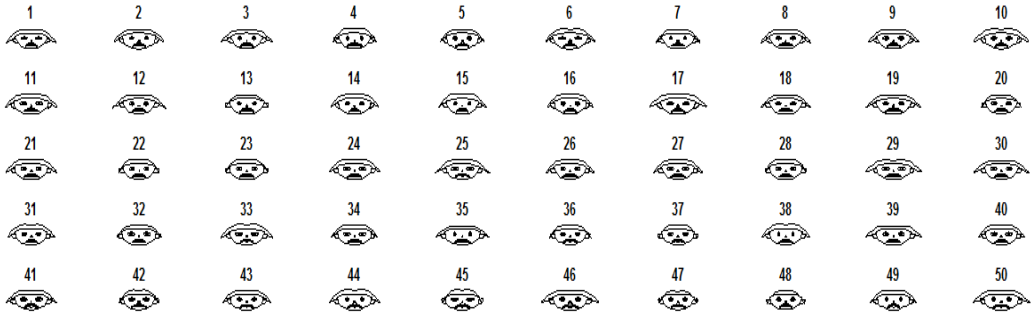
After compilation of individuals from a group, ten (Fig. 3) faces were obtained, each representing a distinct species showing remarkable differences like smile, height and width of eyes (intra-specific variation), with some constant features like height of hair, structure of the nose, and style of hairs. Species of the genus *Bifurcohator* Jain, 1958 exhibited extreme and clear cut distinction from *Cornudiscoides* species in its height and width of hair, eyes, mouth, smile and hairstyle (Inter-generic variation).

Before performing the linear Discriminant Analysis the Shapiro-Wilk's test is used to test the normality of data (p-value<0.001) and Box's M test (3443.47, pvalue<0.001) is performed to check the assumption of homogeneity among variables. Both tests show that assumptions are not fulfilled, i.e., the variables are non-normally distributed as well as were heterogeneous. Therefore Quadratic Discriminant Analysis is also performed along with linear Discriminant Analysis. In both the methods performed with a robust method of estimation is used. By using linear Discriminant Analysis, the classification functions are obtained. The coefficient for this classification functions are represented in Table 5. Using these classification functions, an overall 99.8% correct classification is achieved which is also supported by cross-validation. One species *C. tukarami* which shows 2% variability which is reasonable in determining the intra-specific relationship among *Cornudiscoides* species (Table 6). Using Quadratic Discriminant Analysis the same results (an overall of 99.8% correct classification) was achieved (Table 7). Thus, all the individuals of one species can be separated from other species.

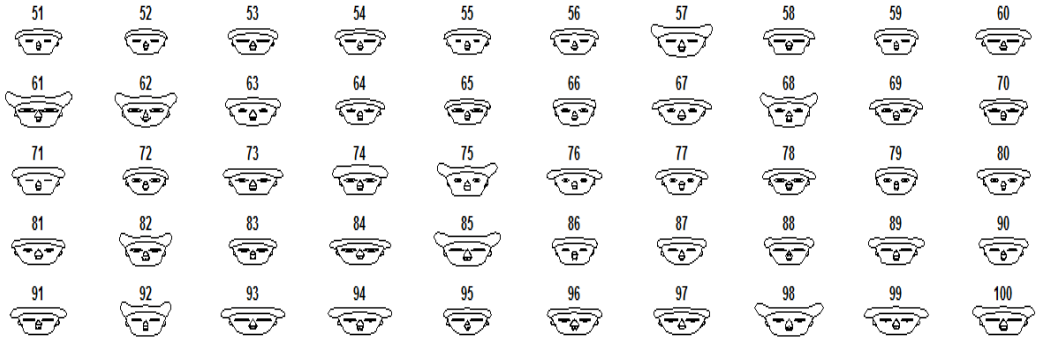
Table 3. Distribution of mean and standard deviation of the variables, with respect to each species in the study

Variables	*C. mystusi*		*C. sclerovaginalis*		*C.* n.sp.		*C. bleekerai*		*C. agarwali*		*C. longicirrus*		*C. geminus*		*C. aori*		*C. tukarami*		*B. indicus*	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
DAIL	36.79	1.31	57.30	5.19	40.84	1.10	47.05	2.80	41.26	3.70	45.90	1.03	39.24	0.87	37.24	0.93	37.35	0.84	**276.64**	10.51
DAOL	31.97	2.12	48.66	5.80	32.39	0.74	40.30	3.67	31.51	3.17	37.62	2.11	28.76	1.58	30.99	1.90	31.82	1.25	**299.31**	7.63
DARP	21.99	1.02	26.74	1.20	23.44	0.78	21.94	1.42	23.58	1.05	25.35	1.63	20.79	1.25	24.76	0.99	23.26	0.85	**50.75**	6.02
VAIL	22.75	0.92	15.31	0.79	17.32	0.81	14.26	0.80	18.10	1.56	27.51	0.92	12.91	0.73	22.61	0.91	17.23	0.93	**36.74**	2.06
VAOL	24.05	1.02	15.71	0.72	19.35	0.83	15.53	0.86	19.09	1.61	23.48	0.88	14.84	0.47	18.44	0.73	15.19	0.98	**33.32**	2.45
VARP	7.49	0.79	23.21	0.78	20.64	1.16	19.71	0.96	20.37	1.63	15.16	0.58	17.18	0.65	14.57	0.87	17.50	1.14	**31.67**	2.84
Dorsal_bar	33.34	1.60	25.94	1.94	30.31	0.89	19.09	0.64	27.10	1.49	37.70	4.20	25.17	1.24	30.03	1.84	22.67	1.00	**86.92**	4.33
Ventral_bar	33.23	1.54	36.49	2.05	32.35	1.46	31.09	2.52	28.18	2.18	83.38	9.41	26.42	0.93	85.03	8.73	29.30	1.59	**124.45**	8.22
Hook_small	13.61	0.83	13.91	0.79	13.29	1.58	11.91	0.55	13.05	0.69	14.14	1.08	12.96	0.63	11.89	0.59	13.84	0.67	**18.50**	1.52
Hook_large	25.57	1.03	24.39	0.73	32.74	0.72	24.34	0.74	27.19	0.82	27.18	1.25	21.62	0.88	19.40	1.18	24.13	1.29	**18.11**	1.23

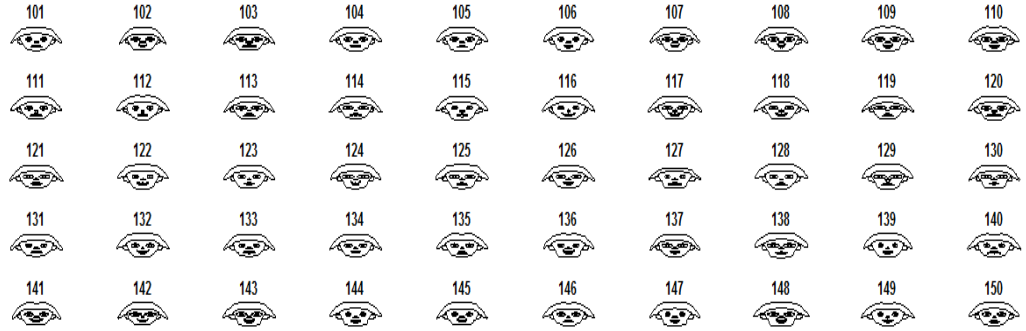
Group I: *C. mystusi*



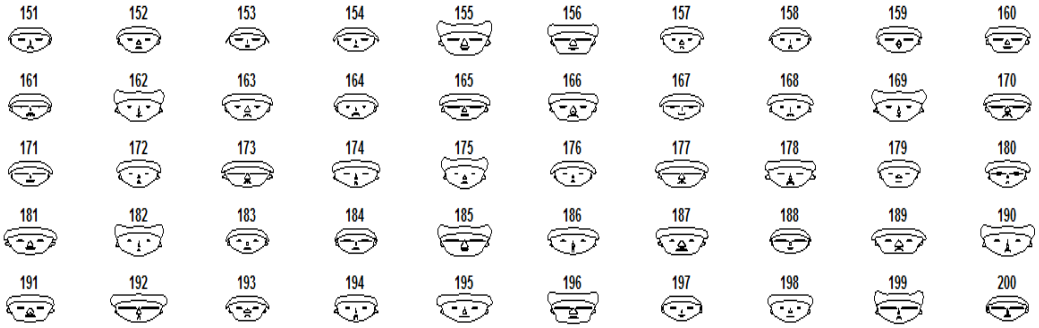
Group II: *C. sclerovaginalis*



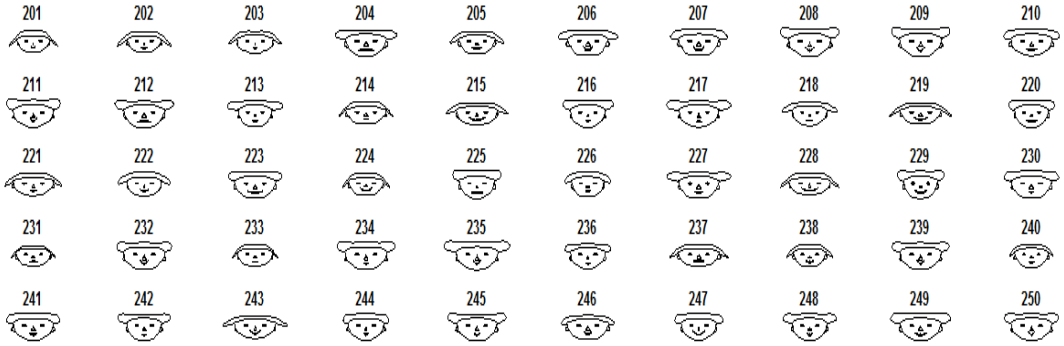
Group III: *C.* n. sp.



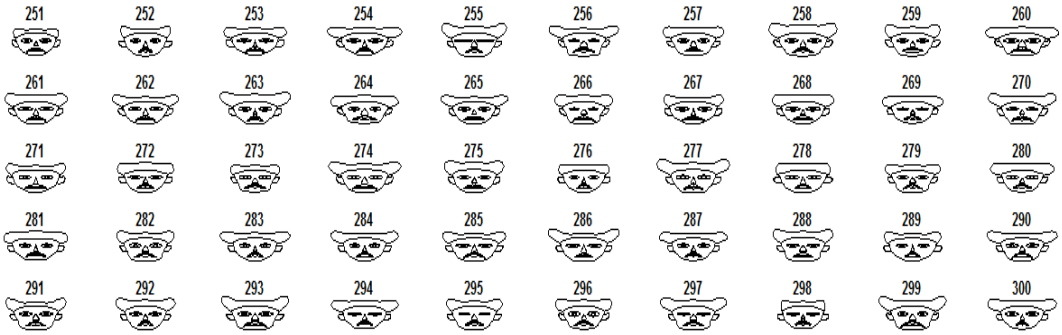
Group IV: *C.bleekerai*



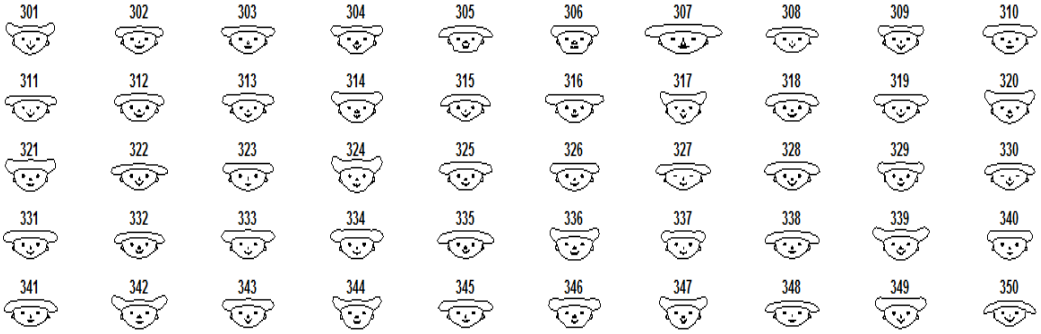
Group V: *C. agarwali*



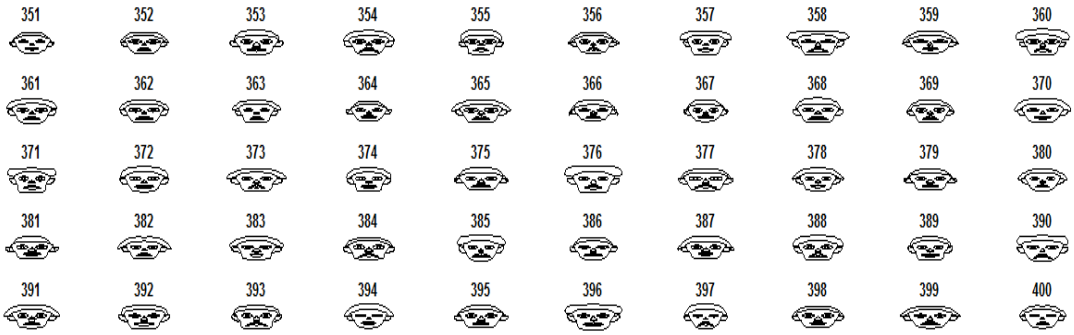
Group VI: *C. longicirrus*



Group VII: *C. geminus*



Group VIII *C. aori*



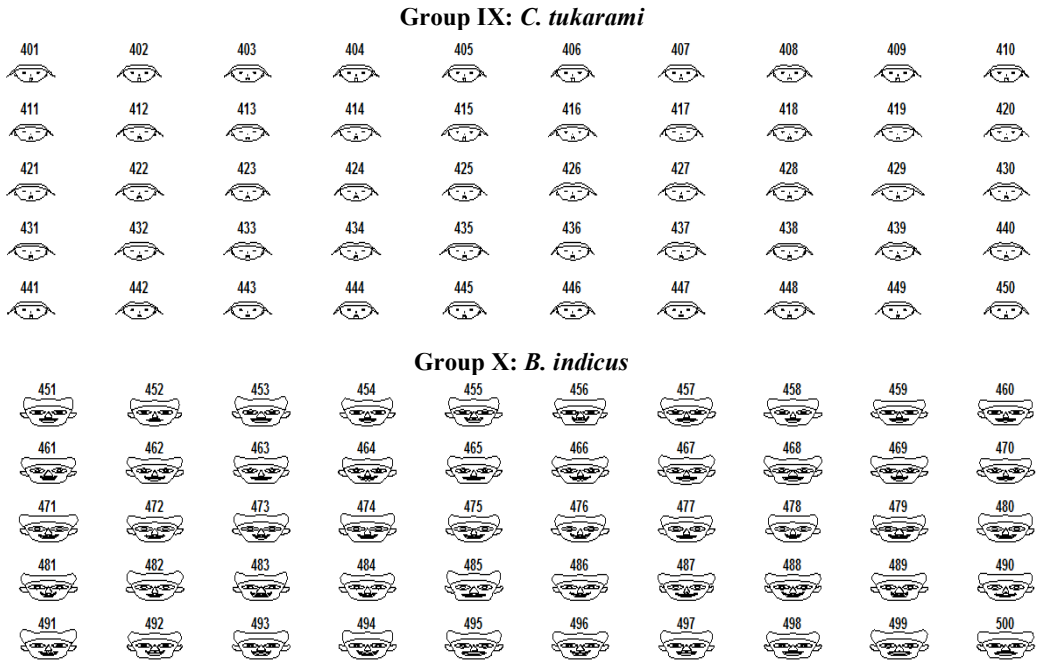


Fig. 2. Depicted 500 Chernoff faces of nine *Cornudiscoides* species (groups I-IX) and one *Bifurcohaptor* species (Group X)

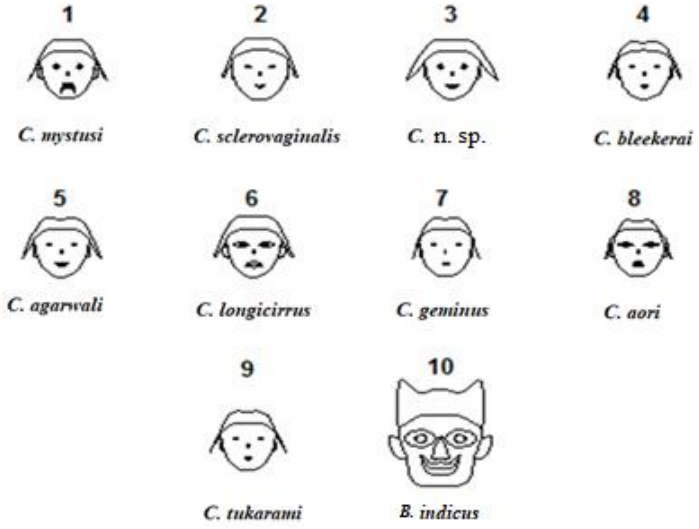


Fig. 3. Chernoff faces, representing graphical summary showing inter/intraspecific relationship among them

Table 4. The number of individuals of different groups, showing differences among their group members

Groups	Cases
Group I:	4, 13, 14, 20, 22, 23, 42, 45, 47
Group II:	57, 61, 62, 68, 75, 82, 85, 98
Group IV:	155, 162, 169, 182, 185, 190, 196
Group V:	201, 202, 203, 205, 214, 215, 219, 221, 224, 228, 233, 237, 238, 240
Group VI:	251, 276, 278, 281
Group VII:	307
Group VIII:	358, 365, 373,376, 396

Table 5. Linear discriminant analysis function by variable of *Monogenean Species* in the study

Variable	Species									
	C. mystusi	***C. sclerovaginalis***	***C.* n. sp.**	***C. bleekerai***	***C. agarwali***	***C. longicirrus***	***C. geminus***	***C. aori***	***C. tukarami***	***B. indicus***
DAIL	0.12	1.56	0.60	1.17	0.74	0.41	0.91	0.29	0.48	10.00
DAOL	0.67	1.37	0.27	1.01	0.26	0.72	0.31	0.52	0.59	17.73
DARP	1.58	1.98	1.70	1.41	1.67	1.66	1.65	2.22	1.96	-2.11
VAIL	14.97	9.80	11.60	9.55	12.30	18.26	8.12	14.71	11.98	18.58
VAOL	11.50	5.28	7.67	5.95	7.99	10.17	5.96	7.87	5.58	6.20
VARP	5.90	14.76	14.29	12.83	13.77	10.54	11.34	9.42	11.64	16.31
Dorsal_Bar	4.15	3.63	4.19	2.32	3.53	5.03	3.56	4.08	2.83	14.16
Ventral_Bar	0.28	0.33	0.03	0.18	-0.02	2.12	0.12	2.46	0.09	3.16
Hook_small	12.63	11.87	11.61	10.34	11.59	11.84	11.69	9.71	12.68	8.71
Hook_large	23.27	23.11	31.66	23.21	26.19	24.26	20.59	16.83	23.20	8.05
(Constant)	-820.48	-812.00	-1020.36	-675.45	-848.41	-1093.25	-580.61	-737.56	-692.02	-5654.20

Keys: DAIL= Dorsal anchor inner length, DAOL= Dorsal anchor outer length, DARP= Dorsal anchor recurve point, VAIL= Ventral anchor inner length, VAOL=Ventral anchor outer length, VARP= ventral anchor point, Dorsal Bar, Ventral Bar, Hook small, Hook large

Table 6. Summary of actual and predicted group of species of *Cornudiscoides* Kulkarni, 1969 and *Bifurcohaptor* Jain, 1958

		Classification Results										
	Species	Predicted Group Membership										Total
		C. mystusi	***C. sclerovaginalis***	***C.* n.sp.**	***C. bleekerai***	***C. agarwali***	***C. longicirrus***	***C. geminus***	***C. aori***	***C. tukarami***	***B. indicus***	
Original	*C. mystusi*	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. sclerovaginalis	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. n.sp.	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. bleekerai	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. agarwali	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. longicirrus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. geminus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. aori	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. tukarami	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (2.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	49 (98.0%)	0 (0.00%)	50 (100.0%)
	B .indicus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	50 (100.0%)
Cross-validated[b]	*C. mystusi*	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. sclerovaginalis	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. n.sp.	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. bleekerai	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. agarwali	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. longicirrus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. geminus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. aori	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. tukarami	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (2.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	49 (98.0%)	0 (0.00%)	50 (100.0%)
	B. indicus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	50 (100.0%)

Table 7. Summary of actual and predicted group of species of *Cornudiscoides* Kulkarni, 1969 and *Bifurcohaptor* Jain, 1958 using quadratic discriminant analysis

		Classification Results										
	Species	**Predicted group membership**										**Total**
		C. mystusi	***C. sclerovaginalis***	***C.* n.sp.**	***C. bleekerai***	***C. agarwali***	***C. longicirrus***	***C. geminus***	***C. aori***	***C. tukarami***	***B. indicus***	
Original	*C. mystusi*	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. sclerovaginalis	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. n.sp.	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. bleekerai	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. agarwali	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. longicirrus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. geminus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. aori	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	0 (0.00%)	0 (0.00%)	50 (100.0%)
	C. tukarami	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (2.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	49 (98.0%)	0 (0.00%)	50 (100.0%)
	B. indicus	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	50 (100.0%)	50 (100.0%)

4. DISCUSSION

Monogenoidea is a diversified group of parasites. According to Whittington [18] about 4000 monogenoidean species are known in which 25,000 species exist in the environment. They have undergone a wide range of adaptive radiation [19] along with evolutionary pressure resulting into the heterogeneity of parasites with special effects on attachment organs or haptor [20], which is of generic importance are generally used to distinguish species. The haptor is equipped with highly sclerotized structures (anchors, bars, hooks and/or clamps etc.). These sclerotized structures are major attachment organ in monogenoids, where the whose number, morphology, and morphometry is of generic importance [21,22] and used as a diagnostic character in taxonomy [23,24]. On the contrary, male and female copulatory organs of monogenoideans are considered as important for evaluation of intraspecific variations among the species of a genus along with haptoral parts.

Two statistical tools Chernoff and LDA were applied to morphometric data set (Five hundred individuals: 50 specimens of each species) obtained from hard parts (anchor, bars and hooks) of the nine *Cornudiscoides* species and one *Bifurcohaptor* species and successfully discriminate the five hundred individuals into ten groups corresponding to distinct species. Our preliminary results of Chernoff faces grouped all 500 species divided into 10 groups look somewhat similar (group III, IX, X) but some of them do not completely do justice as they show less similarity with other group members. After compilation of individuals from a group, ten (Fig. 3) faces were obtained, each representing a distinct species namely *C. mystusi*, *C. sclerovaginalis*, *C.* new species, of *C. bleekerai*, *C. agarwali*, *C. longicirrus*, *C. geminus*, *C. aori*, *C. tukarami* and *B. indicus*, showing remarkable differences like smile, height and width of eyes (intra-specific variation), with some constant features like height of hair, structure of the nose, and style of hairs. Species of the genus *Bifurcohaptor* Jain, 1958 exhibited extreme and clear cut distinction from *Cornudiscoides* species in its height and width of hair, eyes, mouth, smile and hairstyle (Inter-generic variation).

LDA was applied to avoid any discrepancy and based on nine variables, which are summarised (Table 5). LDA gives excellent classification using the present data set of variables, depicting the percentage of correctly identified individual of each species is 100%. This highest classification percentage (100%) varies in case of one species i.e. *C. tukarami* which scores 98% demonstrating some insignificant variation. The assignment of new species to the genus *Cornudiscoides* was also done using Chernoff faces and LDA. Thus proving powerful classification tool. Earlier reports [8,25,26] also showed that these statistical tools are very effective in monogenoid species discrimination.

Nowadays, the molecular analysis using nuclear (18S, 28S and ITS) [27,28,29,30,31] and mitochondria gene (COI) [32] are being used along with the inclusion of systematically important morphometric data set of sclerotized parts to resolve various problems dealt with systematic placement of monogenoids. Alternatively, statistical implication on morphometric data obtained from the hard parts of monogenoideans seems important for differentiation of parasites at a generic and specific level. Here, morphometry of haptoral parts of parasite seems significant for facial expression of Chernoff faces, where the structure of the face is represented by recurved point of dorsal anchor, smile by recurved point of ventral anchor, height and width of eyes by dorsal bar and ventral bar respectively. These facial expressions are crucial for species differentiation, while all the parameters together are useful for generic separation. Apart from quantitative analysis, qualitative (shape and position of sclerotized parts) analysis is equally important for intra-and/or /interspecific variations. Earlier studies have also showed that Quadratic Discriminant Analysis and Linear Discriminant analysis using morphometric variables are efficient tools for identification of monogenoidean parasites at generic/species level.

5. CONCLUSION

The statistical implication on morphometric data obtained from the hard parts of monogenoideans provides graphical representation, using which one can find relevant information quickly and differentiate parasites at generic and specific level and complement morphological attributes.

ETHICAL APPROVAL

For the care and use of animals all the applicable institutional, national and/or international guidelines were followed. The procedures followed in this study with animal specimens were in accordance with the ethical standards of the institution.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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