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Research Article

Resolving Issues at Host Level Using Variable Reduction Methods: Case of Cornudiscoides Spp. (Platyhelminthes: Monogenoidea)

Nirupama A[1], Jyoti V[1], Girdhar GA[2] and Ashok KSingh[3]*

[1]Department of Zoology, India

[2]Department of Statistics, India

[3]RGG Department, University of Nevada, USA

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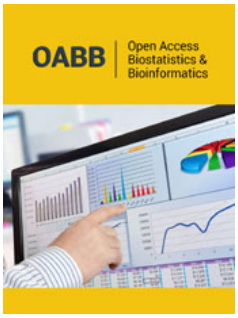

***Corresponding author:** Ashok K Singh, RGG Department, William F Harrah College of Hospitality, Las Vegas, USA

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Abstract

Taxonomy deals with delineating and classifying organisms, and traditionally relies on morphological characters only. We have used morphological characters, and molecular biology to distinguish monogenoids at generic and specific levels. Principal Component Analysis provided a magnifying glass to resolve the taxonomic issues as well as their host levels.

Introduction

Taxonomy is the branch of science which deals with delineating and classifying organisms [1]. It plays an important role in conserving biodiversity [2]. Traditionally, the approaches of taxonomy are based on morphological characters only. According to Dayrat [1] morphospecies (species established on the basis of morphology) are hypotheses, that can be proven by different kinds of approaches and researchers develop several new techniques and tools for testing species hypothesis [3-5]. However, the morphological study has its own limits resulting in misidentification and incorrect placement of several dactylogyrid genera and species [6]. In recent era, molecular taxonomy (nuclear DNA and Mitochondrial DNA) has been used successfully to complement morphologically based taxonomy. DNA molecules have also been utilized to study parasites as well their inter/intra-relationship [7-10]. Apart from molecular analysis researchers also have employed several statistical methods e.g. linear discriminant analysis (LDA), principal component analysis (PCA) and multivariate analysis of variance (MANOVA) to explain inter-specific and intra-specific relationships in monogenoideans [6,11-13]. The monogenoid genus [14] infects two members of the family bagridae *Mystus Scopoli*, 1777 and *Sperata Holly*, 1939. The species infecting the two fishes are quite similar characters. Supporting evidence from one additional discipline is also required and earlier discriminant analysis proved useful in distinguishing dactylogyrid monogenoids at generic and specific level [6].

In the present work, the PCA is used to resolve the taxonomy of a single genus at specific level and the species infecting two hosts. For comparison, species of another dactylogyrid genus *Bifurcohaptor* [15] was also taken into consideration.

Material and Methods

Data collection

Fish hosts (commonly available freshwater food fishes for which ethical clearance is not required) were collected from different water bodies of Lucknow (26º51ºN 80º57º E), Barabanki (26.92ºN 81.20ºE), Gorakhpur (26.7588ºN 83.3697ºE) and Basti (27º 15ºN83º00ºE) and identified with the help of Fish base (Froese and Pauly, 2018) and sacrificed. Gills were removed and transferred in glass Petri-dishes, containing water. Live worms are observed under binocular microscope. A total of 500 specimens of nine species of [13-17] *Cornudiscoides* viz. *C. n.sp.* and one species of *Bifurcohaptor* i.e. *B. indicus* [12]

thus collected are identified with the help of "An Encyclopaedia of Indian Monogenoidea" [10]. Temporary slides (glycerine mount) are prepared for the study of hard parts. The morphometric data is recorded from temporary and permanent specimens. All the measurements were taken in µm. In present 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 are measured in µm (Figure 1), by Olympus BX 51 image analysis software. Needless to mention here that species under study have distinct copulatory complex, being peerless, therefore not included in the present study.

Methods

Principal component analysis (PCA) is a latent variable method that reduces the dimensionality of the data while explaining most of the variation in the data set. It accomplishes this reduction by identifying directions, called principal components, along which the variation in the data is maximal. By using a PCA, each sample can be represented by relatively few principal components instead of possibly thousands of variables. PC scores of samples can then be used in clustering or grouping the species into meaningful groups. The morphometric data of the hard parts (haptor) of 500 specimens (fifty specimens per species), belonging to nine species of the genus *Cornudiscoides* and one species of the genus *Bifurcohaptor* were prepared and statistically analyzed.

K-Means cluster analysis was used on PC-scores to form K groups of observations ($1 \leq K \leq n$), and the plot of within cluster sum of squares vs. K was used to determine the optimal number of clusters K [17]; optimal K is the smallest value of number of clusters after which the within cluster sum of squares does not improve (i.e., does not decrease) much.

Result

Descriptive statistics and visual summary

For exploratory data analysis of the dataset, the sample mean, and standard deviation (sd) were computed, and Box-plots were used to provide a five-point visual summary of all variables by species [18-23]. This gives an insight into the characteristics of variables and helps in our inferential analysis. (Table 1) shows that the sample mean and sd of the 10 variables described in (Figure 1). It can be seen from (Table 2) that the Species B. indicus has the largest mean of all measurements except for Hook Large.

Table 1: Mean and sd of Inner.Length.D, OuterLength.D, and RecurvePoint.D by Species.

	InnerLength.D		OuterLength.D		RecurvePoint.D	
Species	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
B. indicus	276.64	10.51	299.31	7.63	50.75	6.02
C.agarwali	41.26	3.7	31.51	3.17	23.58	1.05
C.aori	37.24	0.93	30.99	1.9	24.76	0.99
C.bleekerai	47.05	2.8	40.3	3.67	21.94	1.42
C.geminus	39.24	0.87	28.76	1.58	20.79	1.25
C.n. sp.	40.84	1.1	32.39	0.74	23.44	0.78
C.longicirrus	45.9	1.03	37.62	2.11	25.34	1.63
C.mystusi	36.79	1.31	31.97	2.12	21.99	1.02
C.sclerovaginalis	57.3	5.19	48.66	5.8	26.74	1.2
C.tukarami	37.34	0.84	31.82	1.25	23.26	0.85

Table 2: Mean and sd of InnerLength.V, OuterLength.V, and RecurvePoint.V by Species.

	InnerLength.V		OuterLength.V		RecurvePoint.V	
Species	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
B.indicus	36.74	2.06	33.32	2.45	31.67	2.84
C.agarwali	18.1	1.56	19.09	1.61	20.37	1.63
C.aori	22.61	0.91	18.44	0.73	14.57	0.87
C.bleekerai	14.26	0.8	15.53	0.86	19.71	0.96
C.geminus	12.91	0.73	14.84	0.47	17.18	0.65
C.n.sp.	17.32	0.81	19.35	0.83	20.64	1.16
C.longicirrus	27.51	0.92	23.48	0.88	15.16	0.58
C.mystusi	22.75	0.92	24.05	1.02	7.49	0.79
C.sclerovaginalis	15.31	0.79	15.71	0.72	23.21	0.78
C.tukarami	17.23	0.93	15.19	0.98	17.5	1.14

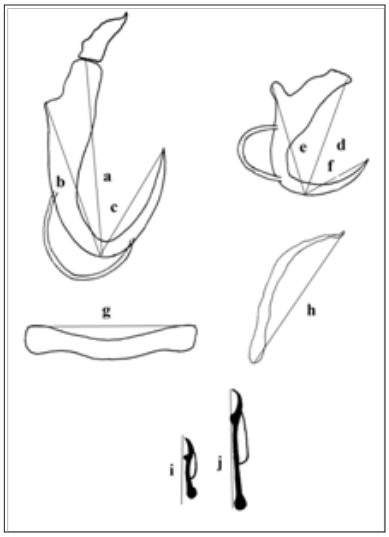

Figure 1: Schemes for nomenclature and measurement of the sclerotized structures used in study. a- dorsal anchor inner length, b- dorsal anchor outer length, c- dorsal anchor,d- ventral anchor inner length, e- ventral anchor outer length, f- ventral anchor recurved point, g- dorsal bar length, h -ventral Bar length, i- small hook length,j- large hook length.

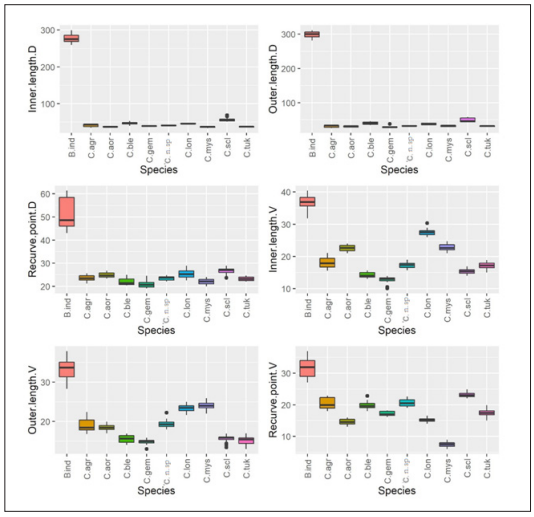

Figure 2(a) and 2(b): Shows that all of the observations of Inner.Length.D, OuterLength.D, RecurvePoint.D Inner.Length.V, OuterLength.V, RecurvePoint.V variables in the Species B. indicus fall well above the same for all other nine species; the values of VentralBar and Hook.Small for the Species B. indicus s are generally higher than the values for the other nine species, but some overlap can be seen. The values of the variable Hook.Large, however, are relatively small compared to the other nine species. It shown the boxplots of the 10 variables by Species.

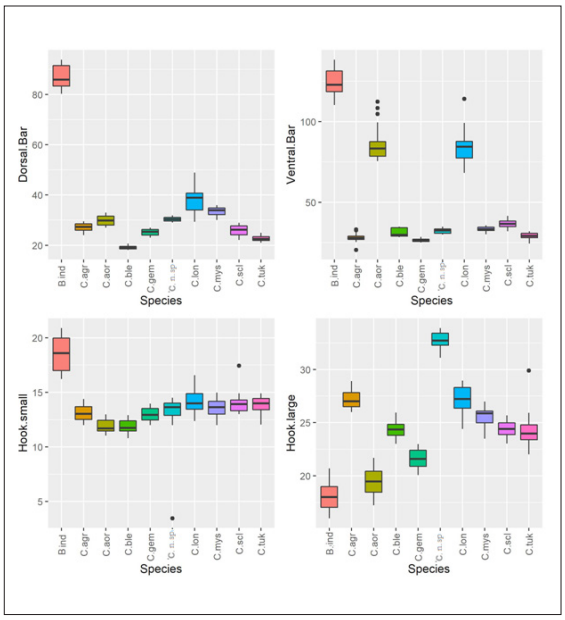

Figure 2(b): Box-plots by Species for Dorsal.Bar, Ventral.Bar, Hook.small, Hook.large, and sample means of all ten variables.

(Figure 2a & 2b) shows that all of the observations of Inner. Length.D, Outer Length.D, Recurve Point.D Inner. Length.V, Outer Length.V, Recurve Point.V variables in the Species B. indicus fall well above the same for all other nine species; the values of Ventral Bar and Hook. Small for the Species B. indicus s are generally higher than the values for the other nine species, but some overlap can be seen. The values of the variable Hook. Large, however, are relatively small compared to the other nine species.

Results of variable reduction from PCA

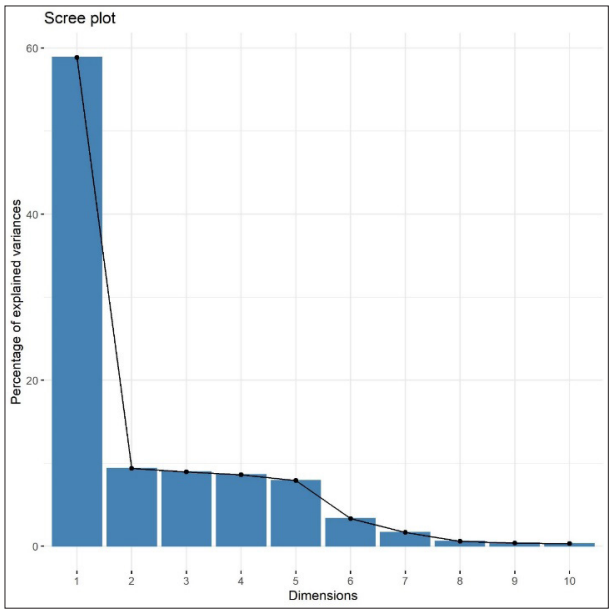

Figure 3: Scree plot showing % of variation explained vs. number of PC components.

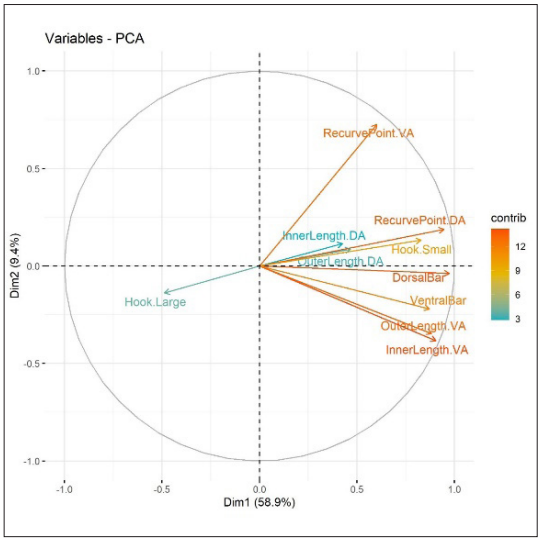

Figure 4: Plot of PCA loadings.

PCA was performed on the correlation matrix of original variables. The scree plot (Figure 3) suggests using the first two components (Table 3). PCA plots of the first two components allowed visualization of the data and to establish whether there were any intrinsic (part of haptor) differences in the anchors, bars and hooks. The PC loadings (Figure 4) were examined in order to determine which variables contributed most to the PC in which separation was observed and hence to indicate which variables were most dominating in separating classes. It can be seen from Figure 3 that the first PC is essentially a contrast between Dorsal Bar, Hook. Small, Ventral Bar, Recurve Point.D, Outer Length.V on the positive side and Hook. Large on the negative side. The second PC is mostly a contrast between RecurvePoint.V on the positive side and Inner Length.V, Outer Length.V Ventral Bar, and Hook. Large on the negative side. (Figure 3) also shows that the first component explains 58.9% of total variability, the second component explains 9.4% of total variability, and the first two components together explain 68.9% of total variability in the data.

Table 3: Mean and sd of DorsalBar, VentralBar, Hook.Small, Hook.Large by Species.

	DorsalBar		VentralBar		Hook.Small		Hook.Large	
Species	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**	**Mean**	**Sd**
B.indicus	86.92	4.33	124.45	8.22	18.5	1.52	18.11	1.23
C.agarwali	27.1	1.49	28.18	2.18	13.04	0.69	27.19	0.82
C.aori	30.03	1.84	85.03	8.73	11.89	0.59	19.4	1.18
C.bleekerai	19.09	0.64	31.09	2.52	11.91	0.55	24.34	0.74
C.geminus	25.17	1.24	26.42	0.93	12.96	0.63	21.62	0.88
C.n.sp.	30.31	0.89	32.35	1.46	13.29	1.58	32.74	0.72
C.longicirrus	37.7	4.2	83.38	9.41	14.14	1.08	27.18	1.25
C.mystusi	33.34	1.6	33.23	1.54	13.61	0.83	25.57	1.03
C.sclerovaginalis	25.94	1.94	36.49	2.05	13.91	0.79	24.39	0.73
C.tukarami	22.67	1	29.3	1.59	13.84	0.67	24.13	1.29

Plots of PCA scores

Plots of second and third PC-scores vs. the first PC-score are shown in (Figure 5); it can be seen from (Figure 5) that the PC-scores are able to discriminate between the ten species.

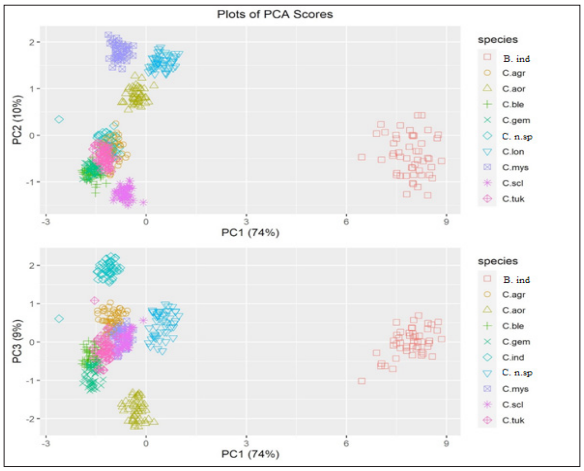

Figure 5: Plots of PC2 and PC3 scores vs. PC1 scores

Results of K-means cluster analysis

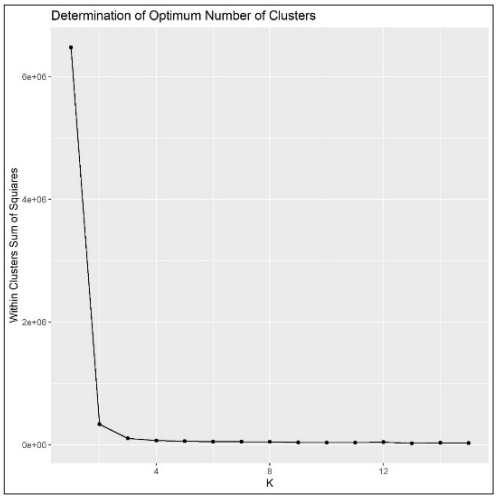

Figure 6: Plot of within clusters sum of squares vs. K (optimum K=3).

The plot of within clusters sum of squares vs. K (number of clusters) shows that K=3 is the optimum number of clusters, since any further increase in K does not improve the within clusters sum of squares (Figure 6). (Figure 7) shows a plot of PC2 vs. PC1 by Cluster Number for the cluster analysis results using 3 clusters. It can be seen from (Figure 7) that Cluster 3 consists of samples with higher PC1 values (large Dorsal Bar, Hook. Small, Ventral Bar, Recurve Point.D, Outer Length.V, and small Hook. Large). Clusters 1 and 2 are characterized by relatively lower values of PC1; the second component PC2 separates Clusters 1 and 2 quite well, with large PC2 values (large Recurve Point.V and low Inner Length.V, Outer Length.V, Ventral Bar, Hook. Large) falling in Cluster. All computations and visualizations in this article are done in the statistical software environment R (2020).

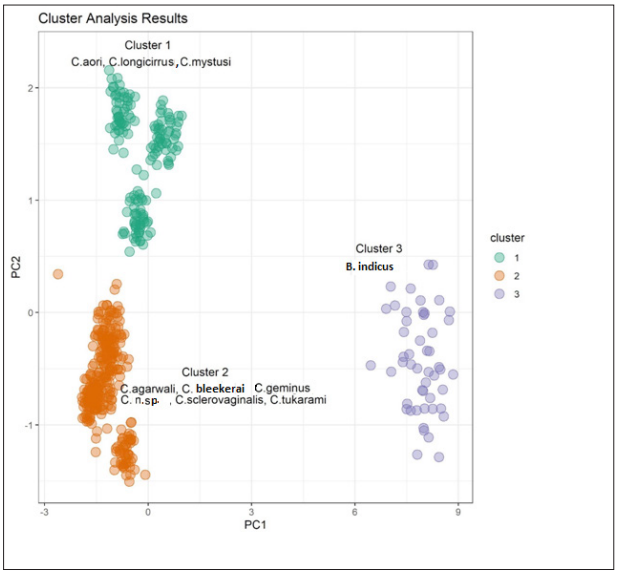

Figure 7: Plot of PC2 vs. PC1 by clusters number.

Discussion

Different species of *Cornudiscoides* are very similar to each other in terms of morphology of dorsal anchor, ventral anchor, bar, hooks. It is difficult to distinguish between closely related species. Because of strong morphological similarities, morphometric identification of closely related species requires use of characters with high diagnostic power, that is, characters that have minimum overlap among species. The statistical tools applied here provide alternate solution of this problem. PCA is able to separate 450 individuals of *Cornudiscoides* and 50 of the genus *Bifurcohaptor* into 3 distinct groups. Scatter plots between PC1 and PC2 revealed that all *Cornudiscoides* species clustered in linear fashion and showed distinct relationship with the genus *Bifurcohaptor* indicus. Apart from this, it is worth noticing that *Cornudiscoides* species clustered on the basis of their host i.e., we can observed three clusters, one, in which all species that reported from *Mystus (C. geminus, C. agarwali, C. tukarami, C. sclerovaginalis, C. n. sp., C. bleekerai)* is clustering together. In second cluster, species reported from *Sperata (C. Mystusi, C. longicirrus, C. aori)* clustered together while B. indicus reported from *M. vittatus* clustered separately, being a different genus.

These analyses indicate the suitability and effectiveness of using the present set of morphometric variables for species distinction at host level. This results also supports the molecular study by Verma et al. [9] in which *Cornudiscoides* species reported from *Sperata* clustered together and forming sister clade with species reported form *Mystus*. In the cluster of *Cornudiscoides* species reported from *Mystus* there is overlapping, except one i.e. C. *sclerovaginalis* the reason behind in *C. sclerovaginalis* shaft of dorsal anchor is comparatively large while other species have similarity in their haptor part with minute differences but. In case of species reported from *Sperata*, there are differences in size of shaft of dorsal anchor and ventral bar. In the present study, PCA has been successful in detecting the presence of groupings or intra-specific morphometric variants among *Cornudiscoides* species and the groups also showing their relationship with their host. Our statistical analyses indicate that main predictors for species discrimination are Inner. length.V, Hook. large, Ventral Bar, and Outer. length.V. Moreover, PCA of 500 individuals showed three distinct clusters, corresponding to ten species. Of these three clusters, one cluster, corresponding to the species of a genus *Bifurcohaptor* sp. is quite separate from the other two. It is worthwhile to mention here that *Cornudiscoides* species, clustered separately even on the basis of their hosts as well (from *Mystus* and *Sperata*). In conclusion, we suggest that since the taxonomy relied traditionally on morphological characters only, at least three data sets should be included: morphology, molecular biology and supporting evidence from one additional discipline. Help of molecular tools was earlier taken. In the present study statistical tool PCA was applied to distinguish monogenoids at generic and specific level. It also proved helpful to differentiate species infecting different of fish host.

Acknowledgement

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