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Statistical Analysis of Indian *Thaparocleidus* (Monogenea: Dactylogyridae) Species based on Morphometric Characteristics

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Abstract: ***The present communication proposes statistical analysis of the morphometric data of haptoral parts of seventeen species belonging to genus Thaparocleidus Jain, 1952 described from different piscine hosts of India using principal component analysis (PCA), linear discriminant analysis (LDA) and multivariate analysis of variance (MANOVA). These statistical methods were applied to morphometric measurements made on anchors, transverse bars and marginal hooks for discrimination among the seventeen species. Initially, PCA showed the multivariate distinction between the seventeen species and able to differentiate the seventeen specimens into four groups. Thereafter, LDA confirmed the results of PCA and gave better classification. Both PCA and LDA showed that there are two morphometric parameters: dorsal outer length (DOL) and ventral inner length (VIL) which fairly contributed to the discrimination between all seventeen species. This analysis of seventeen species indicated the presence of intra-specific morphometric variations and was subjected to more accurately distinguish their placement in respective genus. Findings are discussed in detail.***

Keywords: Principal component analysis, Multivariate analysis, Monogenea, *Thaparocleidus*, India.

1. Introduction

Parasitism is one of the most common and successful modes of life, displayed by living organisms [1]. Parasites evolved independently in the animal kingdom and has diversified, such as in Platyhelminthes [2]. Many parasite species still await the discovery of their true number, correct identification and their relationships. Parasitic organisms are problematic because they are variants in form and structures [2]. Parasites are highly conserved in their morphology and they show very little variations within a group. Sometimes biological and environmental variables like host age, host species, temperature of water may induce changes in morphological structures of parasites [2]. The problem faced in identification of parasites with morphological characters can be cope with the use of statistical analysis. Most of the parasites of genus *Thaparocleidus* harboring ray-finned fish, belongs to order Siluriformes and its of considerable commercial importance, many of the larger species are farmed or fished for food. This order includes 13 genera and about 100 species of freshwater fishes and has a wide geographical distribution, being found in Africa, Syria, and southern and western Asia [3].

Most of the monogeneans are gill parasites of aquatic organisms and are known to be highly host specific [4-6]. The identification of monogeneans is generally based on the morphological criteria (i.e. qualitative and discrete characters) and morphometric analysis allows a quantitative approach in the analysis of several body parts of these parasites. Most of the species of *Thaparocleidus,* in terms of high infestation, is harbouring the fishes of Gangetic riverine system in India, where they caused significant damage in both, ecological and economical terms. The species of *Thaparocleidus*, is morphologically very similar and morphometric discrimination of species is possible only with the use of sophisticated statistical tools. Improper identification often causes problems with their classification which leads to incorrect taxonomy. Improper identification often causes problems with their classification which leads to incorrect taxonomy. Status of the species of the genus *Thaparocleidus* in India is inadequate and hence many Indian species of this genus were originally misplaced [7]. Sometimes, morphologically similar congeneric species that exhibit substantial morphometric variations within their populations may cause difficulties in taxonomy, raising questions as to whether the observed variations are intraspecific or interspecific [8]. The morphometric variations within monogenean populations have been showed by a number of researchers [8, 9-16]. Statistical methods, like, principal component analysis (PCA), linear discriminant analysis (LDA), one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) have been used by various authors to reveal interspecific and intraspecific morphometric variations in monogeneans [8,11, 17-23].

Genus *Thaparocleidus* was proposed by JAIN, 1952 [24] for the species *T. wallagonius* from the gills of *W. attu* (Bloch & Schneider) at Lucknow, India. Subsequently, GUSEV, 1976 [25] proposed the genus *Silurodiscoides*, to which many species of the genus *Thaparocleidus* were transferred. However, LIM, 1996 [26] pointed out that *Thaparocleidus* is a senior synonym of *Silurodiscoides* and listed 80 species of *Thaparocleidus* JAIN, 1952 [24]. LIM et al. (2001) [7] listed dactylogyrid monogeneans of siluriform fishes of the Old World and tentatively considered 77 species of *Thaparocleidus* valid and questioned the validity of certain Indian species, emphasizing the need to ascertain the status of some species from Indian fish. The analysis of seventeen described species of *Thaparocleidus* from India demonstrated significant morphometric differences. These

observed differences are interspecific or intraspecific variations in order to establish that whether statistical tool is useful for characterization of seventeen different species of this genus. The validity of certain species of this genus is still disputed and the use of statistical tools in order to validate the species of genus *Thaparocleidus* for accurate analysis. PCA and LDA are used to group the different species of genus *Thaparocleidus*, based on their morphometric data. MANOVA is performed to access the significance difference among the observed species groups. In the morphometric parameters currently used for *Thaparocleidus* taxonomy, we explored to assess new morphometric characters would increase the discriminatory power. This study aims to improve the methodology based on morphometric characters as a tool for discriminating among species of *Thaparocleidus.*

2. Materials and Methods

The morphometric data for this study were compiled from the published records and from the work of PANDEY & AGRAWAL (2008) [27]. The underwritten variables choose for morphometric analysis follows the parameters which are easily measurable, not be geometrically redundant, repeatable and representative. The list of the analyzed species of *Thaparocleidus* reported from India is given (Table 1). The parameters that were taken for differentiation among the species: dorsal inner length (DIL), dorsal outer length (DOL), dorsal inner root (DIR), dorsal outer root (DOR), dorsal transverse bar (DTB), ventral inner length (VIL), ventral outer length (VOL), ventral anchor recurved point (VPT), ventral transverse bar (VTB) and marginal hooklets (MH). The data were analyzed by PCA, LDA and MANOVA using R-software (version 2.7.1).

The purpose of PCA is to find the best low-dimensional representation of the variation in a multivariate data set. Therefore, first of all we performed principal component analysis to investigate whether we can capture most of the variation between ten measurement parameters using a smaller number of new variables (called principal components), where each of these new variables is a linear combination of all or some of the these parameters (measurements of anchors, bars and hooks). Secondly, LDA was used to find the linear combinations of these parameters that give the best possible separation between the different species groups in our data set. LDA can be treated as the reversed of MANOVA. In MANOVA, the independent variables are the groups and the dependent variables are the predictors whereas in LDA, the independent variables are the predictors and the dependent variables are the groups. LDA is used to predict group membership on the basis of the attributes of the cases indicating which ones contribute most to the discrimination between groups. MANOVA is a statistical test procedure for comparing multivariate (population) means of several groups. It uses the variance-covariance between parameters in testing the statistical significance of the mean differences. Once group means are found to be statistically significant, one can proceeds to see which of the parameters have significantly different means across the groups.

Table 1: List of different species of the genus *Thaparocleidus* Jain, 1952 [24], abbreviation used for this study, host, their family, locality in India and authors reported them.

Species	*Abbr. used*	*Host*	*Author*
T. wallagonius	TWAL	*Wallago attu*, *Sperata seenghala*	Jain, 1952 [24]
T. gomtius	TGOM	*Wallago attu*	(Jain, 1952) Lim, 1996 [24, 26]
T. seenghali	TSEE	*Sperata seenghala*, *Rita rita*,	(Jain, 1961) Lim, 1996 [24, 26]
T. indicus	TIND	*Clupisoma garua* *Wallago attu*, *Eutropiichthys vacha*	(Kulkarni, 1969) Lim, 1996 [33, 24]
T. malabaricus	TMAL	*Ompok malabaricus*, *Ompok bimaculatus*	(Gusev, 1976) Lim, 1996 [25, 24]
T. pusillus	TPUS	*Mystus vittatus*, *Mystus bleekeri*	(Gusev, 1976) Lim, 1996 [25, 24]
T. multispiralis	TMUL	*Silonia silondia*, *Pseudotropius garua*	(Jain, 1957) Lim, 1996 [34, 24]
T. pangasi	TPAN	*Pangasius pangasius, Eutropiichthys vacha*	(Tripathi, 1959) Lim, 1996 [35, 24]
T. vachius	TVAC	*Eutropiichthys vacha*	(Jain, 1961) Lim, 1996 [36, 24]
T. octotylus	TOCT	*Ompok pabda*	(Kulkarni, 1969) Lim, 1996 [33, 24]
T. aori	TAOR	*Mystus aor*	(Rizvi, 1971) Lim. 1996 [37, 24]
T. parvulus	TPAR	*Mystus vittatus*	(Gusev, 1976) Lim, 1996 [25, 24]
T. devraji	TDEV	*Callichrous malabarichus*	(Gusev, 1976) Lim, 1996 [25, 24]
T. vaginalis	TVAG	*Clupisoma garua*	(Gusev, 1976) Lim, 1996 [25, 24]
T. speratai	TSPE	*Mystus aor*	Agrawal et al. 2005 [38]
T. longiphallus	TLON	*Wallago attu*	Chaudhary and Singh 2012 [29]
T. siloniansis	TSIL	*Silonia silondia*	Chaudhary and Singh 2012 [39]

3. Results

The correlation matrix shows that the ten measurement parameters (DIL-MH) seem to hang together in two distinct clusters (Table 2). The parameters (DIL, DOL, DIR, DOR and MH) show fairly strong correlations with one another. Hence all these parameters are measured the same construct. Similarly the parameters (VIL, VOL, VPT, VTB and DTB) correlate strongly with one another. Given this apparent redundancy, it seems that the ten parameters of the data set not actually measuring ten different constructs.

Subsequently, these two constructs as predictor parameters were used for further statistical analysis.

Table 2: Correlation matrix of different morphometric parameters

Variables	*DIL*	*DOL*	*DIR*	*DOR*	*DTB*	*VIL*	*VOL*	*VPT*	*VTB*	*MH*
DIL	**1**	-	-	-	-	-	-	-	-	-
DOL	0.943	**1**	-	-	-	-	-	-	-	-
DIR	0.501	0.348	**1**	-	-	-	-	-	-	-
DOR	0.636	0.787	0.182	**1**	-	-	-	-	-	-
DTB	0.366	0.351	0.492	0.184	**1**	-	-	-	-	-
VIL	0.349	0.303	0.350	0.043	0.816	**1**	-	-	-	-
VOL	0.223	0.160	0.325	-0.040	0.703	0.890	**1**	-	-	-
VPT	0.500	0.424	0.381	0.191	0.809	0.816	0.702	**1**	-	-
VTB	0.416	0.554	0.065	0.393	0.728	0.685	0.572	0.649	**1**	-
MH	0.339	0.379	0.095	0.302	0.315	0.294	0.172	0.450	0.343	**1**

In PCA, the Table 3 shows how much of the total variance of the observed parameters is explained by each of the PCs. The first PC is one which explains the largest part of the total variance and accounts 50.71% of the total variance. The second and third PCs contain respectively 20.95% and 10.5% of the total variance. The scree plot in Fig. 1, demonstrates this distribution of variance among the components graphically. The plot reveals that there appears to be marked decrease in downward slope after the second or third PC implying that we can summarize our ten parameters by the first two or three PCs. For simplicity, we assumed that the two-component (holding 71.66 % cumulative variance) solution is adequate.

Table 3: Eigenvalues and variability shown by Principal components.

	F1	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*	*F10*
Eigenvalue	5.071	2.095	1.053	0.751	0.386	0.276	0.208	0.085	0.066	0.009
Variability (%)	50.708	20.949	10.533	7.514	3.863	2.756	2.077	0.853	0.658	0.089
Cumulative (%)	50.708	71.657	82.191	89.704	93.567	96.323	98.400	99.253	99.911	100.00

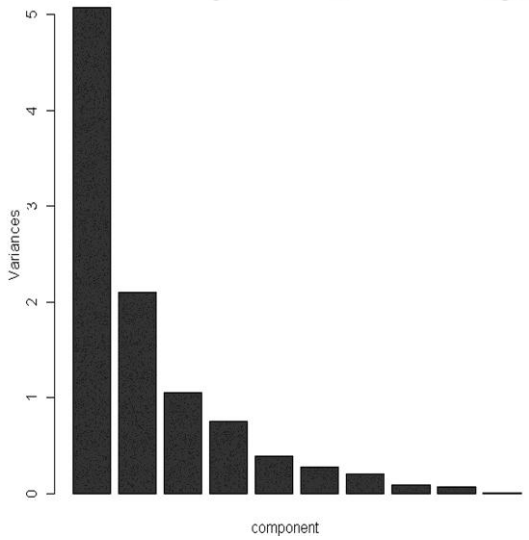

Figure 1: The scree plot demonstrates this distribution of variance among the components graphically.

From Table 4, we observed that the first PC is highly correlated with each of the parameters and is simply the weighted average of all the measurements and provides a measure of overall characteristics of the species. The second PC is positively correlated with parameters DIL, DOL, DOR, and MH and negatively correlated with DTB, VIL, VOL and VPT. The two parameters DIR and VTB respectively have very low positive and negative correlations with second component. Thus, the second PC clearly differentiates the ten parameters into two distinct sets. From the scatter plot of the species in Fig. 2 with PC1 on x-axis and PC2 on y-axis, it is clearly evident that the first PC (which is an indicator of the overall size of the all measurements) makes two separate groups of the seventeen species as shown by vertical line in Fig. 2. Similarly, the second PC separates the *Thaparocleidus* species into two groups as shown by horizontal line in Fig. 2. Thus, the first two principal components are collectively differentiated seventeen species into four distinct groups. We thus define these four species groups as:

Group 1: TVAC, TSEE, TIND, TGOM, TWAL
Group 2: TAOR, TPAN, TSIL, TLON, TDEV
Group 3: TMUL, TPAR, TVAG
Group 4: TOCT, TPUS, TSPE, TMAL

Table 4: Correlation between variables and first three PCs

	DIL	**DOL**	**DIR**	**DOR**	**DTB**	**VIL**	**VOL**	**VPT**	**VTB**	**MH**
PC1	0.717	0.708	0.508	0.466	0.853	0.835	0.717	0.873	0.797	0.492
PC2	0.577	0.667	0.058	0.751	-0.333	-0.471	-0.562	-0.251	-0.071	0.185
PC3	0.216	0.0020	0.794	-0.122	0.059	-0.016	0.048	-0.041	-0.406	-0.434

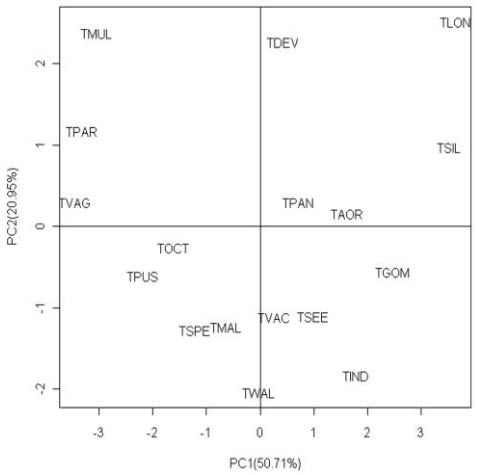

Figure 2: PCA plot of seventeen species belonging to genus *Thaparocleidus* Jain, 1952.

The Biplot of PCA in Fig. 3 exhibits the association between the measurements parameters and the species. The vector length of parameters represents its discriminating ability. The plot reveals that the dorsal outer length (DOL) and ventral inner length (VIL) are the main distinguishing characters of the seventeen species. Now, we performed discriminate analysis to validate the results of PCA. In practice, it was observed that PCA does more on feature classification whereas LDA works on data classification. In PCA, the shape and location of the original data sets changes when transformed to a different space whereas LDA does not change the location but only tries to provide more class separability. For our data set, we required three discriminate functions (the minimum of the degrees of freedom and the number of parameters) that can separate the species using ten measurement parameters. The percentages of variations containing by the first three discriminant functions are 81.75%, 16.85% and 1.40% respectively. The plots of the first three discriminant functions are shown in Fig. 4. The first LD function clearly differentiates Group 1 from Groups 2, 3 and 4; the second LD function separates Group 2 from Groups 3 and 4; and the third LD function reasonably separates Group 3 and Group 4. Table 5 summarizes the actual and predicted group memberships of the species groups. The mosaic plot of the actual and predicted group memberships corresponds to Table 5 is shown in Fig. 5. Through leave-one-out cross-validation method, the respective correct classifications of species of Groups 1, 2, 3 and 4 are observed to be 80%, 80%, 66.7% and 50%. An overall 70.6% of cross-validated grouped species are correctly classified. The misclassified species are TVAC from Group1, TLON from Group2, TMUL from Group 3, TSPE and TMAL from Group 4. The discrimination model predicts that the following species should be classified as: TVAC from group 1 to group 4, TLON from group 2 to group 1, TMUL from group 3 to group 4, TSPE from group 4 to group 1 and TMAL from group 4 to group 3.

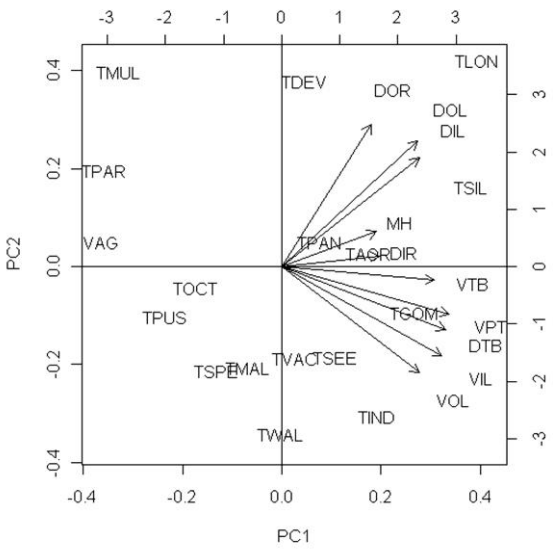

Figure 3: Biplot of the first two principal components for the seventeen species belonging to genus *Thaparocleidus* Jain, 1952.

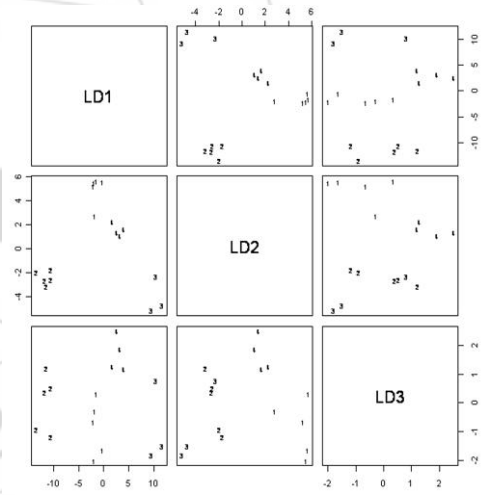

Figure 4: Linear discriminant analysis plots of the first three LD functions, which account for 65%, 21% and 14% of the total variation, respectively.

Table 5: The cross classification table.

		Predicted Group					
		Group 1	Group 2	Group 3	Group 4	Total	% of correct classification
Actual Group	Group 1	4	0	0	1	5	80
	Group 2	1	4	0	0	5	80
	Group 3	0	0	2	1	3	66.7
	Group 4	1	0	1	2	4	50
	Total	6	4	3	4	17	70.6

Further, we performed MANOVA to check the significance of the hypothesis that whether we really have different species groups. For this purpose, we used Wilks lambda test of testing the mean differences in LDA and to select the most significant parameters that contribute toward the classification. Its value ranges from 0 to 1, where 0 stands for group means differ significantly (thus the more the variable differentiates the groups), and 1 implies that all group means are same. In our case, the value of Wilks lambda for group means is .0003 which is much closer to zero (with $p<.001$); hence we can conclude that the four species group means differ significantly at 1% level of significance. From the Table 6, it is observed that the P-values of F-tests for the three measurement parameters DIR, VTB and MH are greater than .05 and hence these parameters do not play significant role in discrimination of the species.

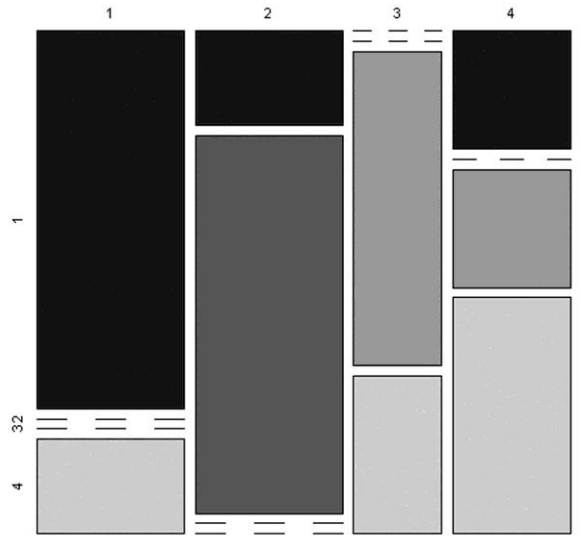

Figure 5: Mosaic plot showing predicted group membership for different species groups

Table 6: Tests for Equality of Group Means.

	df1	df2	Wilks' Lambda	F	P-value
Species group	3	13	.0003	6.0192	.00089
Measurement Variables					
DIL	3	13	.369	7.399	.0038
DOL	3	13	.375	7.225	.0042
DIR	3	13	.607	2.80	.0814
DOR	3	13	.310	9.628	.00129
DTB	3	13	.403	6.418	.0067
VIL	3	13	.174	20.638	.00003
VOL	3	13	.231	14.412	.00019
VPT	3	13	.295	10.368	.00093
VTB	3	13	.580	3.141	.0618
MH	3	13	.605	2.823	.0801

4. Discussion

To describe or identify the taxa, taxonomists are always at lookout for the new tools and for monogenea it is very needful because environmental factors have been shown to influence the morphological variations [28-31]. Statistical tools for discrimination between species were found to be very beneficial [32]. The use of a statistical discrimination system once optimized and validated, it provides a rapid and reliable alternative to traditional methods [17]. Discrimination of the parasites of genus *Thaparocleidus* JAIN, 1952 [24] was necessitating for their critical reexamination and validation based on statistical technique. The discrimination of *Thaparocleidus* species has been achieved to a certain level using multivariate analyses such as PCA applied to morphometric data. This study allow for the discrimination of a species, however, the confidence that one might place upon the discrimination of an individual is dependent upon its relative position in the PCA matrix and thus its position in relation to all other specimens within the analysis. *Thaparocleidus* species discrimination were difficult using conventional taxonomic procedures and discrimination of closely related and morphologically similar species was possible using a variety of statistical classification approaches applied to morphometric data. The classification by the use of statistical methods validated the species and found reliable and cheap alternative to traditional methods and permits the detection of parasites.

Haptoral parts of *Thaparocleidus* provide a valuable tool to discriminate species. The method proposed in the present study based on different seventeen species of Indian *Thaparocleidus* has higher discrimination and validation potential. The classification of these species is based upon morphometric data i.e. 10 morphometric characters (from anchors, ventral bar and marginal hook). During this analysis it was found that in all ten parameters the two parameters are found to be most dominant in overall analysis i.e. the dorsal outer length (DOL) and ventral inner length (VIL) and it would perhaps be possible to obtain better classification performance by using these two parameters. Both PCA and LD analysis are able to separate the seventeen species of *Thaparocleidus* into four groups based on morphometric differences of their anchors, bars and marginal hooklets and indicates the suitability and effectiveness for discriminating species of *Thaparocleidus*. LD analysis using the current dataset gives excellent classification results thus providing a powerful species classification tool for the future. In the present study, PCA has been successful in detecting the presence of groupings within *Thaparocleidus*. The morphometric variants in Group III and IV are less obvious compared to those in Group I and II which could be due to the lack of their proper morphometry or they may be the same species. The species of Group III and IV are difficult to define due to small differences in their morphometric data. The results from this analysis illustrated that higher correct classification score obtained was 70.6%. The classification percentages were equally good in Group I and II (Group I 80%, Group II 80%) but not so for the other groups. The classification results showed that five species out of seventeen were misclassified and hence there is a need to re-examined and further analysis of these five species that are TVAC, TLON, TMUL, TSPE and TMAC. By MANOVA test, we observed that there is a significant difference among group means on the basis of the ten parameters, however, three parameters DIR, VTB and MH are found to be irrelevant in discrimination of the species. It is important to note that this analysis will separate all species equally well and believe that methods as proposed here, represents a

crucial protocol when comparing species. Although the method used in the present study is based on ten parameters of seventeen *Thaparocleidus* species but the two parameters dorsal outer length (DOL) and ventral inner length (VIL) can be used as another set of parameters with a higher classification potential for *Thaparocleidus*. The proposed method in the present study should and most likely will give a high classification potential for all *Thaparocleidus* species. More samples from different localities with same and different environmental conditions are needed for the further establishment of the species of Group III and IV. The hard parts observed in this study were serving as a more effective method of validation of species as they are important part in discrimination between different species. Therefore, such methods should identify morphometric characteristics that best identify species belonging to the genus *Thaparocleidus*. Use of these statistical methodologies will provide further development of novel taxonomic, discriminatory tools for the correct identification of monogenean parasites. This work is promising, but more studies are required in order to be more confident about the accurate interpretation uses as potential tool for the correct framing of parasite taxonomy. The present work has shown that statistical classification based on morphometric characters can differentiate the species of *Thaparocleidus*. We bring to close that despite large overlaps in the measurements of individual morphometric characters, the statistical classification is a powerful tool for validation of species in *Thaparocleidus* taxonomy.

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