

Application of geometric morphometrics to analyse allometry in two species of the genus *Myrmica* (Hymenoptera: Formicidae)

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Abstract

Allometric changes in shape were analyzed in two species of the genus *Myrmica* using geometric morphometrics. The pattern of allometry was visualized by thin plate splines (TPS) analysis. In 291 worker ants, 41 landmarks and 252 semilandmarks were fixed in images from four aspects: dorsal head, frontodorsal clypeus, dorsal mesosoma and lateral petiole. To explore how shape varies with size, a multivariate regression on centroid size was performed using the scores of all partial warps (PWs) from all four aspects. Multivariate analysis of covariance (MANCOVA) was used to compare the pattern of allometry between species, using all PWs from pooled coordinates of the two species as varieties, centroid size as the covariate and species as the grouping factor. For all four aspects in each species, the null hypothesis of isometry was rejected (i.e., allometry was present) since the multivariate regressions were statistically significant. The amount of shape variation accounted for by the regressions differed considerably between the species studied and among the four aspects, ranging from 2.62% for the petiole of *M. vandeli* to 13.95% for the mesosoma of *M. scabrinodis*. There were no significant differences between the two species in the allometric patterns of head and clypeus aspects (MANCOVA test). In a multivariate ordination, removing the allometric effects reduced overlap between species only a little or not at all. Geometric morphometrics allows the visualization of the allometries of particular shape components that would probably remain undetected by a conventional morphometric analysis.

Keywords allometry | *Myrmica scabrinodis* | *Myrmica vandeli* | thin plate splines | ants

1. Introduction

A nearly ubiquitous property of organisms is that individuals of different sizes also have different shapes (Adams 2013). Allometry is the statistical association between size and shape (Mosimann 1970). In a more extended meaning, allometries can be understood as differential change of a quantitative character with variation in overall body size (Seifert 2008).

Shape is the geometric feature of an object except for its size, position and orientation (Dryden & Mardia 1998). Quantifying size is easy when two objects have the same shape – any single length would be suitable – but whenever shape differs there is no unique quantification. Composite size measures may be less affected by shape

changes than a single measurement (Mitteroecker et al. 2013). In geometric morphometric studies, centroid size, the square root of the sum of the squared distances of a series of measured landmarks to their common centroid, is often used as an estimate of overall size (Bookstein 1991).

Geometric morphometric analyses of allometry have been presented in different taxa especially in fish (Loy et al. 1998, Rodríguez-Mendoza et al. 2011, Rosenberg 1997, Sidlauskas et al. 2011), but there has been no geometric morphometric survey of allometry in ants. In this study we used geometric morphometric techniques to address the following subjects:

(1) To analyze the relationship between size and shape in two species of *Myrmica*. *Myrmica scabrinodis* Nylander, 1846 and *M. vandeli* Bondroit, 1919 are very similar and

can be difficult to identify using normal identification keys. *M. vandeli* is apparently a facultative temporary social parasite of *M. scabrinodis* with a weak tendency to disable or kill the host queens (Bagherian et al. 2012). (2) The visualization of allometric patterns. (3) The comparison of patterns of allometry between the taxa and (4) the removal of allometric variance (RAV) to improve species separation in geometric morphometric analysis.

2. Materials and methods

2.1. Samples

187 workers of *M. scabrinodis* sampled from 63 nests and 104 workers of *M. vandeli* from 35 nests were investigated. Localities of samples are listed in Bagherian et al. 2012.

2.2. Taking photographs and digitizing

Z-stack photographs were made with a Leica Z6 Apo photomicroscope equipped with 2.0x planapochromatic objective and the automontage software Leica application suite version 3. The spatial positioning of specimens for imaging was according to the following rules.

Head aspect: maximum head width and maximum head length are in the same image plane (i.e., at same horizontal level). Clypeus aspect: the head is tilted until the posterior clypeal margin (i.e., its border with the frontal triangle) is in the same visual plane as the anterior clypeal margin - this is at roughly a 45° tilt.

Mesosoma aspect: mesosoma in dorsal view with an anterior and two posterior reference points in the same image plane. The anterior reference is the transition point between the anterior pronotal slope and anterior pronotal shield. The posterior reference is the horizontal portion of the upper margin of the propodeal lobes just before they ascend to the subspinal excavation.

Petiole aspect: petiole in lateral view. Landmarks were fixed at unambiguously defined surface points and semilandmarks were determined by the curve-tracing method implemented in the tpsDig software package (Rohlf 2010c). Semilandmarks are points evenly spaced on an outline delimited by two fixed points. From the head aspect, 14 landmarks and 95 semilandmarks were determined. These figures are 9 and 62 for the clypeus aspect, 15 and 63 for the mesosoma aspect, 3 and 32 for the petiole aspect.

2.3. Correlation between shape and tangent space

Correlations between the Procrustes and tangent-shape distances were calculated using tpsSmall software version 1.20 (Rohlf 2003).

2.4. Symmetrizing

In an earlier study (Bagherian et al. 2012), we proved that there was no directional asymmetry in the measurements of the *Myrmica* species analyzed but there was some fluctuating bilateral asymmetry. To correct for this asymmetry, a computation based on a Procrustes superimposition of the landmark configurations with the other side of the body (left side-right side in petiole) or with their reflection (head, clypeus and dorsal view of mesosoma) was used. The Procrustes distance between a shape and its reflection is a measure of asymmetry; it is zero only for perfectly symmetric shapes (Mitteroecker & Gunz 2009). There is no directional asymmetry in the population mean when left-right differences of Procrustes distances from the reference shape are normally distributed with a mean of zero. Procrustes distances were calculated by tpsSmall version 1.20 (Rohlf 2003). Normality and mean value of the differences in each species were tested separately. Differences between left-right shapes were not significantly non-normally distributed in any of the aspects (Kolmogorov-Smirnov $p > 0.05$) and the mean left-right subtracts of Procrustes distances from median shapes were not significantly different from zero (one-sample two-tailed t -test $p > 0.4$, $t < 0.84$). To avoid non-independent data, after symmetrizing, subsequent analyses of the head, clypeus and mesosoma dorsal view were conducted on only one side. Non-independent data were removed in each outline using the tpsUtil 1.46 software application (Rohlf 2010a). However, deformation grids are presented for the bilateral shape configurations of landmarks and semilandmarks, in order to improve the visualization of shape changes.

2.5. Centroid size

Centroid size is a measure of overall size. It is the square root of the summed squared distances of each landmark from the median shape of the landmark configuration. In the absence of allometry it is the only size measurement that is uncorrelated with shape variation (Bookstein 1991). Centroid size was calculated from the raw coordinates (before symmetrizing) of the landmarks and semilandmarks by using tpsRelw version

1.49 (Rohlf 2010b). An independent-samples *t*-test was conducted on the centroid sizes of each aspect to test for size differences between species.

2.6. Partial Warps

The landmarks and semilandmarks were entered into a generalized Procrustes analysis (GPA), then the semi-landmarks were slid along the outline curve until they matched as well as possible the positions of corresponding points in a reference configuration (Bookstein 1997). There are two mostly used criteria to slide points along an outline, minimum bending energy (Bookstein et al. 2002) and perpendicular projection or minimum procrustes distance (Sheets et al. 2004). The program tpsRelw version 1.49 (Rohlf 2010b) was used for superimposition of the landmarks and semilandmarks. This program provides sliding semilandmarks based on minimum bending energy. After computing a mean configuration and aligning the targets, the program moved the semilandmarks of each target to minimize the bending energy of the thin-plate spline, thus describing the deformation of the reference to that target (Zelditch et al. 2004).

2.7. Relationship between size and shape

To explore how shape varies with size, shape variables (all partial warps and uniform components) were entered into a multivariate regression on centroid size (performed with tpsRegr version 1.37: Rohlf 2009). The fit of the regression models was evaluated by the explained variance of the model using Goodall's *F*-test.

2.8. Comparison among patterns of shape changes related to size between two species

Comparison of the patterns of shape changes related to size was performed by multivariate analysis of covariance (MANCOVA) using all partial warps from pooled coordinates of the two species as varieties, centroid size as covariate and species as the grouping factor. This analysis was performed using TpsRegr.

2.9. Visualization of allometric transformation patterns

To visualize allometric transformation patterns in the two species, a multivariate regression of partial warps and

the uniform component on centroid size was performed with tpsRegr, version 1.37 (Rohlf 2009). The size-dependent deformation in shape for all anatomical aspects is illustrated by showing the deformation grids predicted for the smallest and largest specimen. As allometric changes are small in the species, the deformation was exaggerated in the figures to better illustrate them.

2.10. Testing whether correcting for allometry improves ordination and discrimination among groups

To investigate the effect of removing allometries on ordination and species discrimination, MorphoJ version 1.05a (Klingenberg 2011, Klingenberg 2012) was used. Generalized Procrustes Superimposition (GPS) was applied to the symmetrized coordinates. As a baseline for comparison, ignoring the allometry, principle component analyses (PCA) and linear discriminant analyses (LDA) with cross-validation were performed on the Procrustes superimposed coordinates. For the alternative, a pooled within-group allometric regression on log centroid size was performed. The residuals from this regression were subjected to PCA and LDA.

3. Results

3.1. Correlation between shape and tangent space

Variation of the specimens in shape space was perfectly correlated with tangent space for all anatomical aspects. This allows the use of the plane approximation in future statistical analyses and interpretation of results.

3.2. The size

The distribution of centroid size in all four anatomical aspects and in both species did not differ significantly from normal ($p > 0.05$). Means of centroid sizes were significantly different between species ($p < 0.0001$); *M. vandeli* is significantly bigger than *M. scabrinodis* in all four aspects.

3.3. Relationship between size and shape

The null hypothesis of isometry was rejected (allometry was present) in all aspects of the two species since each

multivariate regression was statistically significant (Table 1). The amount of shape variation accounted for by the regressions differed considerably, ranging from 2.62% for the petiole of *M. vandeli* to 13.95% for the mesosoma of *M. scabrinodis* (Table 1).

3.4. Comparison among patterns of shape changes with size between two species

There were no significant differences between allometric patterns of the two species in head and clypeus (MANCOVA, Table 2). However, the MANCOVA for the common allometric model showed significant differences between species in the allometric relationships for the dorsal mesosoma aspect ($p < 0.02$) and the petiole aspect ($p < 0.01$).

3.5. Visualization of allometric transformation patterns

Deformations in coordinate configurations related to centroid size are shown in figures 1 to 4. In head and

clypeus aspects, overall shape changes are relatively similar in the two species, as has been indicated by the MANCOVA analyses.

Differences in petiole shape in larger individuals are dominated by an increase of height relative to length, with the dorsum of the node becoming more pronounced. In *M. scabrinodis* the most striking allometric deformation is in the ventral region of petiole while in *M. vandeli* shape changes are most marked in the dorsal part.

In larger individuals, the anterior head is relatively shorter, the ratio between postocular and preocular head width is relatively larger, and the frontal lobes transform backwards and the eyes show a shift towards the median.

The main changes in clypeal shape with size are a disproportionate increase of distance between anterior and posterior margins whereas the height of the frontal triangle is relatively reduced. In *M. vandeli*, the shape of the clypeal excavation is changed: the proportional width of the excavation is reduced.

The main changes in mesosomal shape with size are a relatively larger spine length and the more parallel orientation of the mesosomal sides at the level of mesonotum and propodeum.

Table 1. Multivariate regressions of shape versus centroid size for each anatomical aspect in the two species.

Species	<i>M. scabrinodis</i>			<i>M. vandeli</i>		
	% Explained variance	Goodall's <i>F</i> -test		% Explained variance	Goodall's <i>F</i> -test	
Aspects		<i>F</i> -value	<i>p</i>		<i>F</i> -value	<i>p</i>
Head	10.74	20.9436	$p < 0.00001$	7.01	5.5051	$p < 0.00001$
Clypeus	4.57	6.3223	$p < 0.00001$	3.03	2.4383	$p < 0.00001$
Petiole	7.36	14.7044	$p < 0.00001$	2.62	3.1227	$p < 0.00001$
Mesosoma	13.95	24.4961	$p < 0.00001$	4.67	5.0487	$p < 0.00001$

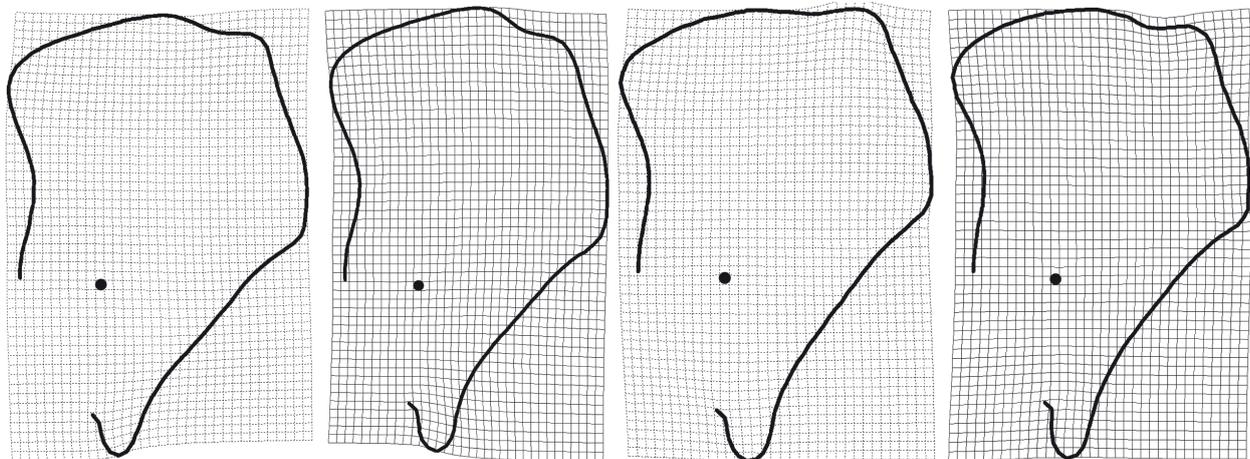


Figure 1. Deformation grids of petioles related to centroid sizes. From left to right respectively: smallest *M. scabrinodis*, largest *M. scabrinodis*, smallest *M. vandeli* and largest *M. vandeli*.

3.6. The effect of the removal of allometric variance (RAV) on discrimination between groups

In all four aspects there is no or little improvement in patterns of overlap and non-overlap among species (Fig. 5 and Fig. 6). The PCA scatter plots for petiole and mesosoma have not been shown because there is no distinction between species by mean PC scores.

Size adjustments reduced within-group Euclidean distances in all PCA factors related to all Euclidean distances (Table 3). The F value of ANOVA test of first

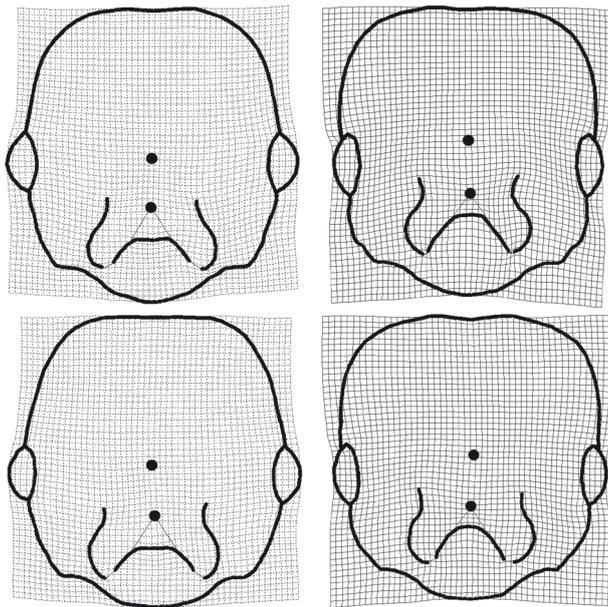


Figure 2. Deformation grids of heads related to centroid sizes. Upper row shows *M. scabrinodis* and lower *M. vandeli*, the left column shows the smallest specimen for each species and the right the largest. Deformation grids are exaggerated three times for better illustration.

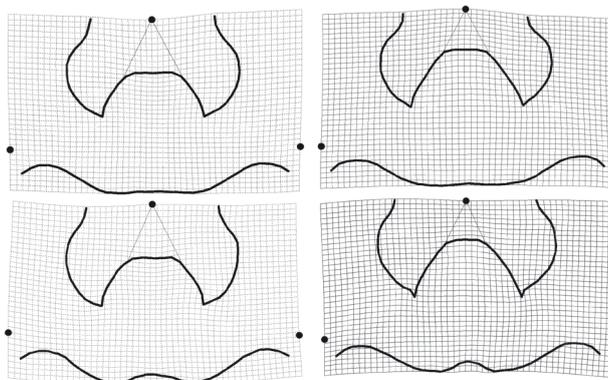


Figure 3. Deformation grids of clypeus related to size in *M. scabrinodis* (upper) and *M. vandeli*, (lower). The left column shows the smallest workers and the right column the largest. The deformation grids have been exaggerated two times.

Table 2. Results of MANCOVA tests for common linear allometric models for the shape and size relationship in two species.

	λ_{WILKS}	F	p
Head	0.01197693	0.928	0.6624
Clypeus	0.09921143	0.890	0.7904
Petiole	0.08278784	1.337	0.0087
Mesosoma	0.00276678	1.665	0.0193

Table 3. Ratio of total Euclidean distances on all RWs to within-group distances and F values of ANOVA test on first PCA factors for the four aspects.

	Within-group Euclidean distances / all Euclidean distances		F value of ANOVA test on first PCA factor	
	Non-corrected data	Size corrected data	Non-corrected data	Size corrected data
Head	0.510	0.495	382	852
Clypeus	0.437	0.431	807	850
Petiole	0.961	0.909	77.3	152
Mesosoma	0.508	0.478	1.17	35.8

factor of PCA increased after removing of allometries (Table 3). This indicates that removal of allometric variance improved species separation.

All workers were grouped into their own species with $p > 0.99$ for head and clypeus aspects for both the non-corrected and size-corrected data (cross-validation LDA). There is no improvement in grouping errors for petiole by removing allometries but a 4.3% improvement in grouping probabilities of $p > 0.99$ (Table 4). For the mesosoma aspect, there is a 1.3% improvement in grouping and a 4.3% improvement in grouping probabilities of $p > 0.99$ after size correction (Table 4). Mahalanobis distances between the species increased in all aspects after size correction (Table 4).

4. Discussion

Linear morphometrics has shown moderate allometries in the two species studied (Seifert 1988) and in related species (Seifert 2011, Seifert et al. 2014). This geometric morphometric investigation confirmed the existence of allometries in these two closely related species of *Myrmica*. Geometric morphometrics allows us to visualize allometries of particular shape components which remained undetected by a conventional morphometric analysis in the two studied species (Seifert

1988). However, similar allometric changes have been found by means of conventional morphometrics in workers of the fire ant *Solenopsis invicta*. Tschinkel et al. (2003) found that across the range of worker sizes, head shape changed from a barrel-profile to a somewhat heart-shaped profile and the mesosoma became relatively shorter and higher.

The allometric deformations of the petiole shape are directed by biomechanical causes – i.e., the increasing petiole height is a consequence of the increased weight of the gaster. The power of a muscle (here the *Musculus elevator abdominalis* and the *M. flexor abdominalis*) depends on its cross-section area and grows as a quadratic function while the weight of the structure (here

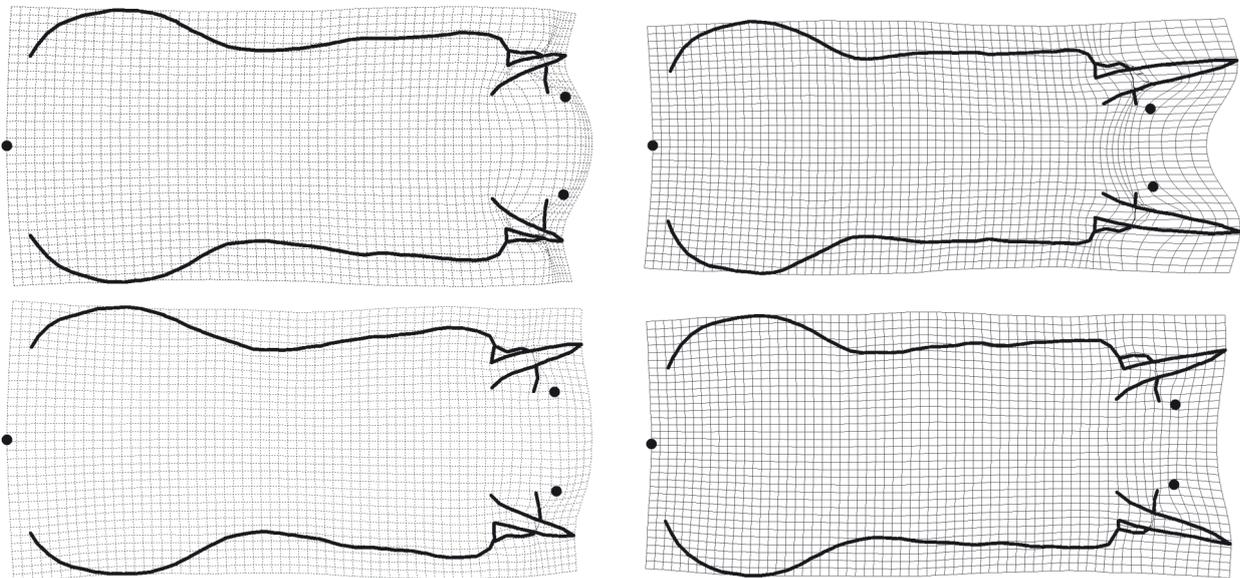


Figure 4. Deformation grids of mesosoma related to size in *M. scabrinodis* (upper) and *M. vandeli*, (lower). The left column shows the smallest workers and the right column the largest. The deformation is exaggerated two times.

Table 4. Mahalanobis distances and correct grouping percentage in LDA for non-corrected and allometries removed data.

	Mahalanobis distance		Correct grouping percentage		Grouping probabilities with $p > 0.99$	
	Non-corrected data	Size corrected data	Non-corrected data	Size corrected data	Non-corrected data	Size corrected data
Head	15.96	17.4	100%	100%	100%	100%
Clypeus	9.40	9.53	100%	100%	100%	100%
Petiole	9.26	9.94	99.3%	99.3%	90.8%	95.1%
Mesosoma	5.98	6.83	97.9%	99.2%	93.4%	97.7%

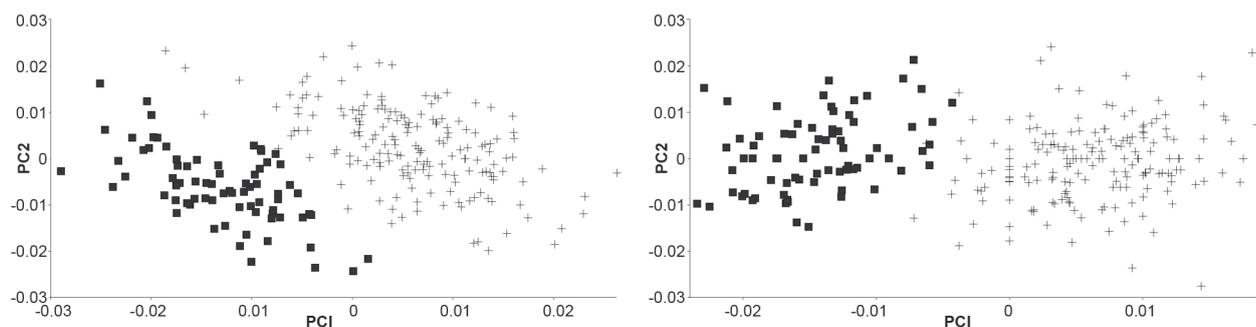


Figure 5. Scatter plot of PC1 and PC2 for head aspect, the left plot is without correction for allometry while the right shows size standardized data. The squares are *M. vandeli* and crosses are *M. scabrinodis*.

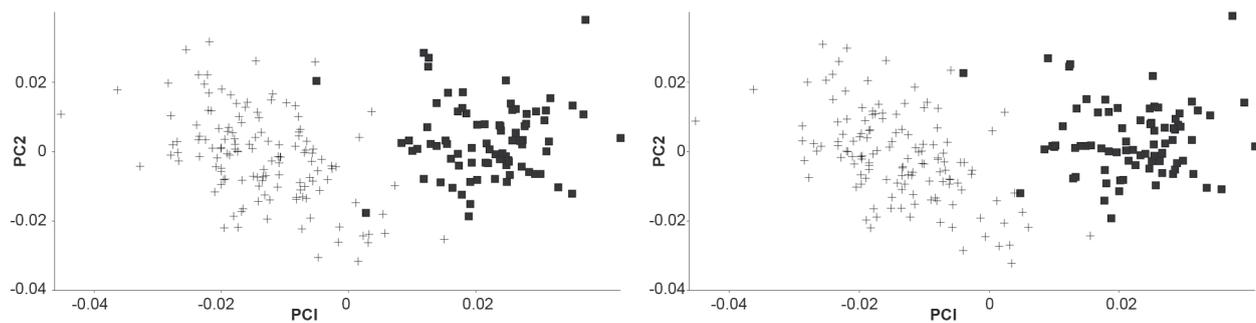


Figure 6. Scatter plot of PC1 and PC2 for clypeus aspect, left plot is without allometric removed and right is size standardized data. The squares are *M. vandeli* and crosses are *M. scabrinodis*.

the gaster) that the muscle has to hold and move grows in cubic function. Accordingly, linear measurements of the petiole have to grow larger than other body measures such as gastral or mesosomal length. Propodeal spines of the mesosoma have a protective function. The longer and stronger the spines are the better the sensitive and thin petiole is saved from mechanical injury during mandible attacks by enemy ants. Furthermore, vertebrate enemies, such as birds, have increased difficulties to swallow the hooked ants. Thus, strongly developed spines are an advantage but they are not essential for the normal function and working performance of a worker ant. In case of food deprivation during larval development, energy and material to produce strong spines can be saved when the development of the essential structures has to be guaranteed. This pay-off is predicted by the imaginal disk interaction growth model of Nijhout & Wheeler (1996), in which a fixed supply of resource during metamorphosis results in competition among imaginal disks, resulting in asymmetrical development of body parts in holometabolous insects. The outcome of this competition may differ with the total amount of resources available within the worker (Matheus & Tschinkel 2010).

The strong performance of species discrimination even without removal of allometric variance (RAV) and the fact that no truly cryptic species (Seifert 2009) are involved did not allow us to use an error analysis to check whether removal of allometric variance affected performance: only for the mesosomal aspect there was a moderate reduction of error. However, the increase of Mahalanobis distances between the centroids and of the grouping probabilities $p > 0.99$ indicates that RAV improves species discrimination. A more severe test situation that examines truly cryptic species is likely to show the effects of RAV more clearly. Cryptic species have been defined by Seifert (2009) as ‘two or more species which are not safely separable by primary visual or acoustic perception of an expert.’

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