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Describing Variability in Mandible Shapes in Selected Workers of the Ant *Diacamma rugosum* (LeGuillou) 1842 (Hymenoptera: Formicidae: Ponerinae)

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Abstract

This study was conducted to determine mandibular shape variation in Diacamma rugosum which still retains the primitive faculties as solitary hunter and as a social worker. The specimens used in this study were collected from Initao National Park, Initao, Misamis Oriental and stored in 70% ethyl alcohol solution. Body size index measurements of each worker were taken. The mandibles of the ants were dissected from 60 randomly selected worker ants and photographed prior to Geometric Morphometric analyses. A total of 100 points were digitized from images of the mandibles using TpsDig ver. 2.12. The X and Y coordinates of the outline points were saved in Matlab format and were subjected to Relative Warp Analysis to remove non-shape components. Results showed that size-dependent shape variation was not observed in the solitary species D. rugosum. Pearson correlation values for shape variables against body size in D. rugosum ranged only from r = 0.006 to 0.197. These results show that age- and size- related changes in the shapes of the mandible may accompany task partitioning in ants and may be important in studying the evolution of sociogenesis in ants.

Keywords: Eusocial ants, *Diacamma rugosum,* solitary ant, mandible.

Introduction

Ants are social insects occupying diverse range of habitats with many species having no morphological differences between functional groups and the division of labour is based on behavioural differences[1]. All ants are mandibulate and within these groups the range of mandibular activities is extended to nest construction and defense of the colony, cut and transport of food and debris, transport of larvae and eggs, and to dig the rolls in the soil or/and wood[2]. Physical castes that are often labeled as minor, media, and major (soldiers) are based on size and have traditionally been defined with reference to peaks in the worker size-frequency distributions of polymorphic species. It is in this group however that typically the female is polymorphic, with an average colony consisting of two basic female castes, the queen (reproductive female) and the worker (sterile female). The queen and worker are connected by intermediate stages which are often stable enough to be recognized as distinct castes. Workers on the other hand may become subdivided into several additional castes[2] and morphological diversity within colonies has its basis in allometry[3].

Division of labor among working individuals in ants is a classical example of adaptive variation in population, like *Formica yenssensis*[4], *Amblyopone pallipes*[5], *Atta cephalotes*[6], *Ectatomma ruidum*[7], *Pachycondyla striata*[8]. Recently, attention has been directed towards the way in which workers actually performed a given task. The so-called task partitioning, whereby a piece of work is divided among two or more workers, like in the collection of a load of forage between a forager and a storer or a transporter has been recognized as an important form of work organization in social insects[9,10]. The aim of the present study is to examine task partitioning in both solitary hunters and social workers of *Diacamma rugosum,* a black, medium-sized ponerine ant about 8 - 10 mm long, with long antennae and the pygidium that terminates in a curved spine and are known predators on a variety of small invertebrates, including termites, and forage singly. The two workers are equally active both day and night forming a small colony from 50 to 100 individuals. They usually nest in open forests at lowland altitudes and apply a basic division of labour in the organization of the colony. Since the mandibles are used by ant workers in foraging behaviour and brood care, it is hypothesized that these structures are important in investigating if there exist differences in mandibular shapes between workers. Likewise, the study will determine if there is age polyethism based on size-dependent shape changes in the mandible using outline-based geometric morphometric analysis (GM). This methodology utilizes powerful and comprehensive statistical procedures to analyse shape differences of a morphological feature[10,11,12]. The techniques uses Procrustes distances to capture shape variation considered to be the most reliable method to determine geometric morphometric relationships among groups or taxa1[3-26]. Rohlf[27,28] developed the tps series of programs which performs the statistics and visualizations of geometric morphometrics and were used in the current study.

Methodology

Sample preparation: Specimens used in this study were collected at Initao National Park, Initao, and Misamis Oriental and was stored in 70% ethyl alcohol solution. Mandibles were dissected from 60 randomly selected worker ants (figure 1). Each pair of mandibles (figure 2a, b) was then photographed on Leica (L350) Stereomicroscope at 30X magnification dissecting microscope.

Morphometric analysis: Body size measurements of the workers were measured from the maximal outer orbital distance and the head length from the posterior margin of the head to the apex of clypeus[4].

Outline Analysis of mandible: A total of 100 points (figure 1D) were digitized from images of the mandibles using TpsDig ver. 2.12[26]. These points covered the internal and external margins of the mandible. These include the masticatory margin, basal margin, and mandulus. The X and Y coordinates of the outline points were saved in Matlab format and were subjected to Relative Warp Analysis to remove non-shape components using TpsRelw ver. 1.46[27]. Using the thin-plate spline equation and the standard formula for uniform shape components, a weight matrix (containing uniform and non-uniform shape components) from the alignned specimens were generated. The RW scores and morphometric measurements were then subjected to Multivariate Analysis of Variance (MANOVA) to test for shape differences in mandible topology in *D. rugosum* using the PAST software[28].

Results and Discussion

Size distribution estimates of Diacamma rugosum workers: Two size classes were observed from among the sixty randomly selected workers from the colony (figure 2). Workers with body size index ranging from 2.1-2.6 mm are the class size I. Workers under class size II have body size index of 2.7-3.0 mm. Based on the taxonomic description of *D. rugosum* as a member of the Ponerinae subfamily, the class size estimates are related to the nature of its worker. Class size I workers are classified as social workers which is said to engage with foraging and maintenance of the nest while class size II workers which is more likely to be larger than the social workers are said to engage in hunting and preying activities and classified as solitary hunters/hunters. Majority of species of ants has the tendency to give the larger workers a more precarious work which involves outdoor activities and the smaller workers the intranidal task[29].

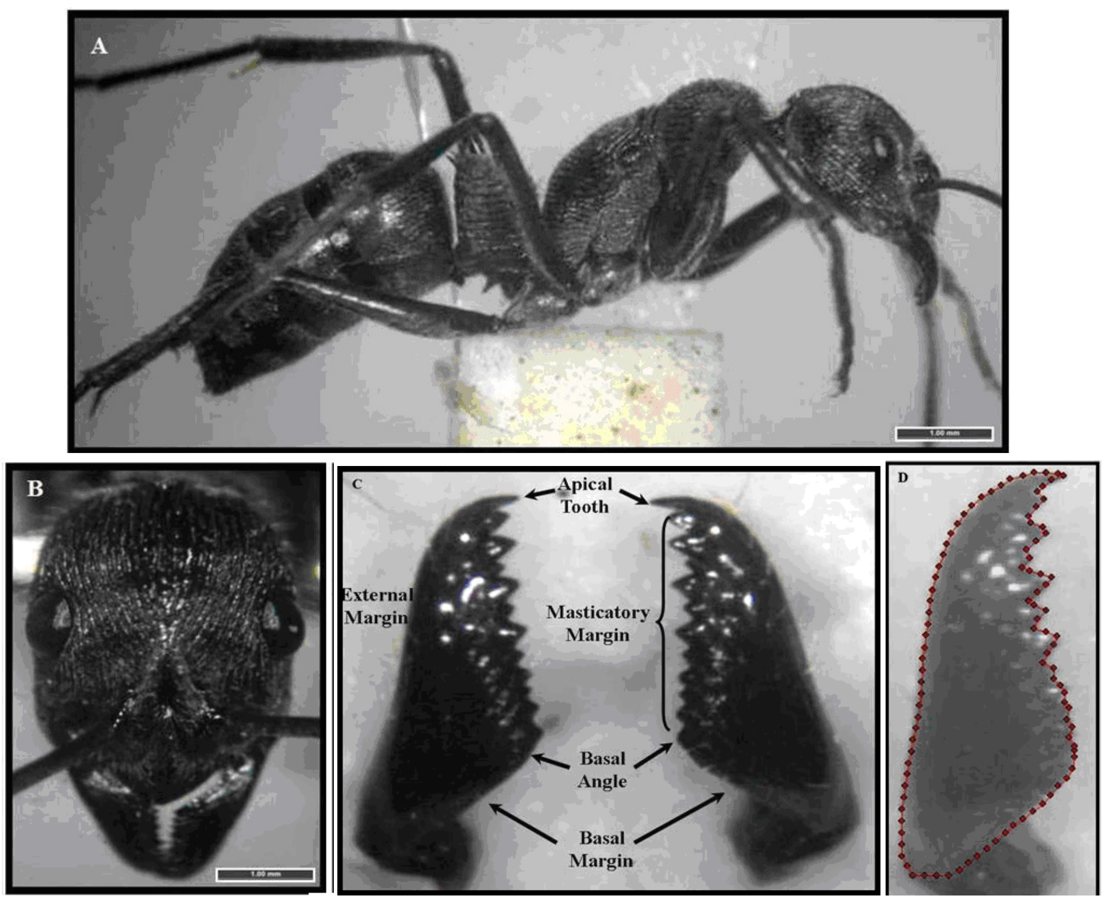

Figure - 1
***Diacamma rugosum*: (A) Lateral view; (B) Head, full face view; (C) Dorsal view of the external mandible morphology and (D) Position of 100 outlines points in the mandible**

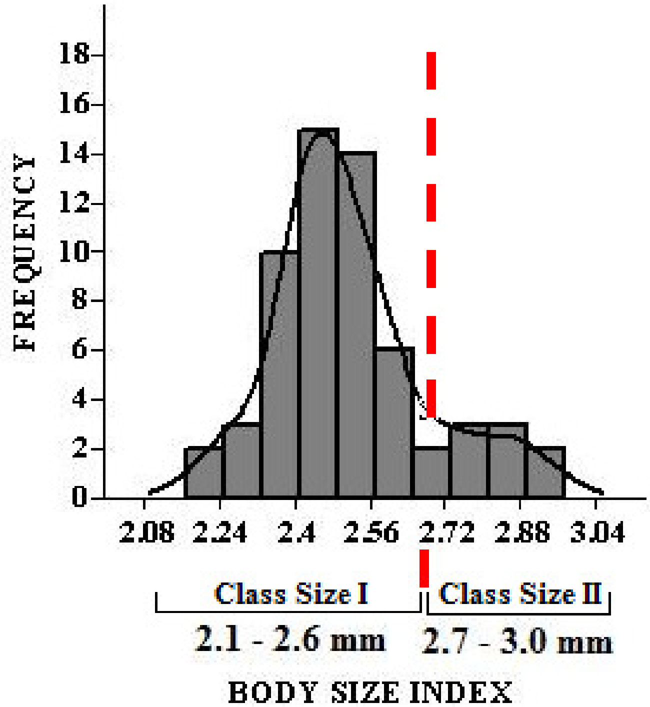

Figure - 2
Body size estimates of *D. rugosum* workers

Mandibular shape variation in the two workers of D. rugosum: As summarized in table 1, 56.42% variations is accounted for the left mandible and 61.31% is accounted for the right mandible. These mandibular shape variations can be attributed to the overall mandible shape of the social workers of *Diacamma rugosum* having small triangular mandibular morphology, elongated masticatory margin with noticeable sharp - pointed curved apical and pre-apical teeth and prominent basal angle to a large triangular mandibular morphology, elongated masticatory margin with blunt apical tooth and sharp - pointed curved pre-apical teeth; prominent basal angle and distinct external margin. These observations on the variations on the mandible shape suggest that these are rather specialized in the task which indicates the infrequent use of mandibles or it maybe a result of their diet for such they are known to be a carnivorous ants and also with the effect of the force of mandible that it exerts during their hunt for their prey and the in looking for the materials in building their nest.

Pearson correlation values for shape variables against body size index in *Diacamma rugosum* ranged only from r = -0.006 to 0.197. Only PC 5 of the left mandible shows significant result in which the age- and size- related changes in the shapes of the mandible was observed (figures 3 and 4). It could be possible that the social workers are the one who is task in foraging and preying while those solitary hunters are in charge in transporting the dead/narcotize prey back to their nest so they can share it to their colony. Solitary hunters of *D. rugosum* would most likely to have lighter task activities than the social workers but they are also in-charge in the protection of their nest. This may result also due its specific function, as the left mandible is use to hold the prey or any materials and it is affected by force of the right mandible in grasping their pray during their transport towards their nest and in cutting their materials for building their nest. Thus, maybe the main reason in the bluntness and the wear off the apical tooth and masticatory margin of the mandible which lead leads in possible task switch among worker ants of the group.

Results of the study have shown that there is the relationship between morphological variation and task partitioning in *D. rugosum*. In nature, division of labor or task partitioning is widespread[30] especially in insect societies such as honey bees, social wasps, ants, and termites where a few individuals (the queen caste) monopolize reproduction, whereas the rest of the colony (the worker caste) performs tasks such as brood care, nest maintenance, and foraging[2]. It defines an important and apparently widespread feature of work organization in social insects. Previous studies on the division of labor among worker ant species revealed that the performance change according to aging and the importance of age and size differences is that it is partly due to the long life span in workers. In a study in *Pachycondyla striata,* temporal polyethism occurs in which the variations of tasks occur according to age[8]. The younger workers stayed on the nest to take care the pupa, larvae, eggs, male and winged female while the older workers (those at more than 56 days of age) performed several activities outside their nest as defense, foraging and exploring and it may be also linked to the maintenance of the colony as measure of protection for the nest and for reproduction. These activities suggest that the division of labor is by age and thus, temporal polyethism may occur in both populations of monomorphic worker and polymorphic worker ants. On the other hand, many ant species exhibit a correlation between task performance and body size. The success of a colony is determined by its workers' ability to distribute themselves efficiently among tasks and to respond, as a group, to environmental conditions[31, 32]. Studies have shown that worker size and morphology correlate with worker behavior in almost all social species[33, 34, 35]. Studies conducted in ants of the genus *Pheidole* with discrete morphological castes, the large "majors" are specialized in carrying large prey items to the nest but rarely engage in other tasks such as brood care, which is typically performed by the small "minors[36, 37]. Task partitioning was also observed in foraging strategy of *Messor bouvieri*, a seed-harvesting ant species that show task partitioning in the form of a sequence of transfer events among workers going from the search area to the nest. The sequence involved workers of different sizes, with seeds being passed along from smaller to larger workers in which small workers are better at finding seeds with less time spend while large workers are better at transporting them back to the nest and lost fewer seeds[38]. It is not too surprising that morphologically differentiated castes behave differently although empirical evidence indicates that behavior of such castes is flexible to some extent[33].

Table - 1
Variance and overall shape variation in the left and right mandible as explained by each of the significant principal components

PC	%	LEFT MANDIBLE	%	RIGHT MANDIBLE
1	**17.18**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with noticeable sharp - pointed curved apical and pre-apical teeth and prominent basal angle to a triangular mandibular morphology with short masticatory margin, blunt apical and pre-apical teeth; distinct basal angle and external margin.	**14.33**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with noticeable long and curved apical tooth and short-sharp pre-apical teeth; prominent basal angle to a triangular mandibular morphology with short masticatory margin, almost lacking noticeable apical and pre-apical blunt teeth; distinct basal angle and external margin.
2	**12.00**	Worker ants having a small triangular mandibular morphology, elongated masticatory margin with short curved apical and pre-apical teeth and prominent basal angle and external margin to a large triangular mandibular morphology with elongated masticatory margin and lacking noticeable apical and pre-apical teeth.	**13.71**	Worker ants having a small triangular mandibular morphology, elongated masticatory margin with noticeable sharp - curved apical tooth and short-sharp pre-apical teeth; prominent basal angle and external margin to a large triangular mandibular morphology with short masticatory margin, almost lacking noticeable apical and pre-apical blunt teeth; prominent basal angle and external margin.
3	**7.82**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with short sharp apical and pre-apical teeth and prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, short and almost lacking noticeable apical and pre-apical teeth; distinct external margin.	**10.38**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with short-blunt curved apical and pre-apical teeth; prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, long noticeable apical and sharp curved pre-apical blunt teeth; prominent basal angle and external margin.
4	**6.92**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with short curved apical and pre-apical teeth and prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, short apical and pre-apical teeth and lacking noticeable teeth along the masticatory margin; distinct external margin.	**9.51**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with noticeable lacking apical and pre-apical teeth; prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, prominent short apical and pre-apical teeth; prominent basal margin and distinct external margin.
5	**6.44**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with noticeable sharp - pointed curved apical and pre-apical teeth and prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, blunt apical tooth and sharp - pointed curved pre-apical teeth; prominent basal angle and distinct external margin.	**7.95**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with short blunt - curved apical tooth and lacking pre-apical teeth; prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, short apical tooth and slightly cleft pre-apical teeth; prominent basal and external margin.
6	**6.06**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with noticeable sharp - pointed curved apical and pre-apical teeth and prominent basal angle to a triangular mandibular morphology with short masticatory margin, blunt apical tooth and blunt, cleft pre-apical teeth; prominent basal angle and external margin.	**5.43**	Worker ants having a triangular mandibular morphology, elongated masticatory margin with lacking apical tooth and slightly cleft short pre-apical teeth; prominent basal angle to a triangular mandibular morphology with elongated masticatory margin, short curved apical tooth and cleft pre-apical teeth; prominent basal and external margin.

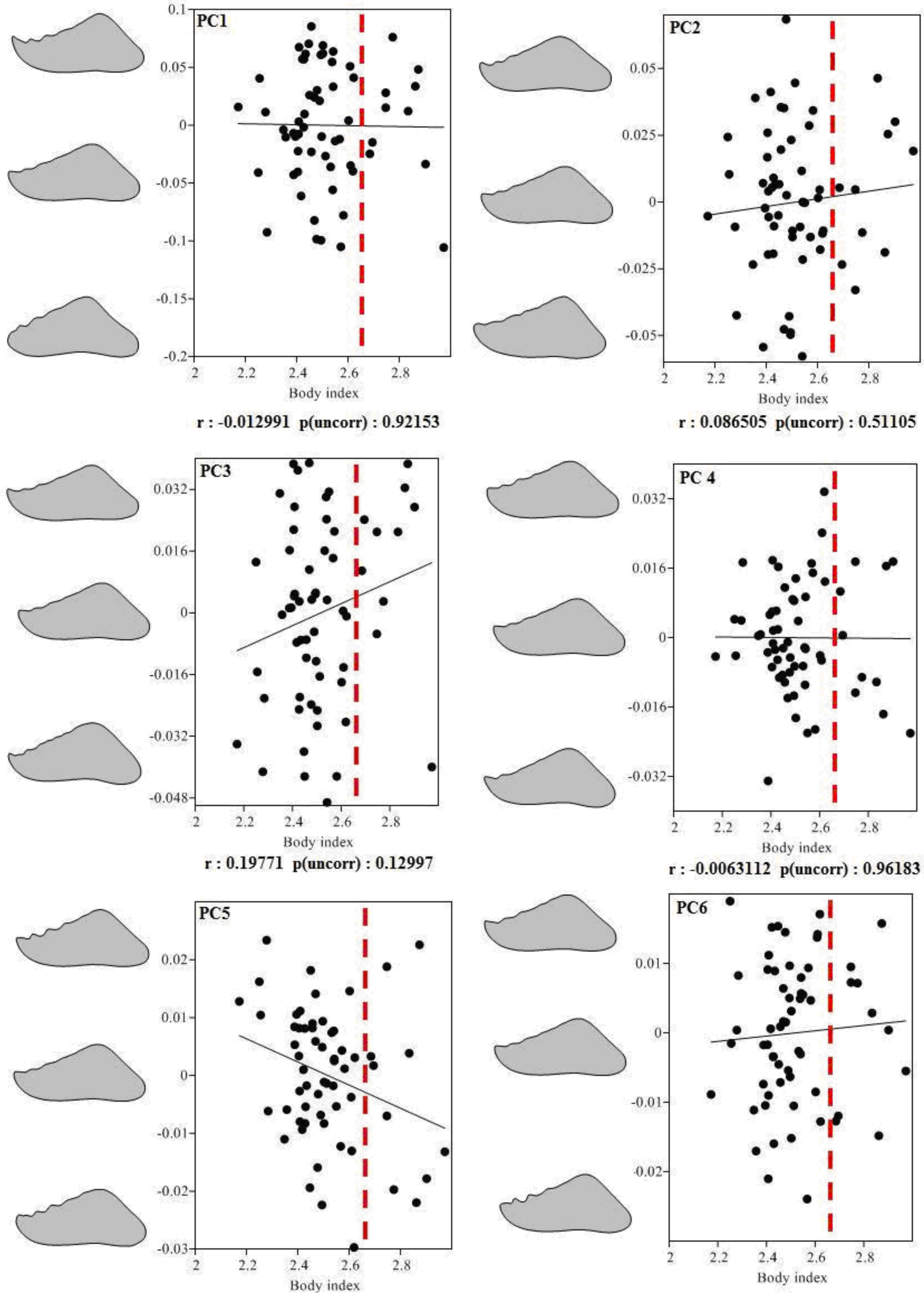

Figure - 3

Results for the test of the size dependent shape changes in the left mandible of *Diacamma rugosum*

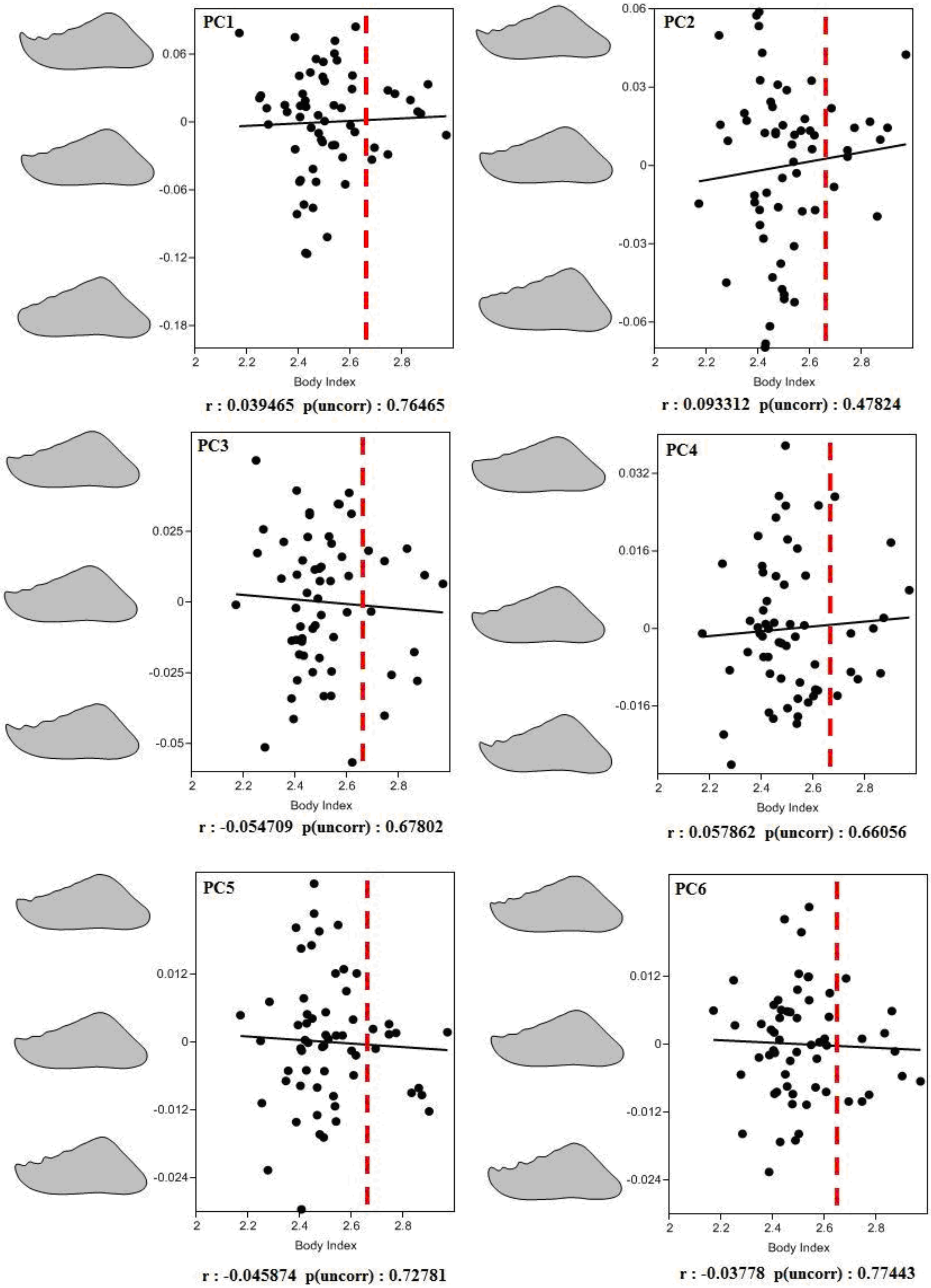

Figure - 4
Results for the test of the size dependent shape changes in the right mandible of *D. rugosum*

Conclusion

The results of the present study on the ant workers of *D. rugosum* based on the body size index in relation to the mandible shape showed that age- and size- related changes in the shapes of the mandible may accompany task partitioning in ants and may be important in studying the evolution of sociogenesis in ants.

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