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RESEARCH ARTICLE

A multivariate approach for developing a dichotomous key for identification and differentiation of *Puntius* (Osteichthyes: Cyprinidae) species in Sri Lanka

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Abstract: Sri Lanka is a global biodiversity hot spot with a rich freshwater fish fauna. Out of a total of 82 freshwater fish species, the genus *Puntius* represents 16 species (19.5%). Ambiguities exist in taxonomic identification of the different *Puntius* species. Hence, in this study a dichotomous key was developed using morphometric and meristic characters to identify and differentiate the *Puntius* species. Altogether 421 specimens representing different Sri Lankan *Puntius* species were collected from 38 sites at four different altitude ranges from five major river basins in Sri Lanka. Fifteen meristic characters, four coded variables and twenty three morphometric characters were recorded for each specimen and characters were analysed using principal component analysis (PCA).

Six principal components were extracted for meristic characters and coded variables that explained 81.5% of the cumulative variance in the dataset. Two meristic characters (number of transverse scales and number of post dorsal scales) and four coded variables (nature of the lateral line, position of mouth, number of barbels and nature of dorsal fin spines) were the variables that contributed most to the variance of the six principal components identified. The six characters were sufficient in isolation to develop a dichotomous key for all, except for two species. Two principal components extracted only for morphometric characters were also able to differentiate *Puntius* species but not to the same level as meristic characters and therefore, they contributed less to the dichotomous key developed here. Based on this approach, 15 *Puntius* species could be differentiated unambiguously.

Keywords: Meristic**,** morphometric, principal component analysis, *Puntius.*

INTRODUCTION

Sri Lanka (5º55'–9º55' N; 79º42'-81º52' E; 65,610 km^2) is a continental island in Asia with a freshwater fish fauna consisting of 82 species, 44 of which are endemic[1]. Of the 82 fish species, 16 species (19.5%) belong in the genus *Puntius*[2] and are members of the family Cyprinidae (Table 1)[1,2,4-15]. The Generic name *Puntius* (commonly known as barbs) is mainly used to represent barbs from the Asiatic region[3].

Many species of *Puntius* are attractive as aquarium fish due to their beautiful colouration, striking body markings, general body shape and small size as well as the ease of rearing in home aquaria. Most are essentially riverine species[15,16] but some are also found in the multitude of irrigation reservoirs across the country. Due to high abundance, they contribute significantly to the trophic dynamics of many reservoir ecosystems in the country[17].

Among the 16 species of *Puntius* in Sri Lanka nine are endemic. Due to over exploitation for the aquarium trade and general habitat degradation, some *Puntius* species have become highly threatened and are prone to extinction (Table 1). Conservation of these species has become a critical issue, and recognition of Sri Lanka as a global biodiversity hotspot has raised their conservation profile. Effective methods for species identification are required to assist their conservation.

Several studies[8,9,11,18] have been carried out on the taxonomy of Sri Lankan freshwater fish including *Puntius* species. Deraniyagala[19] described several new species and published his findings in a coloured atlas, which became a primary reference. Mendis[20] and Munro[21] have made significant contributions to freshwater fish taxonomy in Sri Lanka. Taxonomic reviews by Pethiyagoda have described several new *Puntius* species

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and clarified the taxonomic status of some *Puntius* species where previously ambiguities were present in their identification[4,22-24].

However, there are ambiguities in identification and differentiation of *Puntius* spp.. Currently, identification of *Puntius* spp. is based on several characters that incorporate external morphology, morphometric and meristic characters[3,4,19,21]. In most instances identification at the species level is based on a few specimens that may not adequately represent all intra-specific variations present. It is difficult to obtain some characters (i.e. osteological characters) in a short time period and damage the specimen. Descriptions of colour patterns and markings on the body may fade or may not be clearly seen in preserved specimens. These problems have led to misidentification of *Puntius* species hence the identification of a rigorous set of characters devoid of these failures would enhance the taxonomy of this important fish species. Adopting an approach that screens morphological and meristic characters using large sample sizes and employing appropriate statistical analyses should assist in the identification and discrimination of extant species.

The current study therefore aimed to develop a dichotomous key for Sri Lankan *Puntius* species, to identify and discriminate species using a set of characters with easily identifiable, non overlapping scores that could be recorded within a short time period with high precision.

METHODS AND MATERIALS

A total of 421 fish from 15 described species of *Puntius* (of the 16 species recorded in Sri Lanka) were sampled from 38 sites in 5 major river basins from March 2004 to November 2006 (Figure 1). Fish were caught using gape nets, cast nets and scoop nets. Where particular species were considered to be highly endangered or rare, only 2 or 3 individuals were taken for analysis. Specimens were identified in the field to species level using external morphological characters (colour patterns, specific morphological traits and body shape)[4]. Samples were then preserved in 70% alcohol. Additional identification

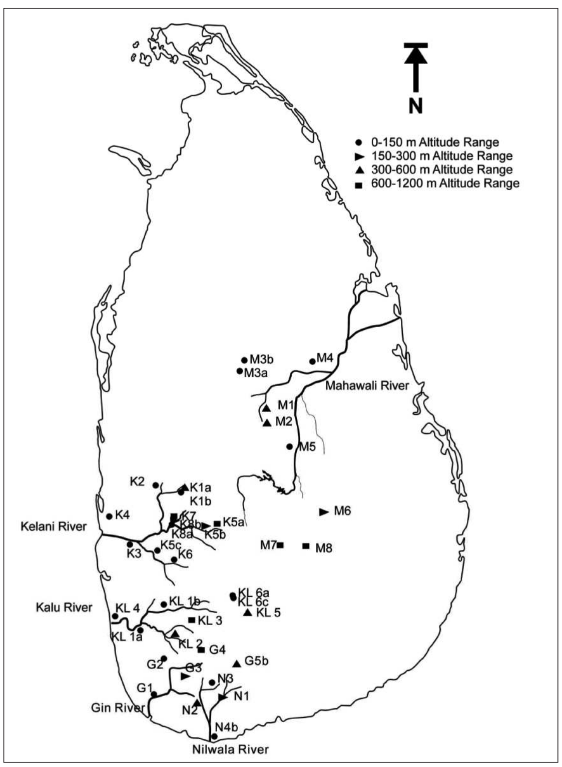

Figure 1: Map of Sri Lanka showing sampling sites, elevations and rivers sampled

Doolgala-K1a, Bulathkohupitiya-K1b, Minimarukolaniya-K2, Pinnawala- K3, Biyagama-K4, Rampadeniya- K5a, Kitulgala- K5b, Dehiowita- K5c, Daraniyagala-K6, Alugala-K7, Peregashandiya-K8a, Yatiyantota-K8b, Illukkumbura-M1, Pallegama -M2, Hiriwadunawewa-M3a, Habarana-M3b, Parakrama Samudraya-M4, Ginnoruwa- M5, Arawa-M6, Welimada- M7, Demodara- M8, Agalawatta- KL1a, Ingiriya- KL1b, Athwaltota- KL 2, Pitigala Kanda (Kalawana)- KL 3, Gamagoda- KL 4, Handurukanda -KL5, Bopath-Ella-KL6a/ KL6c, Wakwella-G1, Hiniduma-G2, Kanneliya-G3 Viharahena-G4, Opatha-G5b, Deyandara-N1, Dediyagala-N2, Mawarala-N3, Godapitiya- N4b

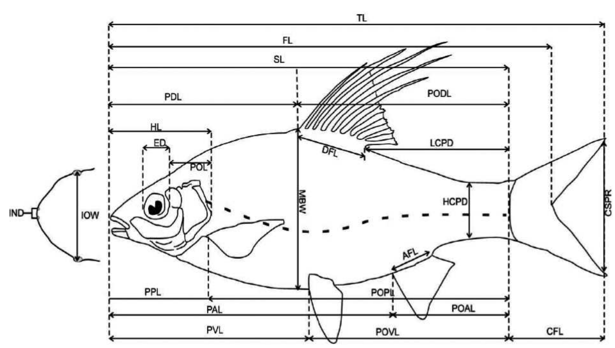

Figure 2: Morphometric characters measured in this study

Total length, TL; Standard length, SL; Fork length, FL; Maximum body depth, MBW; Head length, HL; Eye diameter, ED; Distance between pair of nostrils, IND; Inter orbital distance, IOW; Post orbital length, POL; Dorsal fin length, DFL; Pre dorsal length, PDL; Post dorsal length, PODL; Anal fin length, AFL; Pre anal length, PAL; Post anal length, POAL; Pre ventral length, PVL; Post ventral length, POVL; Pre pelvic length, PPL; Post pelvic length, POPL; Caudal fin length, CFL; Width of the caudal fin when fully spread, CSPR; Caudal peduncle height, HCPD; end of the dorsal fin to end of the caudal peduncle length, LCPD

Table 1: *Puntius* species of Sri Lanka and their status[1,4]

Species	E	V	En	CE	A
Puntius amphibius [5]					Common
Puntius asoka [6]	+			+	Rare
Puntius bandula [7]	+			+	Very rare
Puntius bimaculatus [8]	+				Very common
Puntius chola [2]					Common *
Puntius cumingii [9]	+	+			Common
Puntius dorsalis [10]					Common
Puntius singhala [11]					Common
Puntius martenstyni [7]	+			+	Rare
Puntius nigrofasciatus [9]	+	+			Not yet rare
Puntius pleurotaenia [8]	+	+			Common
Puntius sarana [2]	¬				Common
Puntius srilankensis [12]	+		+		Very rare
Puntius ticto [2]					Common
Puntius titteya [13]	+	+			Common
Puntius vittatus [14]					Common

E – Endemic, V – Vulnerable, En – Endangered, CE – Critically Endangered, A – Abundance.
* – Uncommon in wet zone
Superscript numerals indicate the relevent reference of identification of the fish species.

Table 2a: Meristic measurements scored for *Puntius* species

Characters	
Scale counts	Acronyms
Number of lateral line scales	lls
Number of transverse scales*	tr
Pre dorsal scales- counted from the edge of the operculum to the beginning of the dorsal fin	prds
Post dorsal scles- counted from the end of the dorsal fin to the beginning of the caudal fin	psds
Dorsal fin scales- counted from the beginning of the dorsal fin to the end of the dorsal fin	dfsc
Scales around the caudal peduncle	cped
Fin ray counts	
Number of dorsal fin rays	dfr
Number of anal fin rays	afr
Number of pelvic fin rays	pfr
Number of caudal fin rays	cfr
Number of ventral fin rays	vfr
Fin spine counts	
Number of dorsal fin spines	dfs
Number of anal fin spines	afs
Number of pelvic fin spines	pfs
Number of ventral fin spines	vfs

[b] Numbers one to nine were used in the analysis. Transverse scales were divided into 9 categories according to the arrangement (1) 3.5/2.5; (2) 3.5/3; (3) 3.5/3.5; (4) 4.5/2.5; (5) 4.5/3; (6) 4.5/3.5; (7)5/3.5; (8) 5.5/2.5; (9) 5.5/3.5

Table 2b: Coded variables scored on *Puntius* species

Characters	Acronyms
Nature of lateral line*	nll
1) Complete lateral line	
2) Incomplete lateral line	
Position of mouth*	pom
1) sub terminal (When the mouth is opened it directs towards downward)	
2) terminal (When the mouth is opened it directs front of the head and points forward)	
Nature of dorsal fin spines*	ndfs
1) smooth	
2) serrate	
Barbles	
Number of barbels	nb
0) no barbells or a pair of rudimentary barbels	
1) one pair of barbels	
2) two pairs of barbels	

*Characters were quantified as 0, 1 and 2 on a nominal scale and this number was used in the analysis.

Table 3: Component loadings of each morphometric variable obtained for two principal components extracted by PCA

Variable	Component 1	Component 2
TL	.300	.950
FL1	.921	.388
MBW1	.937	.340
HL1	.941	.335
ED1	.956	.267
POL1	.915	.394
DFL	.385	.916
PDL1	.939	.341
PODL1	.919	.391
AFL1	.908	.401
PAL	.925	.377
POAL	.888	.454
PVL	.934	.353
POVL	.909	.413
PPL1	.949	.303
POPL1	.910	.413
CFL1	.887	.446
CSPR	.866	.476
HCPD	.942	.323
LCPD	.935	.335
IND	.912	.335
IOW	.933	.334
Eigen Value	20.67	1.1
% Variance	94	5

Table 4: Component loadings of each variable obtained for each principal component (highlighted values are the most contributing variables to each principal component)

Variable	Component					
	1	2	3	4	5	6
nll	-.371	**-.661**	.340	.385	.015	-.099
lls	**.848**	.419	-.041	.041	.149	-.009
tr	.246	.516	-.076	.383	**.530**	.143
nb (pairs)	**.858**	.188	-.213	.189	.007	-.112
pom	.174	.074	.092	**.869**	.019	-.203
ndfs	.048	.040	.110	-.052	**.935**	-.085
dfs	.391	**.667**	.176	.227	-.027	-.045
vfs	-.084	.019	**.874**	.179	.214	.178
vfr	-.005	.069	**-.901**	.031	.062	.040
cfr	.081	.031	.066	-.226	-.020	**.851**
prds	**.777**	.114	.105	-.025	.379	.165
psds	.817	.329	.016	.021	-.174	.074
cped	.271	**.816**	-.103	.012	-.077	.148
dfsc	.153	**.791**	.024	.048	.329	-.128
afr	.049	-.024	-.064	**-.578**	.062	**-.618**
Eigen Value	5.12	2.18	1.40	1.36	1.16	1.10
% Variance	34	14.5	9	9	8	7

was undertaken in the laboratory using standard fish keys and guides[3,4,19,21]. A total of 42 characters of which 23 represented morphometric measurements (Figure 2), 15 represented meristic traits and 4 represented coded variables were scored for all 15 species (Tables 2a & 2b). Particular measurements were made by a single individual to minimize scoring errors. Linear measurements were made using vernier calipers to the nearest 0.01 mm. A Stereo microscope (Wild M5A) and hand lens were used to determine meristic counts and to score coded variables.

Analyses were carried out separately for morphometric and meristic characters as these variables are different from both statistical (morphometric are continuous and meristic are discrete) and biological (morphometric characters can be susceptible to environmental factors while most meristic characters are fixed early during the development) perspectives[25]. Coded characters were converted to a discrete form and also included with meristic characters in the analysis (Table 2b).

As body measurements are often strongly correlated with body length, all morphometric variables were standardized for individual size of each species separately using the following equation[26].

$$Y\square_i = \log_{10} Y_i - b(\log_{10} L_i - \log_{10} X)$$

Where $Y\square_i$ = size corrected morphometric variable value for the ith fish, Y_i = original value, L_i = standard length for ith fish, X = mean standard length for that group of fish and b = slope of regression of $\log_{10} Y$ on $\log_{10} L_i$ for the fish group (species) considered. Effectiveness of the standardization was checked by correlation analysis of each variable with standard length, and no correlation was observed with individual length.

Meristic characters are commonly determined early during development and have often been reported as being independent of individual size[27-29]. Relationship of each meristic character with total length of individual was analysed separately for each species and results showed that the majority of characters were not significantly correlated. A few characters did show significant correlation with total individual length but these characters varied across species. As the present study was focused on inter-specific variation, intra-specific correlations of a small number of variables were not considered to be important.

The general aim of the current study was to identify sets of characters that could differentiate individual *Puntius* species. As a univariate approach cannot address any joint effect (interactions) of variables, each individual was considered to be a single multivariate observation in the analyses[30,31]. Data used in the analyses were assessed using Principal Component Analysis (PCA) and consisted of pooled data from 42 variables on 15 species. Raw meristic data together with coded character data and size adjusted morphometric data were analyzed separately according to the methodological steps and statistical steps suggested by Gorge and Mallery[32]. Component loadings were obtained by a rotation method with Varimax and normalization. Variables that had no variance and those which contributed comparatively low variance in the analysis where principal components (PCs) were extracted, were excluded from the analysis. Maximum, minimum, mean and standard deviation of each meristic variable and morphometric variables were obtained for each species. All calculations were carried out using statistical software SPSS version 16.

RESULTS

PCA was performed using 23 standardized morphometric data measurements for each individual/species. The first two PCs possessing Eigen values above 1 explained 99% of the cumulative variance. All morphometric variables had positive loadings in the first and second principal components (PC1 and PC2), that explained 94% and 5% of the variance, respectively (Table 3). According to previous studies[33] any components having all loadings (coefficients) of the same sign for a PC is indicative of size variation whereas any component having both positive and negative loadings is indicative of shape variation. As both PCs had positive component loadings in the present study, it could be concluded that they accounted for the size variation among species. High scores for PC1 were associated with position and size of the eye, maximum depth of the body, position of the anal fin, pelvic fin, ventral fin, length and depth of the caudal peduncle and spread of the caudal fin, and length of the caudal fin. Total length and dorsal fin length provided the highest contribution to PC2.

Component scores of each individual fish obtained for PC1 and PC2 separated 15 species in a two dimensional matrix (Figure 3). *P. nigrofasciatus*, *P. bimaculatus* and *P. chola* individuals showed negative component scores for both PC1 and PC2 and their plots were highly separated from the rest of the sampled species occupying three different positions in the plot. *P. pleurotaenia* and *P. martenstyni* had positive component scores for both PC1 and PC2 that also separated them from other species but that grouped them closely together. *P. titteya*, *P. ticto*, *P. bandula* and *P. vittatus* also clustered in close proximity and formed a separate group.

Separation of species in the biplot was mainly influenced by the length and depth of the body. Individuals possessing small values for maximum body depth (MBW) and large values for total length (TL), width of caudal fin when fully spread (CSPR), caudal peduncle height (HCPD) and distance between end of dorsal fin to end of the caudal peduncle (LCPD) represented slender/ longer bodied species and grouped above the PC1 axis. Individuals with high values for MBW and small values for TL, CSPR, HCPD and LCPD represented deeper/ shorter bodied species and grouped below the PC1 axis (Figure 3). A Spearman rank correlation between component scores (for PC1 and PC2) and the standardized total length of each individual also indicated that the separation of species was highly dependent on individual size of fish (r^2 = 0.799; p = 0.000. and r^2 = -0.631; p = 0.000 respectively for PC1 and PC2). This separation was not sufficient to discriminate between most species. A comparative analysis of the maximum, minimum values and means of morphometric variables among species (not shown) showed that the values overlapped in most species.

A similar analysis performed with meristic characters with number of pelvic fin spines was removed from the analysis because it did not vary among species, while the number of pelvic fin rays (pfr), number of dorsal fin rays (dfr) and number of anal fin spines (afs) were removed due to comparatively low variance contributions.

Six PCs (Eigen values above 1) were obtained with 81.5% of the cumulative variance explained (Table 4). PC1 explained 34% of total variance. The variables that contributed most to PC1 were the number of lateral line scales (lls), number of barbels (nb), number of pre dorsal scales (prds) and number of post dorsal scales (psds). PC2 explained 14.5% of the variance with nature of lateral line (nll) showing negative component loadings and number of dorsal fin spines (dfs), number of scales around the caudal peduncle (cped) and number of dorsal fin scales (dfsc), positive loadings to the variance. The remaining principal components contributed the remaining 33% to the variance.

A plot of component scores obtained for each individual for PC1 and PC2 showed marked separation of the fifteen species in two dimensional space. Number of lateral line scales determined the grouping of species on the negative and positive sides of the plot along the PC1 axis (Figure 4). Incomplete nature of lateral line determined species that were grouped on the lower quarter of the left side of the biplot. A number of barbels contributed to PC1 and determined the grouping of species between the left and right side above the PC1 axis. Accordingly, *P. titteya*, *P. vittatus* and *P. cumingii* possessed incomplete lateral line, lower scores for lls, psds and prds and were grouped in the lower negative quarter of the biplot. *P. nigrofasciatus*, *P. srilankensis*, *P. chola* and *P. dorsalis* that possessed one pair of barbels, lower scores for lls and a complete lateral line were grouped in the upper quarter of the left side (Figure 4). *P. bandula* and *P. ticto* were also grouped with them but the two species possess an incomplete lateral line. *P. sarana*, *P. pleurotaenia* and *P. martenstyni* possessed two pairs of barbels, a terminal mouth position, complete lateral line and high scores for lls, psds and prds, and

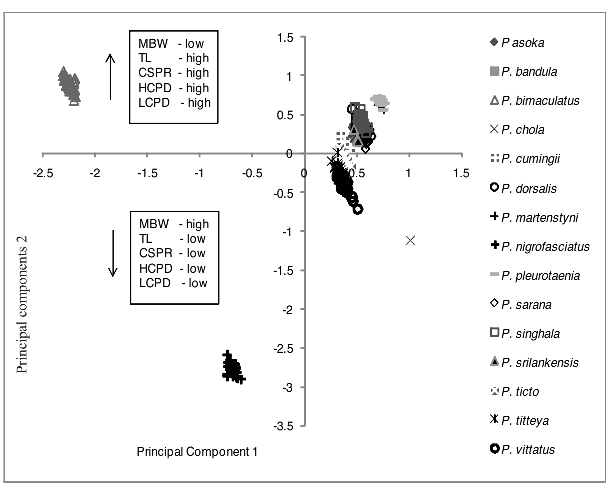

Figure 3: Scatter plot showing individual component scores obtained for PC1 and PC2 for morphometric characters

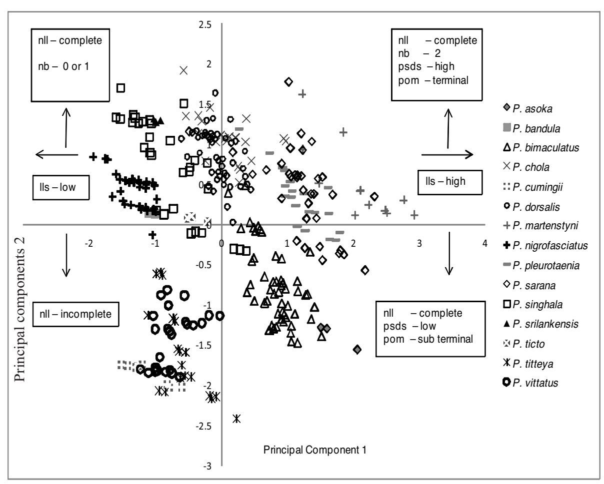

Figure 4: Scatter plot showing individual component scores obtained for PC1 and PC2 for meristic and coded characters

were grouped in the upper quarter on the positive side of the biplot. *P. bimaculatus* and *P. asoka* also had high scores for lls, a complete lateral line and high psds were placed in the lower quarter on the positive side due to sub terminal position of the mouth and a single pair of barbels. The positions of *P. dorsalis*, *P. sarana* and *P. singhala* were scattered due to high variation in some meristic characters (Figure 4).

Comparison of descriptive statistics for meristic characters (maximum, minimum, mean and standard deviation) and coded variables showed that there were noticeable differences for certain variables among the fifteen species sampled (Tables 5 and 6). Variables with clear differences between species contributed the major part of variance in determining the six principal components (Table 5). This outcome was not evident however, for morphometric variables with most characters showing overlapping values among species. Number of barbels showed factor loading of 0.86 for PC1, while psds and number of transverse scales (tr) showed factor loadings of 0.82 and 0.53 for PC1 and PC5 and explained 34% and 14.5% of the variance, respectively. Nature of lateral line (nll), position of mouth (pom) and nature of dorsal fin spines (ndfs) are variables that possessed comparatively high factor loadings for the second, fourth and fifth principal components, respectively. These six characters were considered therefore as important characters for differentiation of *Puntius* species. Number of lateral line scales (lls), number of scales around the caudal peduncle (cped), number of pre dorsal scales (prds), number of pre dorsal fin scales (dfsc), number of ventral fin rays (vfr), number of anal fin rays (afr) and number of caudal fin rays (cfr) also showed high factor loadings but provided only limited utility for developing the taxonomic key as different species possessed overlapping values (Table 6). Length ratios pre dordal length/standard length (DFL/SL and head length/ standard length HL/SL) also showed some utility for differentiating specific species where meristic characters either were similar or overlapped between species that limited their use (Table 7).

Table 5: Variability of characters used in developing the key of 15 *Puntius* species

Species	N	nll	nb	pom	ndfs	tr	psds			
							min	max	mean	std
P.chola	34	Complete (1)	1	Terminal (2)	Serrate (2)	5.5/3.5	10	13	11.00	0.70
P.cumingii	19	Incomplete (2)	0	Sub terminal (1)	Serrate (2)	3.5/3.5	8	9	8.42	0.50
P.bandula	2	Incomplete (2)	0	Sub terminal (1)	Serrate (2)	4.5/3.5	9	9	9.00	0.00
P.bimaculatus	63	Complete (1)	1	Sub terminal (1)	Smooth (1)	3.5/2.5				
						3.5/3.5	9	13	11.11	0.99
P.asoka	3	Complete (1)	2	Sub terminal (1)	Smooth (1)	4.5/2.5	11	11	11.00	0.00
P.martenstyni	13	Complete (1)	2	Terminal (2)	Smooth (1),					
					Serrate (2)	5.5/2.5	13	16	14.61	0.96
P.nigrofasciatus	53	Complete (1)	0	Sub terminal (1)	Serrate (2)	4.5/3.5	8	10	9.06	0.66
P.pleurotaenia	26	Complete (1)	2	Terminal (2)	Serrate (2)	4.5/2.5				
						5.5/2.5	11	13	11.92	0.39
P.sarana	40	Complete (1)	2	Terminal (2)	Serrate (2)	4.5/3.5				
						5.0/3.5				
						5.5/3.5	9	14	11.75	0.98
P.singhala	40	Complete (1)	0,1	Terminal (2)	Smooth (1)	4.5/2.5	9	11	10.20	0.56
P.srilankensis	3	Complete (1)	0	Sub terminal (1)	Smooth (1)	4.5/2.5	11	11	11.00	0.00
P.ticto	16	Incomplete (2)	0	Terminal (2)	Serrate (2)	4.5/3.5	10	11	10.13	0.34
P.titteya	24	Incomplete (2)	1	Terminal (2)	Smooth (1)	3.5/3.5	8	9	8.70	0.46
P.vittatus	31	Incomplete (2)	0	Terminal (2)	Smooth (1)	3.5/3.5	9	10	9.71	0.46
P.dorsalis	54	Complete (1)	1	Sub terminal (1)	Smooth (1)	3.5/2.5				
						4.5/2.5				
						4.5/3.0	10	13	11.1	0.56

min-minimum; max-maximum; std-standard deviation

Table 6: Maximum, minimum, mean and standard deviation of meristic characters of 15 *Puntius* species

Species				prds				dfsc				cped	
	N	min	max	mean	std.	min	max	mean	std.	min	max	mean	std.
P. chola	34	8	10	8.97	0.46	5	6	5.03	0.17	11	13	12.00	0.49
P. cumingii	19	7	8	7.74	0.45	4	4	4.00	0.00	8	8	8.00	0.00
P. bandula	2	9	9	9.00	0.00	5	5	5.00	0.00	11	11	11.00	0.00
P. bimaculatus	63	8	10	8.86	0.59	4	5	4.27	0.45	9	11	10.33	0.60
P .asoka	3	9	10	9.33	0.58	5	5	5.00	0.00	7	7	7.00	0.00
P . martenstyni	13	8	11	9.54	0.88	5	6	5.15	0.38	11	12	11.92	0.28
P. nigrofasciatus	53	7	9	7.92	0.33	5	5	5.00	0.00	9	11	10.62	0.53
P. pleurotaenia	26	7	10	9.08	0.80	5	6	5.62	0.50	9	12	10.58	0.70
P. sarana	40	7	11	9.43	0.81	5	6	5.08	0.27	10	14	11.58	1.11
P. singhala	40	7	8	7.45	0.50	4	6	4.95	0.39	10	11	10.43	0.50
P. srilankensis	3	7	7	7.00	0.00	5	5	5.00	0.00	11	11	11.00	0.00
P. ticto	16	9	9	9.00	0.00	5	5	5.00	0.00	10	10	10.00	0.00
P. titteya	24	7	9	7.25	0.53	4	4	4.00	0.00	8	10	9.13	0.85
P. vittatus	31	7	8	7.19	0.40	4	5	4.03	0.18	8	10	9.26	0.58
P. dorsalis	54	7	9	7.96	0.27	4	5	4.98	0.14	10	12	11.41	0.66

Species				lls				dfs				afr	
	N	min	max	mean	std.	min	max	mean	std.	min	max	mean	std.
P. chola	34	22	26	25.09	1.19	3	4	3.97	0.17	6	6	6.00	0.00
P. cumingii	19	17	19	18.68	0.67	3	3	3.00	0.00	6	7	6.84	0.37
P. bandula	2	20	21	20.50	0.71	4	4	4.00	0.00	6	6	6.00	0.00
P. bimaculatus	63	22	26	23.70	1.14	3	4	3.10	0.29	5	8	6.75	0.65
P. asoka	3	26	28	27.00	1	4	4	4.00	0.00	7	7	7.00	0.00
P. martenstyni	13	28	30	29.08	0.95	4	4	4.00	0.00	7	7	7.00	0.00
P. nigrofasciatus	53	20	22	20.57	0.57	2	3	2.98	0.14	6	7	6.89	0.32
P. pleurotaenia	26	26	30	27.88	1.18	4	4	4.00	0.00	6	7	6.54	0.51
P. sarana	40	25	30	26.98	1.05	4	4	4.00	0.00	6	7	6.78	0.42
P. singhala	40	20	23	20.95	0.78	3	4	3.72	0.45	6	7	6.68	0.47
P. srilankensis	3	22	23	22.33	0.58	4	4	4.00	0.00	7	7	7.00	0.00
P. ticto	16	21	22	21.94	0.25	4	4	4.00	0.00	6	6	6.00	0.00
P. titteya	24	17	20	18.63	0.82	2	4	3.10	0.50	5	7	6.25	0.61
P. vittatus	31	18	20	19.19	0.65	2	4	2.68	0.54	6	8	6.87	0.50
P .dorsalis	54	21	26	23.26	1.14	3	5	4.00	0.27	6	8	6.67	0.58

Species				vfs				vfr				cfr	
	N	min	max	mean	std.	min	max	mean	std.	min	max	mean	std.
P.chola	34	1	1	1.00	0.00	9	9	9.00	0.00	19	22	20.53	0.86
P.cumingii	19	1	1	1.00	0.00	9	9	9.00	0.00	20	21	20.11	0.32
P.bandula	2	1	1	1.00	0.00	9	9	9.00	0.00	20	20	20.00	0.00
P.bimaculatus	63	1	1	1.00	0.00	7	9	8.83	0.42	18	24	20.92	1.73
P.asoka	3	1	1	1.00	0.00	9	9	9.00	0.00	20	20	20.00	0.00
P.martinstyni	13	1	1	1.00	0.00	9	9	9.00	0.00	19	21	20.08	0.49
P.nigrofasciatus	53	1	1	1.00	0.00	9	9	9.00	0.00	18	21	20.38	0.66
P.pleurotaenia	26	1	1	1.00	0.00	9	9	9.00	0.00	19	22	19.73	0.87
P.sarana	40	1	1	1.00	0.00	9	9	9.00	0.00	19	22	20.08	0.57
P.singhala	40	1	1	1.00	0.00	9	9	9.00	0.00	18	21	20.03	0.73
P.srilankensis	3	1	1	1.00	0.00	9	9	9.00	0.00	20	21	20.33	0.58
P.ticto	16	2	2	2.00	0.00	8	8	8.00	0.00	21	22	21.19	0.40
P.titteya	24	1	1	1.00	0.00	9	9	9.00	0.00	18	20	19.21	0.66
P.vittatus	31	1	1	1.00	0.00	8	9	8.84	0.37	19	21	19.84	0.64
P.dorsalis	54	1	1	1.00	0.00	8	10	8.98	0.24	19	28	20.50	1.91

[e] min-minimum; max-maximum; std-standard deviation

Table 7: Descriptive statistics of *P. dorsalis* (N = 54) and *P. bimaculatus* (N = 63) (Highlighted values were used in developing the key)

Species	Character	min	max	mean	std
P. dorsalis	TL/SL	1.17	1.36	1.24	0.05
P. bimaculatus		1.12	1.29	1.20	0.04
P. dorsalis	FL/SL	1.08	1.20	1.13	0.03
P. bimaculatus		1.08	1.20	1.13	0.02
P. dorsalis	MBW/SL	0.26	0.34	0.30	0.02
P. bimaculatus		0.18	0.31	0.27	0.02
P. dorsalis	MBW/TL	0.20	0.28	0.24	0.02
P. bimaculatus		0.15	0.27	0.22	0.02
P. dorsalis	ED/HL	0.24	0.38	0.30	0.03
P. bimaculatus		0.21	0.37	0.28	0.04
P. dorsalis	HL/SL	**0.26**	**0.32**	**0.29**	0.01
P. bimaculatus		**0.19**	**0.28**	**0.23**	0.01
P. dorsalis	POL/SL	0.10	0.14	0.12	0.01
P. bimaculatus		0.09	0.14	0.11	0.01
P. dorsalis	DFL/SL	**0.14**	**0.25**	**0.16**	0.01
P. bimaculatus		**0.09**	**0.16**	**0.12**	0.01
P. dorsalis	PDL/PODL	0.81	1.19	1.00	0.08
P. bimaculatus		0.83	1.13	0.94	0.06
P. dorsalis	PDL/SL	0.46	0.54	0.50	0.01
P. bimaculatus		0.45	0.56	0.49	0.02
P. dorsalis	PODL/SL	0.46	0.56	0.50	0.02
P. bimaculatus		0.48	0.59	0.52	0.02
P. dorsalis	AFL/SL	0.07	0.13	0.09	0.01
P. bimaculatus		0.06	0.11	0.08	0.01
P. dorsalis	PAL/SL	0.69	0.77	0.73	0.02
P. bimaculatus		0.66	1.00	0.73	0.04
P. dorsalis	POAL/SL	0.24	0.32	0.27	0.02
P. bimaculatus		0.25	0.33	0.28	0.02
P. dorsalis	PAL/POAL	2.16	3.20	2.67	0.22
P. bimaculatus		1.98	3.79	2.59	0.26
P. dorsalis	PVL/POVL	0.85	1.09	0.98	0.06
P. bimaculatus		0.35	1.09	0.95	0.09
P. dorsalis	PVL/SL	0.45	0.52	0.49	0.02
P. bimaculatus		0.26	0.54	0.48	0.03
P. dorsalis	POVL/SL	0.48	0.55	0.50	0.02
P. bimaculatus		0.47	0.74	0.51	0.03
P. dorsalis	PPL/POPL	0.33	0.47	0.40	0.03
P. bimaculatus		0.25	0.37	0.31	0.03
P. dorsalis	PPL/SL	0.24	0.32	0.29	0.02
P. bimaculatus		0.20	0.27	0.24	0.01
P. dorsalis	POPL/SL	0.68	0.77	0.71	0.02
P. bimaculatus		0.71	0.82	0.76	0.02
P. dorsalis	CFL/SL	0.17	0.33	0.25	0.04
P. bimaculatus		0.12	0.31	0.21	0.04
P. dorsalis	CFL/CSPR	0.37	1.17	0.64	0.16
P. bimaculatus		0.35	1.50	0.69	0.26
P. dorsalis	HCPD/LCPD	0.55	0.90	0.68	0.06
P. bimaculatus		0.38	1.81	0.63	0.17

min-minimum; max-maximum; std-standard deviation

Table 8: Dichotomous key to separation of 15 *Puntius* species in Sri Lanka

1 Lateral line complete 6
– Lateral line incomplete 2
2 Number of barbels 0 3
– Number of barbels 1 pair .... *P. titteya*
3 Position of mouth terminal 4
– Position of mouth sub terminal 5
4 Transverse scales 3.5/2.5 .... *P. bandula*
– Transverse scales 3.5/3.5.. *P. cumingii*
5 Transverse scales 3.5/2.5 .... *P. ticto*
– Transverse scales 3.5/3.5.. *P. vittatus*
6 Nature of dorsal fin spine smooth 7
– Nature of dorsal fin spine serrate 12
7 Number of barbels 0 or rudimentary 8
– Number of barbels 1 pair 9
– Number of barbels 2 pairs .... *P. martenstyni**
8 Position of mouth terminal .... *P. singhala**
– Position of mouth sub terminal .... *P. srilankensis*
9 Position of mouth terminal; transverse scales 4.5/2.5 ...*P. singhala**
– Position of mouth sub terminal 10
10 Transverse scales 5.5/3.5 .... *P. chola*
– Transverse scales 3.5/3.5,3.5/2.5,4.5/2.5, or 4.5/3 11
11 Ratio between dorsal fin length and standard length 0.14-0.25 (mean 0.16); ratio between head length to standard length 0.26-0.32 (mean 0.29); Transverse scales 3.5/2.5, 4.5/2.5, or 4.5/3 .... *P. dorsalis*
– Ratio between dorsal fin length and standard length 0.09-0.16 (mean 0.12); ratio between head length to standard length 0.19-0.28 (mean 0.23); Transverse scales 3.5/3.5, or 3.5/2.5 ...... *P. bimaculatus*
12 Number of barbels 0 .... *P. nigrofasciatus*
– Number of barbels 2 pairs 13
13 Position of mouth terminal 14
– Position of mouth sub terminal .... *P. asoka*
14 Transverse scales 4.5/3.5, 5.5/3.5, or 5/3.5.................... *P. sarana*
– Transverse scales 5.5/2.5, 4.5/2.5, or 4.5/3 15
15 Post dorsal scales 11-13 ; transverse scales 5.5/2/5, 4.5/2.5, or 4.5/3 .... *P. pleurotaenia*
– Post dorsal scales 14-16 ; transverse scales 5.5/2.5 .... *P. martenstyni**

*Keyed in two places. Explanation in the Discussion

DISCUSSION

Previous studies[34,35] have shown that morphometric characters are often more suitable than meristic characters for describing intra-specific differences. In another study Ihssen *et al.*[36] stated that the discrete nature of meristic data contributed to low ability to discriminate among *Halobatrachus didiactylus* populations. The present study focused discrimination among species and has shown that variation in meristic characters combined with coded characters can be more effective than morphometric characters for differentiating 15 *Puntius* species (Figures 3 and 4). As meristic counts are discrete in nature, they were efficient for developing a dichotomous key for *Puntius* species in Sri Lanka as they gave sharp demarcations between individual species. Some meristic characters overlapped among species however, and were therefore of limited use for distinguishing the species.

Of the 19 meristic characters included in the PCA (inclusive of coded characters), only six characters (nll, pom, ndfs, tr, nb and psds) were used in developing the dichotomous key and these characters could differentiate the 15 Sri Lankan *Puntius* species successfully. The characters can be scored easily, are distinct and had non-overlapping ranges among species (Table 5). Two species (*P. bimaculatus* and *P. dorsalis*) however, could not be fully differentiated using meristic characters in isolation. Combination with the diagnostic morphological characters permitted full separation of all species. Length ratios were employed to remove individual size effects[37], and in combination with the meristic characters distinguished all species and so were incorporated in the key (Table 7).

In step 11 of Table 8 separation of *P. bimaculatus* and *P. dorsalis* were based on two morphological characters (DFL/SL and HL/SL) and one meristic (number of transverse scales) character. HL/SL shows overlaps in the range of 0.26-0.28 and DFL/SL in the range of 0.14-0.16 in these two species (Table 7). Number of transverse scales 3.5/2.5 were recorded in three individuals of *P. bimaculatus* (N=63) and six individuals (N=53) of *P. dorsalis*. A fish having number of transverse scales 3.5/2.5 and overlapping scores for DFL/SL and HL/SL therefore, limits the separation into a species. Analysis of data set of *P. bimaculatus* and *P. dorsalis* indicate that the possibility of this overlap is low, because a fish of 3.5/2.5 transverse scales recorded non overlapping scores for HL/SL or/and post dorsal length/standard length (PDL/SL). Similarly a fish having overlapping values for HL/SL and/or PDL/SL can be differentiated based on having non overlapping scores for transverse scales. In general therefore, having overlapping values for all three characters is unlikely and these characters individually or in combination could be used or to separate the two species.

Formal description of new species is generally based on data from only a few specimens and hence is not able to represent all intra-specific variation. Intra-specific variation associated with geographical and environmental diversity is well documented in fishes[38,39]. The comparatively large sample sizes per species (except for highly threatened or rare species) used here collected from 38 different sites in five major rivers covered a broad geographical range and represented the majority of variation present in the characters assessed.

Data for meristic and coded characters obtained for the 15 *Puntius* species examined in the present study were more or less comparable with the majority of earlier studies [3,4,19,21] in the literature. A difference was evident however, in counts for *P. dorsalis*, *P. pleurotaenia* and *P. sarana* (Table 8). This difference may result from intraspecific geographical variability across the distribution of the species or presence of sub species. Variation in morphological characters in *Puntius* species has been recorded with altitudinal differences in Sri Lanka[40]. Presence of sub species in *Puntius* species has also been reported [3]. A difference in the description of *P. singhala* in the present study compared with earlier reports showed that this species had a terminal mouth (Table 2b). Previous reports suggested that *P. singhala* had a sub terminal mouth[3,22]. The diet of *P. singhala* consists of filamentous algae, crustaceans and diatoms[41] that are generally found in the water column and column feeders often are characterised by a terminal mouth. Descriptions of mouth positions can be subjective however, so this may have contributed to the apparent inconsistency.

In the present study, *P. singhala* individuals were found with no barbels or with a single pair of barbels. According to previous studies[22] this species possess a single pair of barbels but the buccal area also contains many papillae. Therefore, individuals identified as possessing no barbels may possess a pair of rudimentary barbles that may have been be concealed in the papillated area. In the present study *P. martenstyni* was recorded as possessing a serrated dorsal fin spine during their younger stages and smooth dorsal fin spine when mature. Pethiyagoda[4] has also recorded this difference. Therefore, to avoid any misclassification, this variation was considered when developing the key and there are two identification points for *P. singhala* and *P. martenstyni* marked with* in Table 8.

Apart from the 15 *Puntius* species considered in the present study another species, *P. amphibius* has been recorded in Sri Lanka. *P. amphibius* was not included in the current study however, as specimens of this species were not found at any of the 38 sites sampled. *P. amphibius* was first recorded in 1912 by Dunker and was listed as a freshwater species in Sri Lanka [4,19,21]. According to recent studies[23] *P. amphibius* is not found in Sri Lanka but has been misidentified by different authors because it possesses similar morphology to other *Puntius* species.

The dichotomous key developed in this study shows similarities with a key developed by Deraniyagala[19] to identify *Puntius* species in Sri Lanka. Endemic species, *P. bandula*, *P. srilankensis, P. martenstyni* and *P. asoka* were not recorded that time. Nature of lateral line, ndfs, pom and tr were the main characters used by Deraniyagala[19] to develop his key. In the present study, these characters were among the main characters that contributed to principal components and separating taxa were therefore important for developing the new key. In addition, markings (bands and spots of different shapes and sizes) on the body were also traits considered by Deraniyagala[19]. These characters though important in identification of fresh or live specimens, can be lost or modified when specimens are preserved and these features were not considered here.

In multivariate morphological comparisons there are two independent components, namely size and shape. Species grouped in general in the left half of the PCA biplot possessed comparatively deeper and shorter bodies and species that grouped in the right half possessed slender and longer bodies. The two different size morphs showed (Figure 4) two different body forms; fusiform (slender and long) and ovate (deeper and short). This shape variation may result from adaptation to the different aquatic habitats they occupy [42-44] and also to their feeding habits[4,45].

Although morphometric variables had less power to differentiate the *Puntius* species when compared with meristic characters, they could differentiate the 15 species to a considerable level (Figure 3). Accordingly, *P. pleurotaenia*, *P. martenstyni* and *P. bimaculatus* grouped above the PC1 axis formed the slender and long bodied group. *P. ticto*, *P. titteya*, *P. vittatus*, *P. nigrofasciatus* and *P. chola* grouped below the PC1 axis and formed the deep and short bodied group. The remaining species possessed intermediate morphology and could not be differentiated using these characters.

The results show that meristic characters with coded variables are more effective than morphometric characters for discriminating the 15 *Puntius* species. To identify a *Puntius* individual at the species level using the key developed here requires only 2 to 6 steps. The steps need to be followed in a precise manner. In general, this key can assist accurate quantification and assessment of the genus *Puntius* in Sri Lanka and contribute to their long term conservation.

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