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Carapace shape differences between subterranean and aboveground populations of *Macrobrachium sp.* from Marinduque, Philippines

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Abstract. Morphological variations in the carapace structure of the freshwater shrimp *Macrobrachium* sp. collected from the subterranean and aboveground river systems in Marinduque, Philippines were studied using geometric morphometric analysis. Sixteen (16) landmarks were established on the carapace structure of the samples using the MorphoJ software. Variations in carapace shape of the samples were explored using Principal Component Analysis (PCA) and translated to Procrustes coordinates. To compare the differences in carapace shape between the samples, pairwise permutation test Canonical Variate Analysis (CVA) was applied with 10,000 permutations. Discriminate analysis was also used to determine significant difference on the carapace shape. To determine manifestations of allometry, multivariate regression was employed with log of the centroid size as the independent variable and Procrustes coordinate as dependent variable (with 10,000 permutations). Results of the PCA showed some indications of variation in the shape of the carapace that occurred mostly on the rostrum, where the subterranean shrimps generally have longer rostrum, and the aboveground shrimps having a broader rostrum. It also showed that the aboveground samples have a broader carapace compared to that of the subterranean samples. Procrustes pairwise permutation tests using CVA between the carapace shapes of shrimps from the subterranean and aboveground rivers showed a significant difference (Procrustes distance= 0.0418, p=0.0022). Results of the discriminate analysis also showed that carapace of shrimps from the subterranean and aboveground rivers significantly differ (T-square= 3790.74, P-value [parametric] = 0.0064). The multivariate regression showed that the subterranean shrimp samples exhibited allometric relationship between the carapace shape and the long centroid while the aboveground samples did not exhibit allometric relationship. The significant allometry in subterranean shrimps is an indication that the population has a different growth pattern compared to that of aboveground river shrimps population.
Key Words: *Macrobrachium*, geometric morphometrics, allometry, carapace, subterranean, aboveground river.

Rezumat. Variații morfologice în structura carapacei crevеților de apă dulce *Macrobrachium* sp. colectați din rețelele cursurilor de apă subterane și supraterane din Marinduque, Philippines au fost studiate folosind analize geometrice și morfometrice. Șaisprezece (16) trăsături au fost identificate pe structura carapacei probelor folosind softul MorphoJ. Variațiile în forma carapacei probelor au fost analizate folosind Principal Component Analysis (PCA) și transformate în Coordinate Procrustes. Pentru a compara diferențele în forma carapacei, între probe, testul de permutare în perechi Canonical Variate Analysis (CVA) a fost aplicat cu 10000 de permutări. Analiza discriminată a fost de asemenea folosită pentru a determina diferențe semnificative în forma carapacei. Pentru a determina manifestări ale alometriei, regresia multivariabilă a fost utilizată cu *log* al dimensiunii centroide ca și variabilă independentă și coordonate procrust ca și variabilă dependentă (cu 10000 de permutări). Rezultatele PCA au prezentat semne de variație în forma carapacei, care au fost prezente mai ales la rostru, unde crevеții subterani au în general rostru mai lung, iar cei supraterani au un rostru mai larg. S-a constatat de asemenea că la probele supraterane carapacea este mai lată comparativ cu cele subterane. Testele de permutare în perechi Procrustes folosind CVA între formele de carapace ale crevеților din râurile subterane și supraterane au prezentat o diferență semnificativă (distanța Procrustes = 0,0418, p=0,0022). Rezultatele analizei discriminate au arătat de asemenea că diferă semnificativ carapacea crevеților din râurile subterane și supraterane (*T*-pătrat= 3790,74, valoarea- *P* [parametric] = 0,0064). Regresia multivariabilă a arătat că probele subterane prezintă o relație alometrică între forma carapacei și centroida lungă, în timp ce probele supraterane nu au prezentat relație alometrică. Alometria semnificativă la crevеții subterani este o indicație a faptului că populația are un model de creștere diferit, comparativ cu cel al populației de crevеți supraterani.
Cuvinte cheie: *Macrobrachium*, analize morfometrice și geometrice, alometrie, carapace, ape subterane, ape de suprafață.

Introduction. Caves and other subterranean environments are home to large numbers of endemic species which may include relictual taxa from an earlier age (Boulton et al 2003). This can be due to relatively stable environmental conditions in the caves. Sondag et al (2003) monitored temperature and water drip rates in tropical caves and have shown that while drip rates are related to rainfall, they remain very stable during raining season and temperature on the long term is stable. Page et al (2008) also suggested that hydrological and geological events that have created dispersal barriers have structured and isolated many of the populations into distinct geographic communities. Page et al (2008) have also shown that some of the subterranean species of atyids descended from localised surface species.

Macrobrachium species are among the distinct faunas found in the subterranean and aboveground river systems in Marinduque, Philippines. In spite of their potential economic importance and environmental benchmarking potential, it has not been studied.

Macrobrachium species are amphidromous, where they spend their ontogeny in different types of habitats. Zimmermann et al (2012) suggests that amphidromous organisms returning to freshwaters after a marine larval phase, having developed physiological and developmental flexibility, facilitates further adaptation to different environment. The geometric morphometric analysis of *Macrobrachium australe* carapace structure shape variation with samples coming from six populations from Reunion Island freshwaters revealed the occurrence of two morphotypes corresponding to the lentic and lotic habitat. Individuals sampled from lotic habitat exhibit a thick carapace armed with shorter, robust and straight rostrum, while individuals from lentic habitat have a slender carapace armed with a thin long rostrum with upward orientation. This morphological modifications during their ontogenesis often times have lead to misidentification. In a study developed by Rossi & Mantelatto (2013), phylogenetic analysis of the morphologically variable and widely geographically distributed *Macrobrachium olfersii* showed a lower intraspecific divergence than interspecific. Torres et al (2014) conducted a geometric morphometric analysis of *Macrobrachium borellii* at a microgeographical scale in a floodplain system where the variations in body shape within and between populations of the species were influenced by the population dynamics and the characteristics of the flood plain system.

This study compares the geometric morphometrics characteristics of the carapace structure of *Macrobrachium* species collected from subterranean and aboveground river systems and provide the platform for an evolution study with environmental implications. Part of the interest in conducting this study is to establish morphological characteristics of *Macrobrachium* species living in habitats once affected by mine tailing. Marinduque island had experienced one of worst mine tailing disasters in the Philippines (Plumee et al 2000). From a geological perspective, Marinduque Island was once a part of the Luzon island but was separated due to plate subduction and drifting sometime between the Cretaceous and Cenezoic period (Zahirovic et al 2014). *Macrobrachium* species in Luzon mainland has been taxonomically described but not in Marinduque Island.

Methods. A total of 34 adult freshwater shrimps: 17 hypogeal and 17 epigeal were collected from Bagumbungan Cave at Brgy. San Isidro, Sta. Cruz, Marinduque and Kansurot River at Brgy. Tugos, Boac, Marinduque (Figure 1). Bagumbungan Cave is almost 1.8-kilometers in length with some subterranean water pools. The water pool level inside is influenced by rainfall wherein it gets flooded during heavy rains and is at a low level during the dry season. The water habitat inside the cave is deemed as lentic. No natural light is able to penetrate the cave. In the absence of photosynthetic activities, sources of food for the aquatic faunas and other living organisms inside are limited to the organic matter in the sediments brought in by the floods; droppings of bats; and decomposition of the remains of dead organisms inside. Kansurot river, on the other hand, is lotic. It has a continuous supply of water all year round. The river is enriched by organic matter from agricultural and domestic wastes from the upland and along the river communities.

Samples were all representatives of the genus *Macrobrachium* based on the taxonomic key by Chace & Bruce (1993). To prevent damage to the shrimps, the shrimps were collected using scoopnets and when possible by handpicking. The shrimps were preserved in 95% alcohol solution to facilitate the separation of the cephalothorax from the rest of the body. The cephalothorax structure was chosen for the geometric morphometric analysis because of its rigid anatomical piece, which does not deform easily and optimizes the taking of photograph for the morphometric analysis (Bissaro et al 2012). The images of the samples were captured using a digital camera with 10x optical zoom. Sixteen (16) anatomical landmarks were established and digitized using the tpsDig 2.17 software (Rohlf 2013) following with modifications the landmarks established by Torres et al (2014). The landmarks were limited to the left side carapace or the cephalothorax structure (Figure 2). The establishing of the landmarks and the analysis were done using the MorphoJ software (Klingenberg 2011).

Data Analysis. Shape components associated with position, rotation, translation and size were removed by a Procrustes fit procedure prior to any other analysis.

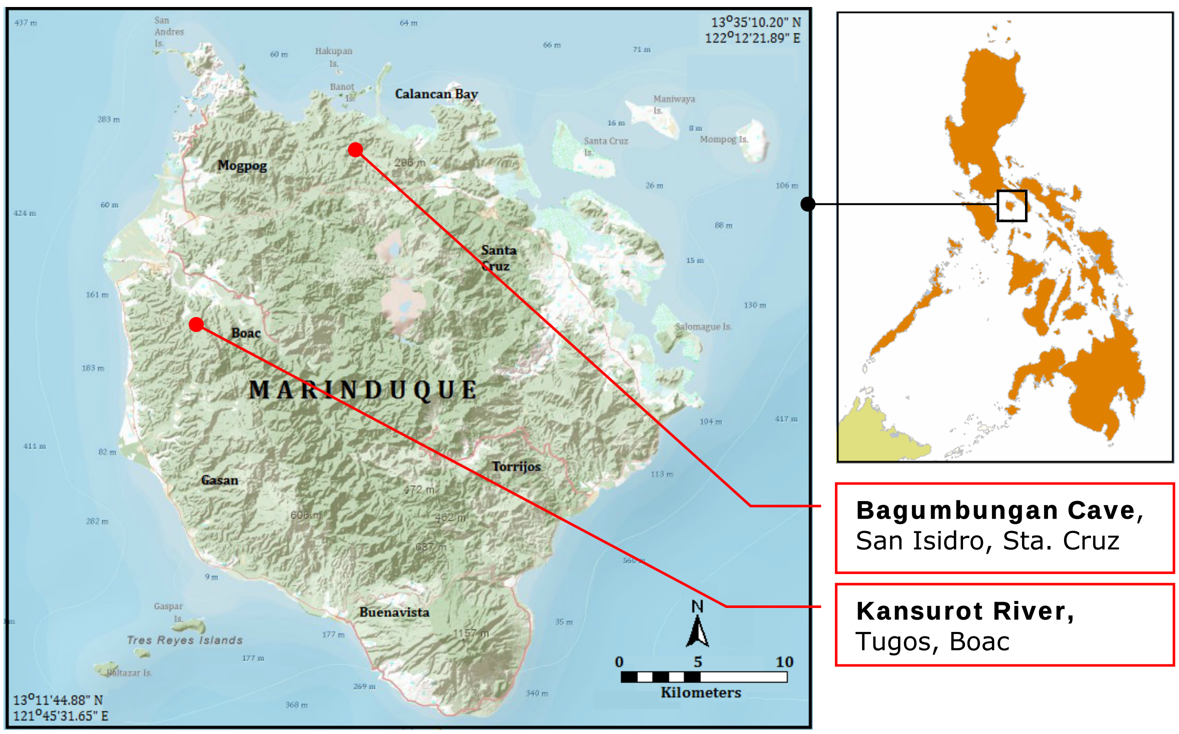

Figure 1. *Macrobrachium* shrimps sampling sites in Marinduque (modified from Mines and Geoscience Bureau Geological Database Information System generated maps).

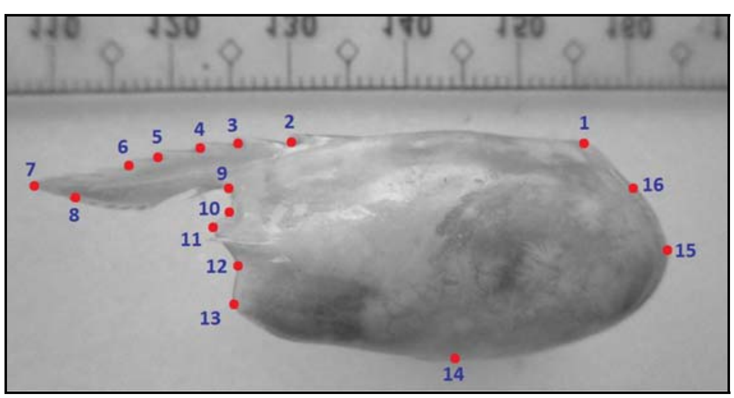

Figure 2. The left side of the carapace of the *Macrobrachium* sp. shrimp with the configuration of the 16 landmarks.

To explore variation in the carapace shape between the samples from subterranean and aboveground river, Principal Component Analysis (PCA) was applied to the Procrustes coordinates.

To compare the differences in carapace shape between the shrimp samples, Pairwise permutation test Canonical Variate Analysis (CVA) was applied with 10,000 permutations. Discriminate analysis was also used to determine significant difference on the carapace shape.

To determine manifestations of allometry, multivariate regression was employed with log of the centroid size as the independent variable and procrust coordinate as dependent variable (with 10,000 permutations).

Result. The subterranean shrimp samples exhibited allometric relationship between the carapace shape and the long centroid while the aboveground samples did not exhibit allometric relationship (Table 1).

Table 1

Intrapopulation multivariate regression (shape-log centroid size) with the carapace shape

Site	*p value*	*% predicted*
Cave	0.0272	17.62
Aboveground River	0.6344	4.29

Results of the principal component analysis showed some indications of variation in the shape of the carapace (Figure 3). As shown in this figure, the variations occur mostly on the rostrum where the subterranean shrimps generally have longer rostrum, and the aboveground shrimps having a broader rostrum. It also showed that the aboveground samples have a broader carapace compared to that of the subterranean samples.

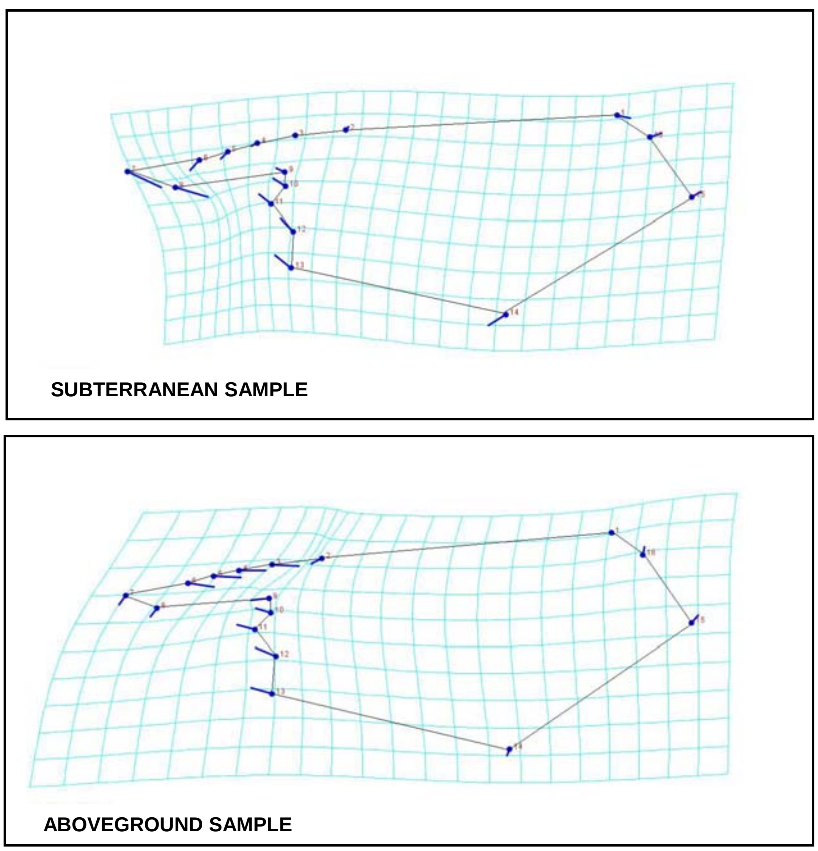

Figure 3. Grids representing the variation in the mean shape of the carapace from subterranean and aboveground river sampling sites.

Procrustes pairwise permutation tests with canonical variate analysis (CVA) between the carapace shapes of shrimps from the subterranean and aboveground rivers showed a significant difference (procruste distance= 0.0418, p=0.0022). Results of the discriminate analysis also showed that carapace of shrimps from the subterranean and aboveground rivers significantly differ (*T*-square= 3790.74, *P*-value [parametric] = 0.0064).

Discussion. The results of the geometric morphometrics analysis showed a significant difference on the shape of the carapace of samples representing subterranean lentic habitat and aboveground lotic habitat. This conforms to the findings of Torres et al (2014) and Zimmermann et al (2012) wherein the rostrum and the carapace of aboveground shrimps are broader compared to those found in subterranean river. This suggests the influence of water flow environment and morphology. Fluctuations in the water levels determine the level of connectivity between lentic and lotic communities, the water residence time in pools determines the rates of exchange of nutrients and the organism (Jose de Paggi & Paggi 2007).

The carapace morphology could also be influenced by the nature and availability of food in the habitat. Montoya (2003) found *Macrobrachium* species have some association with the roots of the water hyacinth (*Eichnornia crassipes*). *Macrobrachium* species are omnivorous and euryphagous where they feed on any food item it comes across especially when food is not in abundance (Jimoh et al 2011). The subterranean habitat in this study is characterized as devoid from any sunlight and therefore does not produce plant materials for food. *Macrobrachium* in the cave may have to rely with organic matter in the sediments brought in by the floods, droppings of bats and decomposition of the remains of dead organisms inside.

The significant allometry in subterranean shrimps may indicate that the subterranean population does have a different growth pattern compared to that of the aboveground river population which may be a function of age and sexual dimorphism (Jayachandran & Joseph 1985).

To establish whether the morphological variation is genetic or ecological morphs, genetic analyses are needed. The *Macrobrachium* species from the cave and the aboveground river system may have genetic variations but because they are prone to show plastic responses to environmental influences it may be just a phenotype variation. This may also be on the other hand an indication that the morphological characters are undergoing convergent evolution as they are under similar selective pressure (Schwander & Leimar 2011; Yang & Ranala 2010). This will also determine if the subterranean water habitat is connected with the aboveground river system or is indeed isolated as currently assumed.

The results of the study have also established a morphological benchmark for *Macrobrachium* to establish the long-term effects of the copper mine tailings in Marinduque Island after commercial mining activities have ceased operating.

Conclusion. Results of the geometric morphometrics in this study have shown significant difference on the carapace morphology of *Macrobrachium* species populations from subterranean and aboveground river habitats in Marinduque Island, wherein the rostrum and the carapace of aboveground shrimps are broader compared to those found in the cave. The results have provided the platform for further evolution and environmental study in area.

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