

Geometric morphometrics species discrimination within the Genus *Nemipterus* from Malaysia and its surrounding seas

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Abstract. Imtiaz A, Md Naim D. 2018. *Geometric morphometrics species discrimination within the Genus Nemipterus from Malaysia and its surrounding seas. Biodiversitas 19: 2316-2322.* High demand of fish protein diet and exposure of marine fish to a challenging marine environment has increased the importance of advanced fisheries research especially in Malaysian waters, particularly research related to taxonomy, biology, diversity, population structure as well as phylogenetic histories of commercially important marine fish species to ensure continuous supply of fish and fish products. This current research aimed to explore the potential of geometric morphometrics method to discriminate among taxa of genus *Nemipterus* through body size and shape differences. A total of 171 individuals representing ten species of genus *Nemipterus* collected from Malaysia and its surrounding seas were examined. The resulting homologous landmarks were subjected to generalized procrustes analysis followed by principal component analysis. Principle component scores were further investigated to cross-validated discriminant analysis to determine the efficacy of landmarks in discriminating by taxon. Our finding shows that species within the genus *Nemipterus* can be differentiated using geometric morphometrics method as strongly depicted by canonical variance analysis. This current study is the first report utilizing geometric morphometrics approach conducted on the genus *Nemipterus* from Malaysian waters.

Keywords: Geometric, morphometric, *Nemipterus*, species discrimination

INTRODUCTION

Family Nemipteridae is one of the most taxonomically complex families of marine fish with a broad range of characteristic traits among and within genus (Russell, 1990). Members of this family live near coral reefs and feed on small invertebrates (Edwards 1992). There are altogether five genera within this family. In all genera, the differences in the anterior part of the body (especially head region) are highly pronounced. The genus *Nemipterus* is discriminated by having a slightly compressed small to moderate body size with variable streaks on fins and trunk and contributes the maximum number of species. Furthermore, it is different from the other genera due to its variable body color patterns that disappear or fade upon being brought out from its natural environment. Its canine teeth are present in the form of bands and the presence of three transverse rows of scales on the operculum is a unique feature that only can be seen in species of this genus (Ambak et al. 2012).

Due to the dependency on marine food in Malaysia, a trend of overexploitation has been observed in many marine fisheries (Ahmad et al. 2003). The Nemipterids or threadfin breems are among one of the most highly landed fish group. According to the Malaysian Fisheries annual data (Department of Fisheries 2013) and Teh (2017), until the year 2010, there have been considerable decreases in fish landing as compared to previous years. Ambak et al. (2012) reported that the fish landings in Malaysia consist of mixed catches with a variety of Nemipterids pooled as a

single group. Color patterns and various other obvious morphometric characters are key parameters for segregation of species in family Nemipteridae (Russell 1990), but these are only accurate in freshly caught specimens. Due to the lack of expertise in taxonomic identification at the landing sites, the mixed catches are rarely segregated into separate species. Thus, in Malaysian markets almost all threadfin breems are locally identified as 'kerisi'. This would be problematical in any management strategies, as the species cannot be prioritized into those that are at risk of decline as compared to those at healthy levels of population sizes (Imtiaz et al. 2016).

This current research aimed to explore the potential of geometric morphometrics to discriminate among taxa of genus *Nemipterus* through body size and shape differences and to access patterns of diversity among members of this genus with reference to current taxonomic classification.

MATERIALS AND METHODS

Sample collections and study area

Fishes from family Nemipteridae were randomly collected from three main geographically important major oceans/seas, i.e., Straits of Malacca (connects the Indian Ocean, IO with the South China Sea), South China Sea (marginal sea of the Pacific Ocean) and the Arabian Sea (AS). These were further divided into seven marine regions (Figure 1) namely Indian Ocean, Straits of Malacca (M), East South China Sea (ESCS), North South China Sea

(NSCS), West South China Sea (WSCS), Sulu Sea (SS), and Celebes Sea (CS). A total of 15 landing sites were chosen from these marine regions for sampling. These include four landing sites of West coast of Peninsular Malaysia; Batu Lanchang, Penang (M); Kuala Kedah, Kedah (M); Kuala Perlis, Perlis (M); Lumut, Perak (M) representing diversity from Straits of Malacca (M), six landing sites from Malaysian Borneo; Kota Kinabalu (ESC), Tawau (CS), Kudat (SS), Sandakan (SS) all in Sabah; Kuching (ESCS) and Mukah (ESCS) in Sarawak and two landing sites from East coast of Peninsular Malaysia (Chendering and Marang, Terengganu (WSCS), two landing sites from Vietnam; Bac Lieu, Can Tho,

(NSCS), all these locations representing diversity of South China Sea (Pacific Ocean) and a single landing site from Pakistan (Karachi) (AS-IO) representing diversity of the Indian Ocean (Figure 1).

Samples from Malaysia were transported to the Molecular Ecology Laboratory, School of Biological Sciences, Universiti Sains Malaysia in a cool box. The specimens were cleaned with running water upon arrival to the lab, drained and placed with the left side on a flat surface with black background for optimum visibility. To capture the proper insertion and origin of fins, all fins were erected using pins (Figure 2).

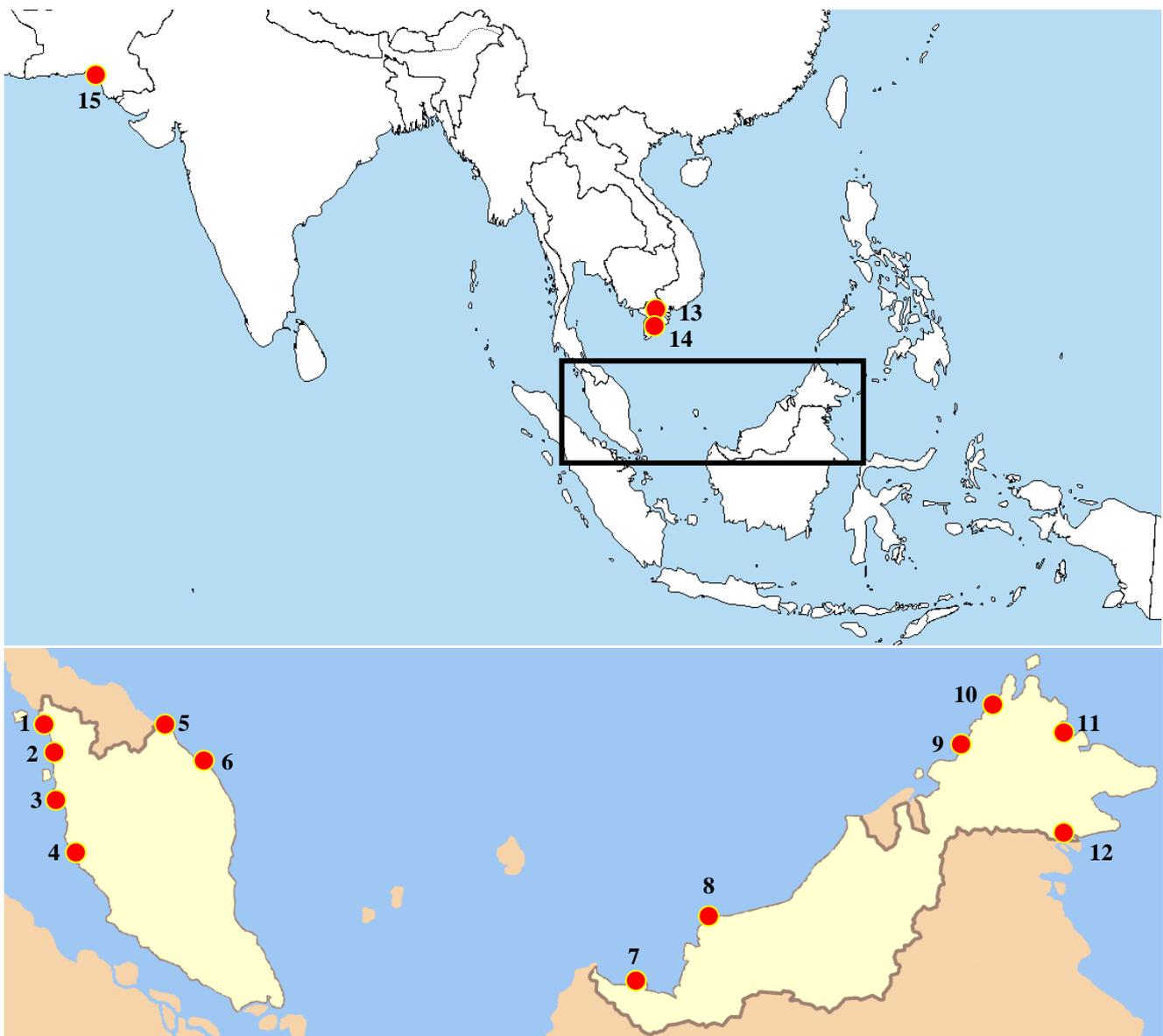


Figure 1. Sampling sites of Nemipterids along the coasts of Malaysia and Neighbouring oceans/seas. Strait of Malacca: 1. Kuala Perlis, Perlis, 2. Kuala Kedah, Kedah, 3. Batu Lanchang, Penang, 4. Lumut, Perak. South China Sea: 5. Marang, Terengganu, 6. Chendering, Terengganu, 7. Kuching, Sarawak, 8. Mukah, Sarawak 9. Kota Kinabalu, Sabah, 10. Kudat, Sabah, 11. Sandakan, Sabah, 12. Tawau, Sabah, 13. Can Tho, Vietnam, 14. Bac Lieu, Vietnam. Indian Ocean: 15. Karachi, Pakistan.

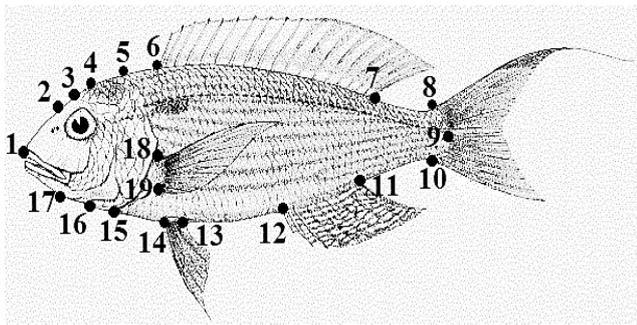


Figure 2. Landmarks location on fish body used to analyze the body shape variations in genus *Nemipterus* (refer to Table 1)

Table 1. Dataset showing landmarks and description of variables

No.	Landmarks	Description of variables
1	1-2	Anterior tip of snout to anterior tip of eye orbit
2	2-3	Anterior tip of eye orbit to middle of eye
3	3-4	Middle of eye to end of eye orbit
4	4-5	End of eye orbit to end of cranium
5	5-6	End of cranium to origin of dorsal fin
6	6-7	Origin of dorsal fin to end of dorsal fin
7	7-8	Dorsal fin insertion to insertion of caudal fin
8	8-9	Insertion of caudal fin to caudal Peduncle
9	9-10	Caudal peduncle to insertion of lower lobe of caudal fin
10	10-11	Insertion of lower lobe of caudal fin to insertion of anal fin
11	11-12	Insertion of anal fin to origin of anal fin
12	12-13	Origin of anal fin to insertion of pelvic fin
13	13-14	Insertion of pelvic fin to origin of pelvic fin
14	14-15	Origin of pelvic fin to base of cranium
15	15-16	Base of cranium to end of lower base of operculum
16	16-17	End of lower base of operculum to end of lower lip
17	17-1	End of lower lip to tip of mouth
18	18-19	Origin of pectoral fin to insertion of pectoral fin

Samples from other countries (Vietnam and Pakistan) were photographed in the field by standard digitizing protocol and the right side of the pectoral fin was clipped and preserved for further analysis. Traditional identification keys (Russell 1990) were used to observe meristic and morphometric characters for confirmation of genera. Only individuals identified as a member of a species from genus *Nemipterus* were then subjected to geometric morphometric analysis.

Geometric morphometric analysis of data sets: *Body shape*

The x and y coordinates of the landmarks was recorded using the tps Dig software v2.10 (Rohlf 2006) on the digital images that were used as a baseline data for further analysis. The differences in shape dimensions due to variations of angle in digitizing images is reduced by using of MorphoJ v1.02h (Klingenberg 2011) that configure landmarks into procrustes superimposition and generates a consensus configuration as described by Zelditch et al. (2004).

The homologous landmarks were chosen along the entire length of fish in order to record maximum variations in all samples examined. Basically, 19 homologous landmarks were chosen on digitized images of all samples so that the landmark configurations on all images describe the same positions (Figure 2, Table 1). The procrustes superimposition is an important part of geometric morphometric because it ensures the minimal differences among landmarks chosen on different images.

The procrustes superimposition removes the degree of freedom from landmark configuration of shape data and also rotates it along its axis to scale all images to get a centroid size. The centroid size is the sum of distances of all configured landmarks from the center of the body and is used to plot configured landmarks in Kendall's space shape (Kendall et al. 1983). The procrustes distance is the distance attained from the square root of sum of squared distances between corresponding superimposed configured landmarks (Slice 1998). A consensus of configured coordinates was also made to show the exact seam points between curves of configured landmarks and was used with procrustes coordinates in PCA analysis to record variations in body shape. Procrustes coordination is necessary to conduct PCA because it simplifies observations in quantitative data and is widely used currently in morphometrics study (Verbeek et al. 2002).

The outlier is any data that deviates such that it is not part of the group. It is diagrammatically presented by a deviation curve with cumulative distribution of difference in entire landmarks positions from average body shape when data is projected in MorphoJ v1.02h (Klingenberg 2011). The quality of landmark data can be assessed with accumulation curve. High deviation in landmark of any sample from mean shape can be traced back and is recommended to be removed from dataset for further analysis. Finally, wireframe was constructed by linking landmarks with each other, measured and recorded in order to analyze the shape variations.

Data analyses

Principal Component Analysis (PCA)

A PCA was conducted in MorphoJ v1.02h (Klingenberg 2011) by generation of the matrix of eigenvectors (B) and eigenvalues (L) from disintegration of covariance matrix (S) (the matrix consisting of shape variables) so that $S = BLB$. The eigenvectors matrix (B) is important to transform the original variables (X) into a set of new variables (Y) of principal components so that $Y = XB$. The change in body shape was done by principal wrap analysis (PWA) to minimize the angle of variations by generation of mean values and the shape changes are observed through the various principal components produced. The analyses were conducted according to species and the mean scores for each species were utilized to perform principal component analysis and all subsequent analyses. Significant eigenvalues from various PCs were used to address the number of variations in different PCs.

Canonical Variate Analysis (CVA)

The canonical variate analysis (CVA) was performed on centroid size to explain discriminations among and within taxa. MorphoJ v1.02h (Klingenberg 2011) program was used along with tps series v2.10 (Rohlf 2010) to analyse data and the procrustes distance of mean shape was used to conduct cluster algorithm for interpreting phenetic relationships. The CVA used covariance matrix among groups (B) and premultiplies with inverse of covariance matrix within group (W^{-1}) to transform it into canonical variate so that $W(W + B)^{-1}$ projected different groups separately in canonical space. The canonical space was different from the original variables space because the W was transformed from proportional to identity matrix and the distances in the transformed spaces was calculated as Mahalanobis distances that was used to measure the differences among species. High values of Mahalanobis distances show greater degree of discriminations and high rate of correct classification (McGarigal et al. 2000). The Wilk's lambda (λ) value is used to assess the effectiveness of CVA and is given as: $\lambda = W/T = W/W + B$; where W is sum of squares within groups and B is total sum of squares. The Discriminant Function Analysis (DFA) was also conducted on all species within genus *Nemipterus* to record their morphological differences based on centroid size.

RESULTS AND DISCUSSION

Samples collection

A total of 210 samples of family Nemipteridae were collected from the Malaysian waters and its surrounding seas as shown in Figure 1. Of these, 171 individuals were identified morphologically into ten different species from genus *Nemipterus* namely *N. tambuloides* (n= 7), *N. bipunctatus* (n= 13), *N. peronii* (n= 4), *N. thosaporni* (n= 10), *N. marginatus* (n= 13), *N. nematophorus* (n= 8), *N. nemurus* (n= 4), *N. hexodon* (n= 14), *N. furcosus* (n= 45), and *N. japonicus* (n= 53).

Shape variations and patterns of body size in *Nemipterus*

The body shape and size in geometric morphometrics are key methods to record morphological variations in particular, shape and size variations and also to assess the relationships among taxa on the basis of changes in body shape (Darlina et al. 2011; Openshaw and Keogh 2014). Costa and Cataudella (2007) suggested that geometric morphometrics has the ability to discriminate even among closely similar species of fish. In this present study, a total of 18 variables were generated from 19 homologous landmarks and were used to differentiate the taxa based on variation in body shapes among 10 species within genus *Nemipterus* (Table 1, Figure 2).

The choice of maximum number of landmarks increases the sensitivity of data so that any minimal differences in body shape could also be recorded throughout the entire length of fish samples. Among the 18 variables, ten variables addressed the shape of anterior region (i.e. head, mouth pectoral fin, body depth). These landmark variables were able to fully discriminate among species of genus

Scolopsis in morpho-space of PCA (other studies by authors; data not shown). However, in threadfin breams of genus *Nemipterus*, the PCA analysis showed a weak differentiation and variations in body shape were not strong enough to fully discriminate the species within this genus. This was further corroborated by the PCA analysis conducted on all individuals examined, which revealed that more than 30 components but with low eigenvalues were resulted from the analysis. The first component with highest variance of 56.87% had an eigenvalue of less than 0.22% (Table 2). This indicates a low significance because eigenvalue > 0.3% is considered as significant (Webster and Sheets 2010; Lombarte et al. 2012). However, the first four PCs showed 56.87% (head, body depth, pectoral fin, body size; PC1), 11.17% (head, body depth, caudal fin; PC2), 6.38% (head, caudal fin; PC3) and 5.55% (head, pectoral fin; PC4) with a combined variance of approximately 80.00% to explain body shape variation in two dimensions (Table 2). The weak variations of body shapes in *Nemipterus* was clearly demonstrated by the overlapping patterns in the scatterplot of PC1 versus PC2 (Figure 3), with a very low eigenvalue (< 0.02) that was not significant to discriminate among species within genus on the basis of mean body shape (Figure 3).

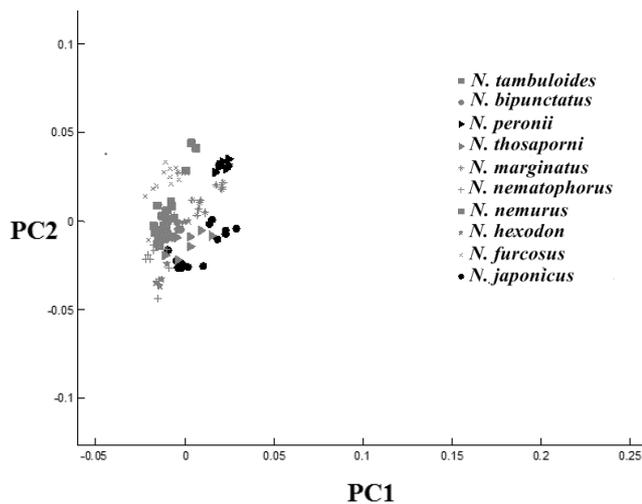
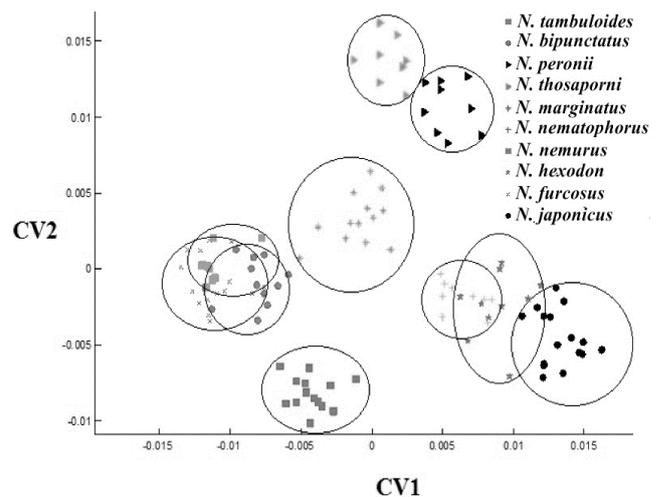
The findings in this study are similar with Claverie and Wainwright (2014) who reported that the first four PCs recovered 75.3% of shape variations in various families of reef fishes and also suggested that in reef fishes, body elongation and fin positions are primary sources of discrimination among the species. Likewise, Geiger et al. (2016) highlighted that the first four PCs addressed a total of 81.3% of body shape variations in the hybrid complex of *Barbus* sp. due to differences in body depth. Thus, the variations in head and body shape detected in this study is influenced by the presence of different species in variable marine zones specifically on the basis of availability of prey.

The variations among species within genus *Nemipterus* were well explained in CVA analysis (Figure 4). The analysis showed that *N. japonicus*, *N. marginatus*, *N. hexodon* and *N. furcosus* were closely related to each other on CV1 but *N. furcosus* has separated from the other group on CV2. Similarly, *N. nemurus*, *N. nematophorus* and *N. thosaporni* form a closely related group on CV1 but *N. nemurus* is separated from the other two by CV2. Likewise, *N. bipunctatus*, *N. tambuloides* and *N. peronii* were grouped together in CV1 but separated on CV2. This analysis provides a better resolution for intra-genera discrimination within *Nemipterus* compare to the PCA.

The body shape variations from CVA partial warp score shows a maximum variation in average body shape with elongated body in CV1 (Figure 5). Meanwhile, CV2 described more variations in head region. This pattern was also obvious in CV4, however, the variations in head region seems more significant in CV2 compared to CV4. CV3 contributed to a maximum rate in the body stretching that resulted in body elongation (Figure 5). Obviously, overall variation was based primarily on the body depth, head orientation and the body length that plays an important role in identification of *Nemipterus* as depicted by CVA.

Table 2. PCA scores of variation among groups, scaled by the inverse of the within-group ($P < 0.05$)

PCs	Eigen values %	Variance %	Cumulative %	Changes in body shape
PC1	0.224	56.875	56.875	Head, body depth, pectoral fin, body size
PC2	0.0442	11.176	68.052	Head, body depth, caudal fin
PC3	0.0252	6.382	74.434	Head, caudal fin
PC4	0.0219	5.559	79.993	Head, pectoral fin
PC5	0.0172	4.365	84.357	Head, Caudal fin, overall body shape
PC6	0.0092	2.326	86.683	Head, Caudal peduncle, overall body shape
PC7	0.0088	2.148	88.831	Head, Trunk stretch, overall body shape
PC8	0.0074	1.883	90.714	Overall body shape
PC9	0.0062	1.573	92.287	Overall body shape
PC10	0.0057	1.441	93.728	Overall body shape
PC11	0.0039	0.993	94.721	Overall body shape
PC12	0.0031	0.802	95.523	Overall body shape
PC13	0.0027	0.699	96.222	Overall body shape
PC14	0.0020	0.522	96.745	Overall body shape
PC15	0.0017	0.452	97.197	Overall body shape
PC16	0.0016	0.417	97.613	Overall body shape
PC17	0.0013	0.339	97.952	Overall body shape
PC18	0.0012	0.304	98.256	Overall body shape

**Figure 3.** Principal Components Analysis of *Nemipterus* shows PC1= 56.87% and PC2= 11.17%, accounting for 68.05% of total variation in 10 species**Figure 4.** Canonical Variate Analysis of genus *Nemipterus* showing CV1= 56.87%, CV2= 11.17%, accounting for 68.05% of total variation in ten species

It is well documented that morphology of many species is related with their habitat (Taylor 2000; Jonsson and Jonsson 2001). The findings of the present study are similar with the findings by Park et al. (2001) who conducted a morphometric study on *Rhynchocypris* sp. and reported that the changes in head morphology is important to discriminate the species. They further explained that changes in head morphology relates with habitat and availability of prey. Our findings are also congruent with the geometric morphometric study by Bilici et al. (2015) on two closely similar species (*Cyprinus kais* and *C. macrostomus*) from family Cyprinidae. Their research reveals that the variations in body shape have significantly

discriminated between the two species. Furthermore, variations in head is more pronounced between the two species where *C. kais* has shorter head (in height) in which insertion of dorsal fin is a bit backwardly directed and has a narrow caudal peduncle as compared with *C. macrostomus*. The differences in head shape of *Nemipterids* as observed in the wireframe PC zone (Figure 5), points out to the possibility of variable preference for prey distributed at various zones of the ocean. Similar result was also reported by Love and Chase (2009), which suggested that head shape differentiations in scup, *Stenotomus* sp. are due to the differences in feeding habits.

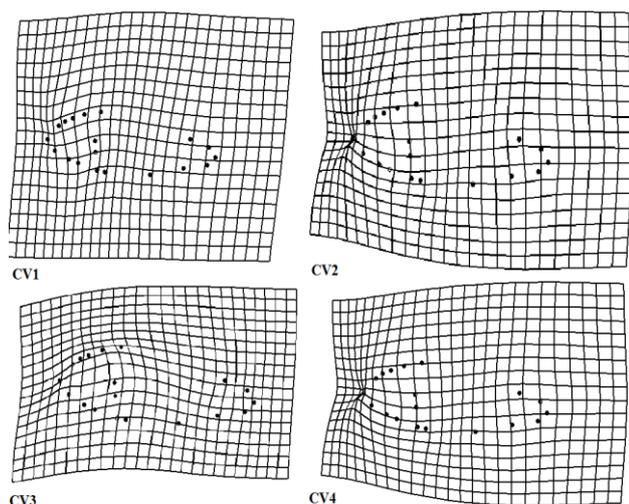


Figure 5. Visualization of shape variation along CV1 to CV4 by partial warp along wire frame showing average shape variations. CV1 contributing a maximum variation along head region. CV2 shows body stretching to maximize body depth and fusiform body. CV3 contributed squeezing of head to form a more streamlined position. CV4 contributed to variations in abdominal region

Pairwise differences among taxa

In this study, *N. peronii* was the most diverge from all other species (Table 3). The maximum value based on Procrustes distance was 7.2% between *N. peronii* and *N. nematophorus* whereas minimum distance of 2.3% was recorded between *N. japonicus* and *N. hexodon*. The later indicates that both species are highly similar in mean body shape whereas the p value for all distances was < 0.01 which shows significance of the results. The maximum procrustes distance recorded in *N. peronii* is due to the reasons that the shape of this taxa is more diverged from the rest of the taxa. Russell (1990) reported *N. peronii* as a deep body fish of the genus *Nemipterus* as compared to other taxa in this genus. The results of Goodall’s test and F statistics are congruent with CVA procrustes distance and are shown with asterisk (*) in Table 3.

Our study shows a significant Procrustes and Mahalanobis distances among species within *Nemipterus* and this strongly suggested that each taxon represents a separate group. The changes in body shape are the basis for habitat diversity and ecological adaptations (Robinson and Wilson 1994). Marine ecosystem contains many zones that differ in several ecological characteristics such as sunlight penetration, food availability and water velocity (Brinsmead and Fox 2002; Adibah and Darlina 2014). The long and shallow body shape with very low body compression support adaptation in a habitat of coastal inshore benthic water habitat in *Nemipterus*. Nemipterids have adapted variable zones of ocean as well as multiple choices of prey that include small crustaceans, fish larva and also juvenile fish (Russell 1990). The presence of differences and ecological adaptations (feeding, swimming etc.) in body shape among closely related marine taxa present in variable marine zones provides evidence of divergences in their genes in molecular studies (Langerhans et al. 2003) that further helps to address phylogenetic question of a particular marine taxa.

Although the significant p value reflects the authenticity of geometric data but the presence of low sample size and less species are limitations of the current study. Ferdous (2013) received low success rate of only 22% to recover phylogenetic signals based on differences in body shape and adipose tissue in a geometric dataset of Family Cyprinidae. Furthermore, the number of individual for some species investigated in this study was limited with only four individuals (*N. peronii* and *N. nemurus*). It is also well understood that the quantitative data such as geometric morphometrics does not predict true phylogeny as what the molecular data can suggests (Imtiaz et al. 2017). However, the question of discrimination among taxa on basis of shape differences can be resolved on the basis of the geometric taxonomy as demonstrated in this current study.

To conclude, this is the first report of geometric morphometrics method conducted on the genus *Nemipterus* aimed to interpret body shape variations within member species of the genus. The results revealed that species of Nemipterids can be differentiated using geometric morphometrics. The body shape variations among the species are clearly shown by CVA analysis and play a key role in species discrimination of the genus.

Table 3. Canonical Variate Analysis (CVA) procrustes distances among different taxa of genus *Nemipterus*. An asterisk (*) shows the confirmation from Goodall’s F test and F statistics

Species names	<i>N. bipunctatus</i>	<i>N. furcosus</i>	<i>N. hexodon</i>	<i>N. japonicus</i>	<i>N. marginatus</i>	<i>N. nematophorus</i>	<i>N. nemurus</i>	<i>N. peronii</i>	<i>N. tambuloides</i>
<i>N. furcosus</i>	0.037								
<i>N. hexodon</i>	0.038*	0.044							
<i>N. japonicus</i>	0.049	0.053	0.023						
<i>N. marginatus</i>	0.053	0.049	0.052	0.043*					
<i>N. nematophorus</i>	0.048	0.057	0.031	0.041	0.053				
<i>N. nemurus</i>	0.039	0.039	0.047	0.054*	0.032	0.049			
<i>N. peronii</i>	0.065	0.038*	0.062	0.062	0.042	0.072	0.048		
<i>N. tambuloides</i>	0.031	0.039	0.033	0.042	0.041	0.061	0.032*	0.064	
<i>N. thosaporni</i>	0.051*	0.063	0.048	0.054	0.048	0.042*	0.048	0.059	0.052

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