REASSESSMENT OF THE SAWFISH ROSTRA TAXONOMY FROM THE NATURAL HISTORY MUSEUM IN SIBIU

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***Abstract**: A total of 7 sawfish (Pristidae) rostral specimens from the Natural History Museum, Sibiu and from the Hunting Museum, Sibiu that were previously identified as Pristis pectinatus Latham 1794, Pristis sp. or Pristis antiquorum Latham 1794 (nomen dubium), are reevaluated using available literature, identification keys and statistical analysis using PAST software. Following the reassessment, all the specimens were classified as either Pristis zijsron Bleeker 1851 or Anoxypristis cuspidata Latham 1794.*
***Keywords**: Pristidae, Anoxypristis, Pristis, rostra, statistical analysis, PAST, Brukenthal Museum.*

***Rezumat.** Un total de 7 rostruri de pește fierăstrău (Pristidae) de la Muzeul de Istorie Naturală din Sibiu și de la Muzeul de Vânătoare din Sibiu anterior identificate drept Pristis pectinatus Latham 1794, Pristis sp. sau Pristis antiquorum Latham 1794 (nomen dubium), sunt reevaluate folosind literatura de specialitate, cheile de identificare și analiza statistică bazată pe programul PAST. În urma reevaluării, specimenele au fost atribuite speciilor Pristis zijsron Bleeker 1851 și Anoxypristis cuspidata Latham 1794.*
***Cuvinte cheie:** Pristidae, Anoxypristis, Pristis, rostru, analiză statistică, PAST, Muzeul Brukenthal*

Introduction

The need for the reevaluation of the sawfish specimens from the Natural History Museum in Sibiu arose from another research subject that involved a comparison between fossil material of the family Pristidae and recent material of the same family. Soon after starting this project it became clear that the recent material is not correctly identified and taxonomical reevaluation was needed.

Six of the evaluated specimens belong to the historic collection of the Transylvanian Society for Natural History in Sibiu (Der Siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt) and were all donated prior to 1900´s. Now these specimens are part of the Natural History Museum in Sibiu.

The rostra usually have two or even three catalog numbers due to the renumbering performed several times in the last 117 years or more since they were donated to the Transylvanian Society.

The first catalog of fishes, written by Moritz von Kimakowicz between 1897 and probably 1909, records the Pristidae material.

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This catalog contains the records of 20 rostra including 4 small, presumably juvenile-sized, rostra. These rostra donations consisted of two of *Pristis antiquorum* (*nomen dubium*) from the Red Sea of Ethiopia ("M. rub. Abessynien") donated by C. F. Czeckelius in 1895 and 1899 (catalog numbers 171 and 172), three rostra of *Pristis antiquorum* from the Aden Gulf of the Red Sea ("M. rub. ins Aden erworben") by M. Schuleri in 1889 (catalog numbers 173-175), and four juvenile-sized rostra from Singapore South China Sea (catalog numbers 176-179). Another eleven rostra were recorded in 1898 under the name *Pristis* sp., donated again by M. Schuleri, with catalog numbers 180-190. Unfortunately, much of the material is now lost and only a few rostra could be located in collections. The latest catalog lists 7 rostra, all under *Pristis pectinata*, with no mention of the old catalog numbers. Fortunately, some of the specimens preserve, inscribed with black ink, the old numbers (see Table 1).

Another rostrum is preserved in the Museum of Hunting in Sibiu but it might not have come from any of the donations discussed above since no label or associated data could be located for it.

No information regarding these specimens was published until 2003 when a list of taxa in the fish collection was compiled (Bănăduc, 2003).

Unfortunately, only one specimen is listed, under the name *Pristis pristis* Linnaeus 1758, but no catalog number is associated with it.

Material and Methods

A combined morphological, morphometrical and statistical approach was taken. The seven rostra were measured and photographed. The length was recorded with a measuring tape while the width of the rostra, the inter-dental distance and the dimensions of the rostral spines were measured with digital calipers. The images were taken with a Nikon D700 camera and 14–24 mm lens.

We use the term 'rostral spines' vs. 'rostral teeth' as established by paleontologists that acknowledge that these odontological formations have a different evolutive origin than the oral teeth, most likely originating from modified dermal denticles (Welten *et al*., 2015).

The measurements were done following Whitty *et al*., (2013) and Seitz & Hoover (2017) and consist of: total specimen length (TSL), the distance measured between the tip of the rostrum to the place where the rostrum was cut; total rostrum length (TRL), the distance measured from the tip of the rostrum to the place where the rostrum begins to flare; standard rostrum length (SRL), the distance from the tip of the rostrum to the midpoint of the line that unites the posterior edge of the most proximal rostral spine from the left and the right sides; proximal rostrum width (PRW), the width of the rostrum between the left most proximal rostral spine base to the opposite edge of rostrum; distal rostrum width (DRW), the width of the rostrum between the left most distal rostral spine base to the opposite edge of rostrum; distal inter-spine distance (DSD), the distance between the bases of the two most distal right rostral spines (DSD has the RD as equivalent in Whitty *et al*., 2013 and DTG in Seitz & Hoover, 2017); and proximal inter-spine distance (PSD), the distance between the bases of the two most proximal rostral spines) (see Fig. 1).

The abbreviations used for the name of collections are NHMS, for the Natural History Museum in Sibiu; and HMS, for the Hunting Museum in Sibiu. Since HMS belongs from the administrative point of view to the NHMS in the title we refer only to NHMS.

For the statistical analysis (Hammer *et al*., 2011) we used the PAST software v.3.11. The approach used in analysis is different from the method of Seitz & Hoover (2017), who included additional data correction, but is based on their method. We used a univariate statistical test with basic descriptive statistics (as gave by the PAST package) for one or more samples of univariate data. The descriptive statistics are: N (number of individuals [48]), minimum and maximum values, the mean, standard error, variance, standard deviation, median, the 25th percentile, the 75th percentile and the geometric mean. The univariate statistics give us the skewness distribution (zero for a normal distribution, positive for a tail to the right) and kurtosis: (zero for a normal distribution). Coefficient of variation (CV), expressed as a percentage, allows for an evaluation for the normality test together with the skewness distribution and kurtosis. In the table of univariate statistic test it can be seen that PRW, DSG or DRW/ TRL*100 have an abnormal distribution, have a high CV value and the average is not one of the representative indicators. For this reason, it was necessary to test for the normal distribution for all characters using the PAST software package.

We analyzed eight rostra preserved as dried specimens, with current catalog numbers: P569, P21, P22, P570, P568, P567, P566, VT738 (see Figure 2 and Table 1).

Discussions

Recent Pristidae include two genera, *Anoxypristis* and *Pristis*. There is currently a single species within the genus *Anoxypristis*, *A. cuspidata*. The genus *Pristis* currently includes four species: *Pristis pristis* that until recently had been represented by three species (*P. pristis*, *P. perotteti* Müller & Henle 1841 [*nomen dubium*], and *P. microdon* Latham 1794 [*nomen dubium*]); and three closely related species having clear genetic and morphological differences; *P. pectinata*, *P. zijsron* and *P. clavata* Garman 1906, (Faria *et al*., 2012).

In order to evaluate the taxonomy of the species form NHMS and HMS we firstly took into account the differences among genera, and then various indicators that distinguish between species: number of rostral spines per side of rostrum, the presence or absence of grooves on the rostral spines, cutting edges of the spines, inter-spine distance ratios, distribution of spines along the rostrum etc. It is important to note that there is some variation in the number of rostral spines per side within a given species based on biogeographic region from where the specimens originate (see Table 3).

The rostra were separated in two morphological categories according to the shape of the rostral spines, disposition of the spines on the rostrum and general shape.

Morphological type A: referred specimens P569 and P570 (see Figure 2 a, b).

The rostral spines are short with a rounded end in dorsal view and both anterior and posterior with sharp margins. Both the length and the thinness of the spines increase from the distal part of the rostrum towards the middle and decrease again towards the proximal part. The density of the spines in the most proximal quarter of the rostrum is obviously lower than in the remaining length. Morphology and morphometry indicates that these rostra belong to *Anoxypristis cuspidata*.

Morphological type B: referred specimens P21, P568, P567, P566 and VT738 (see Figures 2 c, d, e, f, g,)

The spines are slender and pointed, most of them presenting a posterior groove bordered by two edges. The most proximal four or five rostral spines present either a much shallower groove, or a flat posterior surface. The thickness of the rostral spines decreases constantly, starting from the most distal part of the rostrum towards the proximal part. Morphology and morphometry indicates that these rostra belong to *Pristis zijsron*.

We conducted statistical analysis in addition to comparing morphology and morphometry. This analysis intended to confirm the allocation to the species level with the help of a larger data sample analyzed by Seitz & Hoover (2017).

Species analyzed: *Anoxypristis cuspidata*; *Pristis pectinata*; *P. pristis* and *P. zijsron*.

Characters analyzed: TSL, TRL, SRL, PRW, DRW, PRW/DRW, Rostral spines (left), Rostral spines (right), PSD, DSD, SRL/TRL*100, PRW/TRL*100, DRW/TRL*100, PSD/TRL*100, DSD/TRL*100.

Three statistical tests were conducted for normal distribution of one or several samples of univariate data, given in one or more separate columns or with a single data column and a group column. The data in Table 5 were generated by the normal and uniform random number generators in PAST ("Evaluate expression" module). For all three tests, the null hypothesis is H0: The sample was taken from a population with normal distribution. If the given p (normal) is less than 0.05, normal distribution can be rejected. Shapiro and Wilk's W-statistic is a well-known goodness of fit test for the normal distribution.

The Shapiro-Wilk test (PAST Package) returns a test statistic W, which is small for non-normal samples, and a p value. For example, our sample shows that PRW, rostral spines left, DSD, SRL/TRL*100, PRW/TRL*100, DRW/TRL*100, and DSD/TRL*100 each have a p value < 0.05 (accepted threshold). For this reason, these morphological variables were log-transformed using a base-10 logarithm. The resultant values were then compared to a log-normal distribution.

The next step in our interpretation of data was to correlate the morphological characters. The PAST package investigates the dependence between multiple variables at the same time with the correlate test. The correlation was computed by Pearson’s r, which is the most commonly used parametric correlation coefficient. It can be seen that few variables (TSL, TRL, rostral spines left) have high values (>0.95) for the accepted threshold of $p = 0.05$.

The following analyses were conducted without the variables TSL, TRL and no. of rostral spines left because there is a strong relationship between two variables. This means that changes in one variable are strongly correlated with changes in the second variable. In our example, Pearson’s r is 0.994 between overall length and TRL, this number is very close to 1. For this reason, we can conclude that there is a strong relationship between these variables. The same situation is between overall length and SRL and between TRL and SRL. Finally, we concluded that best results utilize TRL and rostral spines (right side) from the following analysis.

The data were further computed with principal component analysis (PCA), which finds variables (components) that account for as much as possible of the variance of multivariate data (Hammer, 2017). PCA may be used for reduction of the effective dimensional of a multivariate data set by producing linear combinations of the original components that summarize the predominant patterns in the data.

In order to provide meaningful interpretations for principal components, it is important to determine which variables are associated with particular components, in our case it seems that SRL characters is very important to separate the data in groups (see the fig. loading plot). The eigenvalue has a very large variance (99.84 %) for PC1

(Table 7) with confidence intervals from 99.769 to 99.906 bootstrapped (computed with PAST).

The coefficient value from loading plot of PCA (almost 1.0) gives the PC coefficients and correlation plot gives the correlation between the best variable (SRL, PTG and DRW) and the PC scores.

The PCA analysis based on matrix of variance-covariance (Fig. 4) shows very little overlap between *P. pristis* and *P. zijsron*, however, *A. cuspidata* and *P. pectinata* have considerable overlap for these two components. From this reason, additional ordinal statistical tests were conducted using the multivariate data.

Linear discriminant analysis (LDA) is a generalization of Fisher's linear discriminant, a classification method where two or more groups are known *a priori* and one or more new observations are classified into one of the known groups based on the measured characteristics (Fig. 5).

A scatter plot of specimens was computed using the PAST package along the first two canonical axes produces maximal and second to maximal separation between all groups. The linear combinations of the original variables in the first axis of PCA shows that SRL variable is the best morphological character for differences between groups, explained by the 23.808 (along axis 1) and 58.082 (along axis 2) coefficient of variation (Table 8).

The data were classifieds with the classifier command (PAST package), assigning each point to the group that gives minimal Mahalanobis distance to the group mean. The Mahalanobis distance is calculated from the pooled within-group co-variance matrix, giving a linear discriminant classifier. The given and estimated group assignments are listed for each point (Table 9).

The group assignment were 97.92 % correct classified in the predicted groups (on columns) (see the table of classifier). It seems that one individual of *Pristis zijsron* was incorrectly classified according to the LDA analysis.

Conclusions

The taxonomic analysis of the rostra in the NHMS and HMS collections confirmed that they were misidentified. Of the seven rostra analyzed, two were reassigned to *Anoxypristis cuspidata* (specimens P570 and P569) and the remaining five specimens were reassigned to *Pristis zijsron* (specimens: P21, P568, P567, P566, and VT738). PCA over imposed on a larger sample (see Seitz & Hoover, 2017), confirmed the morphologic and morphometric observations. The new taxonomic assignment significantly increases the scientific value of these specimens. It opens the opportunity of integration into an international database (e.g., 'Sawfish Conservation Society' or the 'International Sawfish Encounter Database (ISED)' at the Florida Museum of Natural History) in order to allow other researchers the use of data for research projects. One example would be a study of the historic dynamic of the distribution of sawfishes in the Indian Ocean or around the world.

Acknowledgments

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LIST OF ILLUSTRATIONS

LISTA ILUSTRAȚIILOR

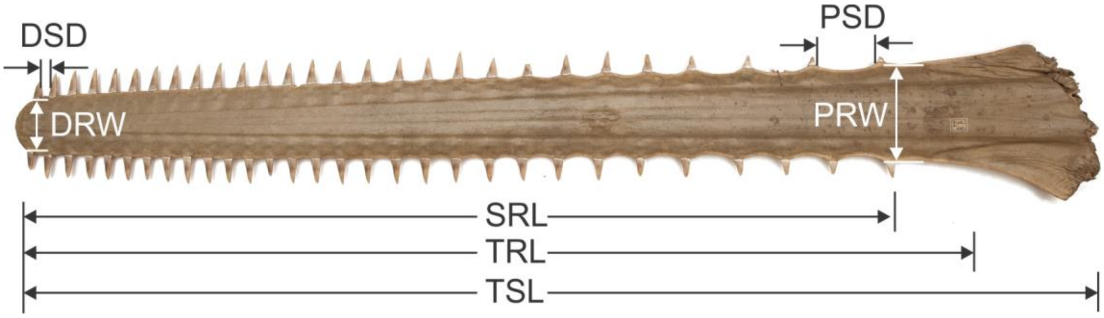

Fig. 1. Morphometric analysis measurements following Whitty *et al*., (2013) and Seitz & Hoover (2017)

Fig. 2. The rostra analyzed: a. P570; b. P569; c. P21; d. P568; e. P567; f. P. 566; g. VT 738;

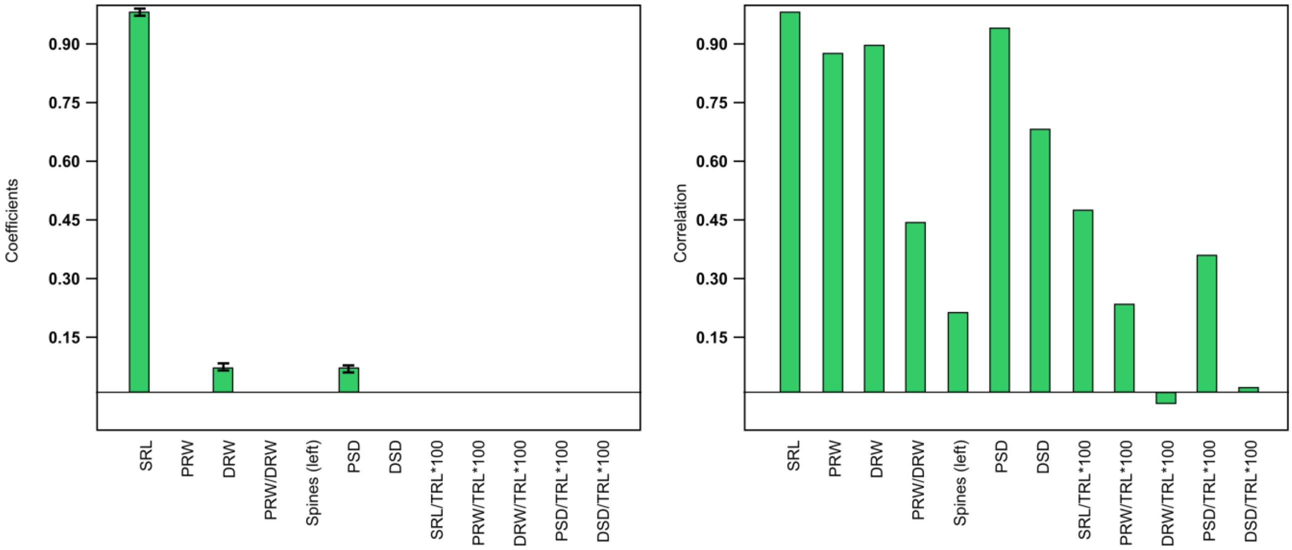

Fig. 3. Loadings plot of PCA with the best morphological caracter along PC1

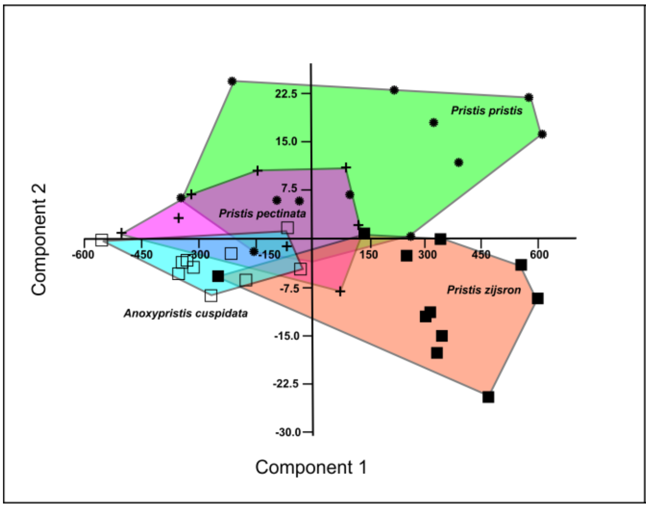

Fig. 4. PCA analysis based on matrix of variance-covariance.

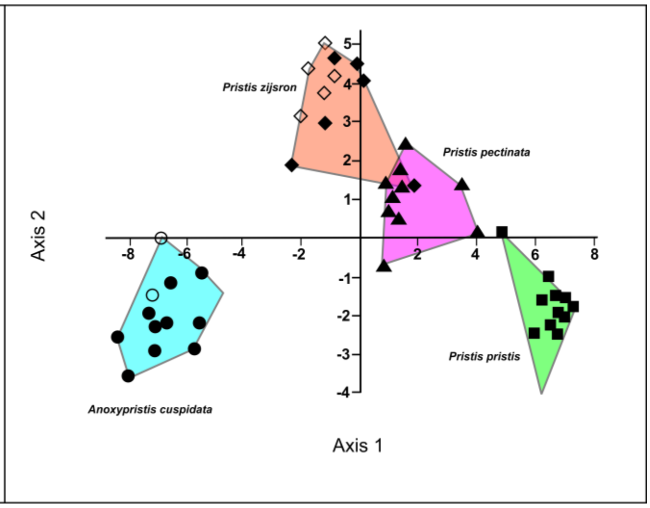

Fig. 5. Plot of linear discriminant analysis (LDA). Filled symbols represent the results of data from Seitz & Hoover (2017). Unfilled circles and rhombuses represent NHMS specimens.

Tab. 1. Correspondence of the old and current catalog numbers for rostral specimens

Old catalog number	Recorded as	Current catalog number	Recorded as	Other number present on specimen	Origin
NHMS					
174	*P. antiquorum*	P21	*P. pectinatus*	2530	Red Sea, Aden
179	*P. antiquorum*	P22	*P. pectinatus*	4063	Singapore
180	*Pristis* sp.	P570	*P. pectinatus*	4061	Singapore
181	*Pristis* sp.	P569	*P. pectinatus*	4960	Singapore
Not found	-	P568	*P. pectinatus*	4062	Unknown
Not found	-	P567	*P. pectinatus*	4059	Unknown
Not found	-	P566	*P. pectinatus*	-	Unknown
HMS					
Not found		VT738	-	-	Unknown

Tab. 2: Morphometry of the rostra. For the abbreviations used in this table see material and methods section.

Specimen (current catalog numbers)	No. of rostral spines (left and right)	TSL (mm)	TRL (mm)	SRL (mm)	PRW (mm)	DRW (mm)	DSD (mm)	PSD (mm)	DSD/ PSD ratio
P570	L29/R30	824	676	602	54	40	6	27	0.22
P569	L30/R27	866	743	637	58	36	6	30	0.2
P21	L31/R28	1189	1062	920	109	60	10	65	0.15
P568	L31/R31	1076	1076	1014	108	51	9	61	0.14
P567	L28/R29	1080	1054	971	108	53	11	66	0.16
P566	L29/R32	1273	1273	1134	117	52	12	83	0.14
VT738	L29/R30	1370	1270	1220	135	73	14	65	0.21

Tab. 3. The reported range in the number of rostral spines per side by species of sawfish

Source:	Biogeographic region	*Anoxypristis cuspidata*	*Pristis pristis*	*Pristis zijsron*	*Pristis clavata*	*Pristis pectinata*
Faria *et al*. 2012	Indian Ocean	22-29	16-21	27-34		
	West Pacific	17-30	14-23	24-32		
	West Atlantic		14-23			20-30
	East Atlantic		14-19			20-27
	East Pacific		15-23			
Morgan *et al*. 2009	Western Australia		17-24		18-24	
Whitty *et al*. 2013	within and outside of Australia	16-33	14-24	23-37	18-27	

Tab. 4: Univariate statistics test table using 48 specimens. Lengths and measurements are in mm.

	Overall length	**TRL**	**SRL**	**PRW**	**DRW**	**PRW/ DRW**	**No. of rostral spines (left)**	**No. of rostral spines (right)**	**PSD**	**DSD**	**SRL/ TRL *100**	**PRW/ TRL *100**	**DRW/ TRL *100**	**PTG/ TRL *100**	**DTG/ TRL *100**
Min.	174	163	114	15	10	1.33	14	14	7	2	69.94	7.11	4.07	3.22	0.79
Max.	1497	1470	1272	268	102	3	37	35	89	54	97.89	20.72	11.63	9.13	4.86
Sum	36913	35381	32096	4723	2163	100.17	1101	1109	2001	746	4295.95	629.71	298.8	263.33	101.09
Mean	769	737	668	98	45	2.09	22.94	23.1	41.69	15.54	89.5	13.12	6.23	5.49	2.11
SE	50.16	48.17	46.25	8.71	3.26	0.06	0.75	0.73	3.23	1.87	1.17	0.65	0.23	0.18	0.18
Variance	120759.6	111392.5	102669.8	3640.33	510.1	0.16	27.34	25.46	500.18	167.57	65.37	20.39	2.59	1.52	1.6
SD	347.5	333.76	320.42	60.34	22.59	0.4	5.23	5.05	22.36	12.94	8.08	4.52	1.61	1.23	1.27
Median	733.5	685.5	619.5	101.5	45	2.05	23	22.5	41	11.5	93.18	12.23	6.07	5.41	1.51
25 percentile	489.75	477	407	52.5	27.25	1.8	18.25	18.25	22.25	7	86.1	9.07	4.88	4.57	1.11
75 percentile	1053.25	1042	958.25	119.5	56.5	2.27	27	27	61	21.25	95.45	17.84	7.42	6.38	3.49
Skewness	0.23	0.24	0.22	**0.99**	0.63	0.37	0.34	0.2	0.29	**1.49**	**-1.15**	0.34	**0.86**	0.63	**0.86**
Kurtosis	-0.78	-0.67	**-0.9**	**0.86**	-0.01	-0.2	-0.57	-0.84	**-0.96**	**1.34**	0.06	**-1.4**	**1.12**	0.75	-0.78
Geom. Mean	680.64	651.17	580.28	80.54	39.29	2.05	22.36	22.56	34.87	11.63	89.11	12.37	6.03	5.35	1.79
Coeff. Var.	45.19	45.28	47.92	61.32	50.12	19.12	22.79	21.84	53.65	83.29	9.03	34.42	25.86	22.45	60.15
Min.	174	163	114	15	10	1.33	14	14	7	2	69.94	7.11	4.07	3.22	0.79

Tab. 5: Normal distribution test.

	TSL	**TRL**	**SRL**	**PRW**	**DRW**	**PRW/ DRW**	**No. of rostral spines (left)**	**No. of rostral spines (right)**
Shapiro-Wilk *W*	0.973	0.975	0.964	0.912	0.956	0.976	0.950	0.966
***p* (normal)**	0.322	0.393	0.141	**0.002**	0.070	0.435	**0.040**	0.170
	PSD	DSD	SRL/ TRL*100	PRW/ TRL*100	DRW/ TRL*100	PSD/ TRL*100	DSD/ TRL*100	
Shapiro-Wilk *W*	0.957	0.799	0.827	0.898	0.938	0.970	0.831	
***p* (normal)**	0.077	**0**	**0**	**0.001**	**0.014**	0.261	**0**	

Table 6: Pearson's *r* correlation coefficient and linear regression statistics

	TSL	TRL	SRL	PRW	DRW	PRW/ DRW	No. of rostral spines (left)	No. of rostral spines (right)	PSD	DS G	SRL/ TRL *100	PRW/ TRL *100	DRW/ TRL *100	PSD/ TRL *100	DSD/ TRL *100
TSL		0	0	0	0	0.01	0.11	0.05	0	0	0.02	0.45	0.39	0.05	0.6
TRL	**0.99**		0	0	0	0	0.12	0.05	0	0	0.01	0.41	0.35	0.03	0.64
SRL	**0.98**	**0.99**		0	0	0.02	0.15	0.07	0	0	0.01	0.13	0.83	0.01	0.97
PRW	0.82	0.89	0.88		0	0	0.62	0.82	0	0	0	0	0.05	0.01	0.01
DRW	0.86	0.86	0.89	0.92		0	0.56	0.79	0	0	0	0	0.02	0.02	0.01
PRW/DRW	0.36	0.39	0.44	0.7	0.49		0.01	0.02	0	0	0	0	0.1	0	0
No. of rostral spines (left)	0.23	0.23	0.21	-0.07	-0.08	-0.35		0	0.337	0.01	0.43	0.01	0.01	0.41	0
No. of rostral spines (right)	0.28	0.28	0.26	-0.03	-0.05	-0.34	**0.96**		0.209	0.01	0.3	0.01	0.01	0.41	0
PSD	0.92	0.93	0.94	0.84	0.83	0.52	0.14	0.18		0	0.01	0.08	0.69	0	0.66
DSG	0.62	0.63	0.68	0.91	0.84	0.76	-0.42	-0.38	0.682		0	0	0.01	0.01	0
SRL/TRL*100	0.34	0.35	0.47	0.67	0.56	0.51	0.11	0.15	0.425	0.6		0	0	0.01	0.01
PRW/TRL*100	0.11	0.12	0.22	0.6	0.52	0.72	-0.46	-0.45	0.252	0.74	0.74		0	0.01	0
DRW/TRL*100	-0.19	-0.14	-0.03	0.29	0.34	0.24	-0.4	-0.4	-0.058	0.46	0.62	0.84		0.31	0
PSD/TRL*100	0.28	0.31	0.35	0.48	0.34	0.58	-0.12	-0.12	0.61	0.48	0.45	0.43	0.15		0.02
DSD/TRL*100	-0.07	-0.07	0.006	0.39	0.34	0.62	-0.75	-0.74	0.065	0.7	0.46	0.88	0.78	0.33	

Table 7: Summary of components used in principal component analysis (PCA)

PC	Eigenvalue	% variance
1	103523	99.847
2	99.0649	0.095547
3	59.5471	0.057432
4	0.351806	0.000339
5	0.112209	0.000108
6	0.012047	1.16E-05
7	0.008358	8.06E-06
8	0.006254	6.03E-06
9	0.001055	1.02E-06
10	0.000108	1.04E-07
11	3.88E-05	3.75E-08
12	2.98E-21	2.88E-24

Table 8. Discriminant analysis loadings of first three axis

Parameters	Axis 1	Axis 2	Axis 3
SRL	23.808	58.082	-136.04
PRW	0.041209	0.02668	-0.08667
DRW	2.6921	1.7302	-7.8178
PRW/DRW	0.061241	-0.01006	-0.05303
No. of rostral spines (left)	-0.007577	0.03141	0.013283
PSD	1.8225	4.3106	-10.075
DSG	0.05293[illegible]	-0.01306	-0.10349
SRL/TRL*100	0.006245	0.006279	0.005389
PRW/TRL*100	0.028565	-0.00665	0.015777
DRW/TRL*100	0.015797	-0.00666	0.024413
PSD/TRL*100	0.12155	0.16126	-0.27145
DSD/TRL*100	0.040292	-0.04639	-0.00104

Table 9. Classifier table with Mahalanobis distance to the group mean.

	Anoxypristis cuspidata	***Pristis pectinata***	***Pristis pristis***	***Pristis zijsron***	**Total**
Anoxypristis cuspidata	14	0	0	0	**14**
Pristis pectinata	0	10	0	0	**10**
Pristis pristis	0	0	13	0	**13**
Pristis zijsron	0	1	0	10	**11**
Total	**14**	**11**	**13**	**10**	**48**