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# The First Morphometric Study of the Horn Morphological Pattern in a Geotrupidae: The Case of the Dor Beetle *Ceratophyus rossii* Jekel, 1865

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Among beetles, thousands of species develop horns, the size of which is often extraordinarily disproportionate with respect to body size. The Scarabaeidae is the family in which horned species are most predominant, but other families, such as the Geotrupidae (dor beetles), also show remarkable horns, although in a more limited number of species. Horn expression mechanisms are well documented in Scarabaeidae but, despite the wealth of studies on this family, the horn morphological pattern of the Geotrupidae, to our knowledge, has never been investigated. In this paper, we describe for the first time the horn expression pattern in a dor beetle. As a study species, we chose *Ceratophyus rossii*, an Italian endemic dor beetle of the protected Mediterranean maquis in Tuscany, which shows remarkable head and pronotal horns in males and a notable cephalic horn in females. We identified and modeled shape and size horn patterns combining traditional and geometric morphometric approaches. We discuss the results in the wider landscape of developmental models described for other, more well-characterized, scarab beetles.

**Key words:** geotrupinae, static allometry, geometric morphometrics, female dimorphism, trade-off

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## INTRODUCTION

Many scarab beetles produce rigid outgrowths of the cephalic and/or thoracic exoskeleton, i.e., horns, typically used for mate choice and intraspecific combat (e.g. Eberhard, 1978; Rasmussen, 1994; Emlen, 2000; Hunt and Simmons, 2001; Iguchi, 2001; Hongo, 2003). These horns are often exaggerated in size and extremely diverse in shape; in exceptional cases, horn expression more than doubles body length and may account for approximately 30% of body mass (Moczek et al., 2006).

Horns or horn-like structures appear to have arisen repeatedly within the scarab beetles, in at least seven families, and are, in general but not always, confined to males (e.g. Arrow, 1951; Grimaldi and Engel, 2005; Emlen et al., 2007; Kijimoto et al., 2012). The Scarabaeidae, and especially the Scarabaeinae (true dung beetles) and Dynastinae (rhinoceros beetles) subfamilies, is the predominant family in terms of the number of species with exaggerated horns. In both subfamilies, thousands of species express horns which have diversified with respect to location, shape, and number of horns expressed. However, other families, such as the Geotrupidae (dor beetles), also show remarkable horns, though in a more limited number of species (Emlen et al., 2007; Emlen, 2008). Scarabaeinae, Dynastinae and Geotrupidae are phylogenetically widely separated within the scarabs as a whole, leading to the view that scarab

horns must have evolved independently many different times, and that they are easy to gain or lose (Arrow, 1951; Emlen et al., 2007).

The developmental mechanisms of horns and their intra and interspecific expression patterns are well studied especially in Scarabaeidae: within this family, due to its large number of species and its morphological variability, *Onthophagus* is the most studied genus (Emlen, 1997; Emlen and Nijhout, 1999; Emlen et al., 2005; Moczek, 2011; Palestini et al., 2000; Pizzo et al., 2006a, b, 2012; Macagno et al., 2009, 2011a). Despite the wealth of studies on the Scarabaeidae, the horn expression pattern of the Geotrupidae, to our knowledge, has never been investigated.

From studies carried out on Scarabaeidae, it has been shown that horns can scale with body size through two main modes: linearly, in which horn length increases with respect to body size at a relatively constant (and faster) rate, or in a sigmoidal fashion, often implying a mechanism of threshold-dependent development. In this latter case, only males that exceed a certain critical body size express fully developed horns; if they remain smaller, they may show only rudimentary horns, or fail to develop them at all. (Moczek and Emlen, 1999; Moczek, 2011; Kijimoto et al., 2009). The two types of development may therefore underlie different ontogenetic regulatory mechanisms (Emlen and Nijhout, 2000; Nijhout and Grunert, 2002; Kijimoto et al., 2012).

Moreover, some studies have also demonstrated that the growth of a horn is metabolically expensive and occurs in the virtually closed system of the pre-pupal stage, after the developing larva has stopped feeding (Emlen, 2001; Simmons and Emlen, 2006). The shared and limited pool of resources available at this stage can trigger competition for

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nutrients, hormones, growth factors and morphogens between developing structures. This can result in morphological trade-offs in which the development of the exaggerated trait (the horn) can result in other structures (e.g. other horns, genitalia, other functional traits, such as eyes or wings) growing proportionally smaller (Emlen, 2001; Kawano, 1995, 1997; Klingenberg and Nijhout, 1998; Moczek, 2009; Moczek and Nijhout, 2004; Nijhout and Emlen, 1998; Nijhout and Wheeler, 1996; Okada and Miyatake, 2009; Painting and Holwell, 2013; Parzer and Moczek, 2008; Pizzo et al., 2012; Simmons and Emlen, 2006).

In this paper, we describe for the first time the horns morphological pattern in a dor beetle. We focused on the genus *Ceratophyus*, as its members bear two horns occurring in the same basic body regions as the horns of more “typical” horned scarabs; the anterior surface of the pronotum, and the dorsal surface of the head (Grimaldi and Engel, 2005; Kijimoto et al., 2012). Moreover, the females in this genus also bear a cephalic horn. Due to the great similarity of the species within this genus, as representative for the genus we chose the Italian endemic *C. rossii* Jekel, 1865. We hope that beginning the study of horn expression in the Geotrupinae by starting from a species at risk of extinction may serve as an incentive for the investigation of other aspects of its biology, ecology, behavior and life history related with horn expression pattern highlighted here, although these probably not available for a long time yet.

As horn expression pattern can be represented by means of a static allometry based on measurements of conspecific individuals at the same life stage (Eberhard and Gutierrez, 1991; Emlen and Nijhout, 2000; Moczek and Nijhout, 2003; Shingleton et al., 2007), we analysed the scaling relationship between body size and horns in adult *C. rossii* along a wide range of different body sizes. A landmark-based approach was also adopted to identify and quantify horn shape modifications along with body size variation.

Because males of *C. rossii* develop horns on both head and thorax, this species also provides an interesting opportunity for investigating whether the relative expression of one horn type is affected by the development of another horn type in another location in the same individual (*trade-off*). To explore developmental correlation resulting in a trade-off between head and pronotal horns, we tested for possible negative correlations between measures of the relative investments in each horn type by each individual.

We then discussed our results in the framework of the results highlighted from other best-studied scarab beetles, mainly the *Onthophagus*, for a more complete understanding of the meaning and the evolution of the secondary sexual traits in beetles.

## MATERIALS AND METHODS

### Study species

The Geotrupidae are thought to represent an early offshoot of the superfamily Scarabaeoidea that may have diverged from the remaining dung beetles at least during the Lower Cretaceous according to fossil data (Krell, 2007). *Ceratophyus* is a monophyletic genus composed of few species (Zunino, 1973; Cunha et al., 2011; Bisby et al., 2011), all with a scattered Palearctic distribution (along the ancient northern margins of the Tethys) with the excep-

tion of one, isolated Nearctic species (Cartwright, 1966). Species within this genus are phenotypically very similar; certain identification of each species is often impossible when only external morphology is considered (Zunino, 1973).

*C. rossii* is an endemic species (IUCN national status) found only in an extremely restricted Tyrrhenian coastal region (Carpaneto, 1976; Audisio, 2003; Agoglietta et al., 2012). This rare and localized species is an uncommon member of the winter beetle fauna of the protected Mediterranean maquis shrubland and it is considered to be at risk of extinction and protected by regional law (All. B, L.R. 56/00) due to its extremely reduced and fragmented distribution area, and for the progressive loss of suitable habitats (Nistri et al., 1991). It is found only in a few relict dunes covered with sub-coastal woodland and consolidated dunes between Tombolo di Pisa, San Rossore and the Oasis of Burano near Grosseto (Tuscany) (Carpaneto, 1976; Audisio, 2003). *C. rossii* has previously been reported occurring in Sardinia (Bargagli, 1872) and Corsica (Mulsant and Rey, 1871; Barthe, 1901; Boucomont, 1912; all cited by Dellacasa, 2004), but these records have not been confirmed by subsequent surveys.

In males, the clypeus triangularly extends forward and upward in a long, acute horn, which is sculptured with close elongate punctures; the pronotal horn, long and acute, faces forward and, in the larger individuals, can reach the tip of the cephalic horn.

The female head horn, on the contrary, is short, laterally flat, and expanded in anterior-posterior direction to form a crest not overhanging the anterior edge of the clypeus. Females do not have a pronotal horn, but the median anterior area of the pronotum forms a small blunt, squared protuberance (Fig. 1).

Although there are no specific data on the use of the horn by individuals of *C. rossii*, Klemperer (1984) observed that in *C. hoffmanseggii* beetles of either sex resist intruders in the entrance to their burrows by a head-to-head pushing contest, without injury to either opponent, similarly to males of several species of rhinoceros and dung beetles (e.g. Rasmussen, 1994; Emlen, 1997; Moczek and Emlen, 2000). The recurrent association between horns and fights over burrows led to the suggestion that tunnelling behavior may have been an important ecological prerequisite for the evolution of horns in beetles (Emlen and Philips, 2006).

### Sample and data acquisition

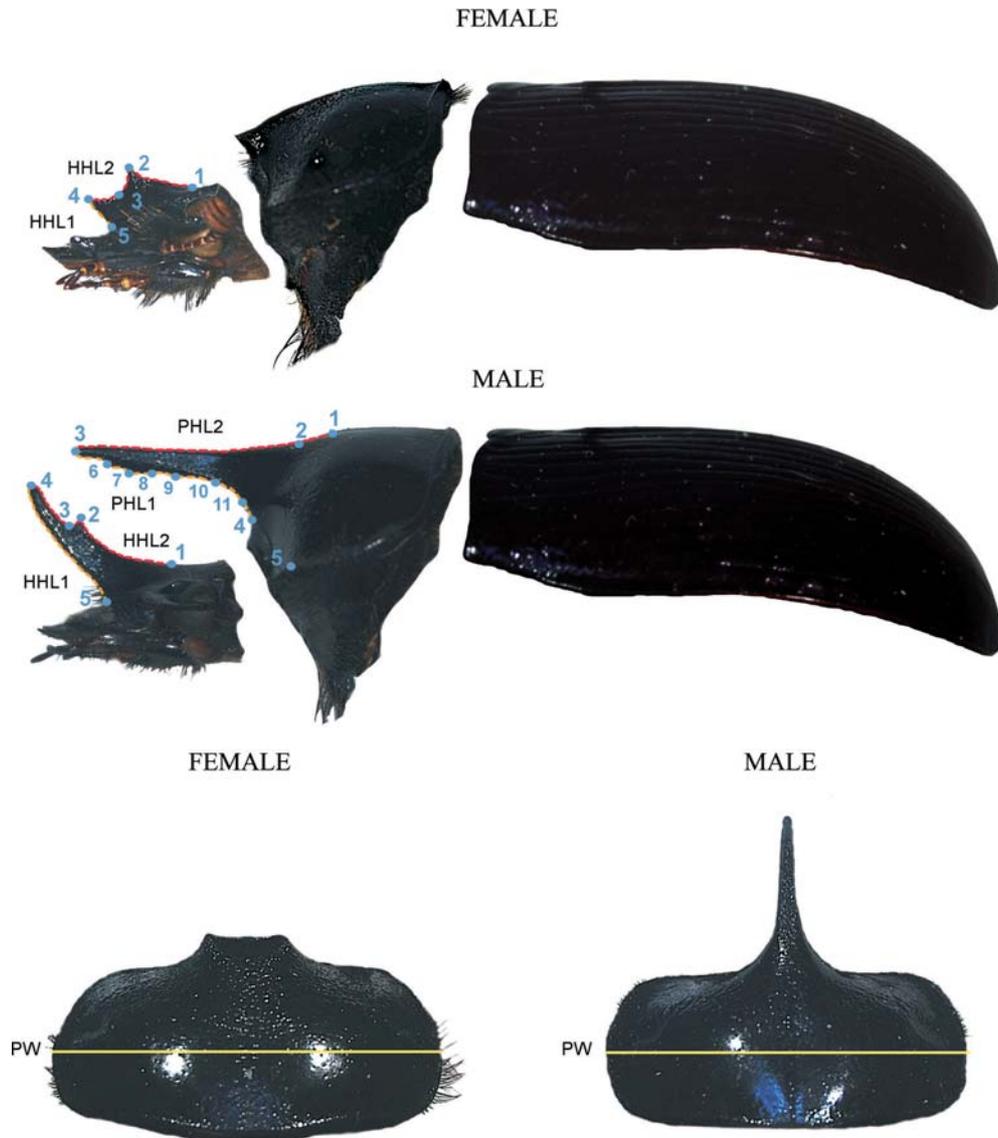
Due to the rarity and the extreme localisation of the species, we avoided new field collection and we analysed a sample of 63 males and 42 females from San Rossore courteously provided by G. Dellacasa and by Dr. E. Barbero (University of Turin, Italy); due to the scientific value of these specimens, we photographed only entire animals without dissecting them. We photographed heads and pronota using a Leica Z16Apo stereoscope connected to a Leica DFC320 digital camera at a magnification of 7.81x (Leica Microsystems AG, Wetzlar, Germany).

### Linear measurements

Morphological measures were taken on the two-dimensional images with the software LAS v 2.5.0 (Leica Application Suite) as in Fig. 1. Some papers (Tomkins et al., 2006; Moczek, 2006) have suggested that some differences in the patterns of horn allometries can arise from the method used to measure the beetles' horns. In general, those measurements that reflect the actual shapes of the structures (i.e. those able to follow curves, tips and depressions) are preferable.

Where landmarks have been used that include a proportion of some other body part, the slope will always be affected. For this reason, on the head horns we measured separately front (HHL1) and back (HHL2) horn edge lengths, as they have two significantly different shapes, while on the pronotal horns we measured ventral (PHL1) and dorsal (PHL2) horn edge lengths.

On the dorsal view, we measured pronotum width (PW) which



**Fig. 1.** Measures, landmark, and semilandmark configuration on horns, head and pronotum in *Ceratophrys rossii*. Top: lateral view of the cephalic horn, with landmark configuration, front (HHL1) and back (HHL2) horn length; lateral view of the pronotal horn, with landmark (1–5) and semilandmark (6–11) configuration (male only), ventral (PHL1) and dorsal (PHL2) pronotal horn length. Bottom: dorsal view of the pronotum, with pronotum width (PW).

was used as a proxy for the overall body size (Emlen, 1994; Moczek, 1998; Parzer and Moczek, 2008; Pizzo et al., 2012).

### Static allometries

We first inspected the trend of the natural variation in pronotum width and horn length measurements, making frequency histograms. The number of classes for the size frequency distribution histograms of each sex was chosen using Sturges' rule ( $k = 1 + \log_2 n$ ), which has been found to be relatively effective for sample sizes that are smaller than 200 (Gouws, 2011). We tested for approximation to a normal distribution by running a Kolmogorov–Smirnov test (Sugiura et al., 2007). Kernel density curves were drawn on the histograms (Munguía-Steyer, 2012), by using SigmaPlot version 13.0, from Systat Software, Inc., San Jose California USA, [www.sigmaplot.com](http://www.sigmaplot.com), to help the readers to better visualize the differences between the distributions.

We then graphed the log-log scaling relationship between horn lengths and body size (static allometries). We first excluded the pos-

sible presence of segmented regressions by running Segreg (Oosterbaan, 1994) software (<http://www.waterlog.info/seg-reg.htm>); then, the scaling relationships were determined by fitting to the log-scaled data both a simple linear ( $y = y_0 + bx$ ), and a four-parameter non-linear regression in the form of the four-parameter hill equation:

$$y = y_0 + \frac{ax^b}{c^b + x^b}$$

(Moczek et al., 2004) where  $x$  is body size,  $y$  is trait size length,  $y_0$  specifies minimum trait size,  $a$  defines the trait size range in the sample,  $b$  is the slope coefficient and  $c$  is the body size at the point of inflection of the sigmoid curve. A recent study (Nijhout and German, 2012) suggested that most growth in biological systems approximately follows a kinetic of growth well described by a Gompertz curve. We therefore also fitted this equation to our data and compared the results with those coming from the former approaches. To discriminate which model is the most appropriate to describe the scaling relationship found, we first compared  $r^2$  values generated by the two regressions. A very small increase in fit when using the non-linear with respect to the linear regression model is expected in most cases, even when this doesn't underlie, in fact, a sigmoid scaling relationship. This may be due to the larger number of parameters available in a sigmoidal with respect to in a linear model (Moczek et al., 2004). Thus, to determine if the two models differed significantly in how well they fitted the data, the residuals from the two regressions types were also compared by means of an Extra-Sum-of-Squares F-test (Moczek et al., 2004; Macagno et al., 2009). However, some authors have expressed concerns in the use of  $r^2$  values to compare the goodness of fit between linear and non-linear models, even when corrected for the number of parameters taken into account (as  $r^2$  values cannot properly evaluate non-linear models) (Spiess and Neumeyer, 2010); for this reason, the corrected Akaike's information criterion (AICc) (Akaike, 1973; Motulski and Christopoulos, 2003; Knell, 2009) was used to select the most appropriate models, in addition to the  $r^2$  values comparison. AIC is based on the principle of parsimony and helps to identify the model that accounts for the most variation with the fewest variables; the model that best explains the data is that with the lowest AIC. AICc was used rather than AIC as this is preferred when the number of regressions' parameters is large relative to sample size (Burnham and Anderson, 1998). The AICc value was calculated as in Iguchi (2013).

Kolmogorov–Smirnov tests, linear and non-linear regression

fits,  $r^2$  values and Extra Sum of Squares F-tests, sum of square of residuals (SSR) used for the AICc calculation were computed using SigmaPlot version 9.0, from Systat Software, Inc., San Jose California USA, www.sigmaplot.com.

### Geometric morphometrics

Landmark configurations were digitised by using TpsDig 2.16 (Rohlf, 2010) as shown in Fig. 1: 5 landmarks on the lateral view of the male and female head horns and 5 landmarks (1–5) and 6 semilandmarks (6–11) on the lateral view of the male pronotal horn. These were chosen for their relative ease of identification and ability to capture the general shapes. Superimposition of landmark configurations and subsequent principal component analyses of shape variables (PCA) were performed using MorphoJ (Klingenberg, 2011).

To assess the effect of body size on head and pronotal horn morphology, a series of multivariate tests for a general linear model predicting shape variation as a function of body size was computed (Bookstein, 1991); the overall significance of multivariate regressions was assessed by Wilks-Lambda and Goodall F-tests (Goodall, 1991). All tests and resulting deformation grids were performed in tpsRegr 1.37 (Rohlf, 2009).

### Trade-off between head and pronotal horns

To investigate the developmental correlations between head and pronotal horns, we calculated the expected head and pronotal horn lengths for each individual's body size: to calculate the

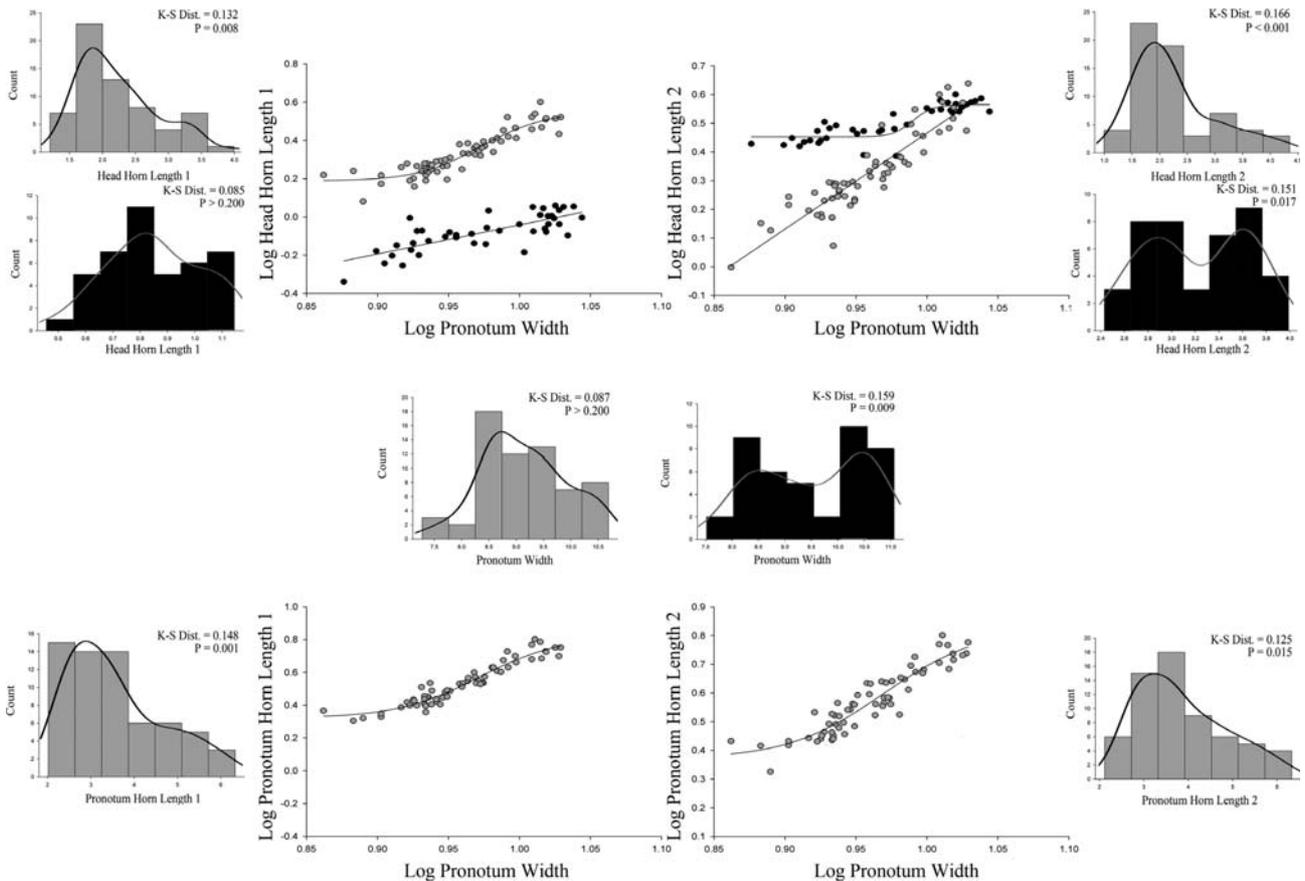
expected horn length we used the regression models obtained earlier that best described the horn length-body size scaling relationship. We calculated horn length residuals (the difference between observed and expected values) for each horn type, and we then tested for possible correlations between them by using a standard linear regression model (Pizzo et al., 2012). Resource allocation trade-offs during development should be evidenced by a negative correlation between horn length residuals (Emlen, 2001).

## RESULTS

### Static allometries

In males, pronotum width (PW) showed a normal distribution; both head (HHL1 and HHL2) and pronotal horn (PHL1 and PHL2) length distribution failed Kolmogorov-Smirnov tests. However, the visual inspection of the frequency histograms for those traits advocated a left-skewed distribution, but applying a log transformation to the data (May, 1981; Gouws et al., 2011), all of them showed a log-normal distribution (log-transformed HHL1: K-S Dist. = 0.111 and p-value = 0.053; log-transformed HHL2: K-S Dist. = 0.101 and p-value = 0.108 ; log-transformed PHL1: K-S Dist. = 0.104 and p-value = 0.086; log-transformed PHL2: K-S Dist. = 0.079 and p-value > 0.200).

In females, HHL2 and PW failed Kolmogorov-Smirnov



**Fig. 2.** Static allometries of log-log cephalic (HHL1, HHL2) and pronotal (PHL1, PHL2) horn lengths versus body size for females (black circles) and males (grey circles). For each allometry, the best fitting regression model, resulting from Extra Sum of Squares F-test, is shown. Frequency histograms of the distribution of female body size, HHL1 and HHL2 (black histograms) and male body size, HHL1, HHL2, PHL1 and PHL2 measures (grey histograms). K-S distance values and p values from Kolmogorov-Smirnov tests are shown for each graph.  $P > 0.200$  suggests a normal distribution for the data. Kernel density curves on frequency histograms of the distribution of each trait size are shown in black (on grey histograms of male traits) and in grey (on black histograms of female traits).

tests (see Fig. 2), even when log-transformed (log-transformed HHL2: K-S Dist. = 0.165 and p-value = 0.006; log-transformed PW: K-S Dist. = 0.164 and p-value = 0.006).

**Table 1.** R<sup>2</sup> values resulting from both linear and non-linear regression fitted to each allometry, F and p values from Extra Sum of Squares F-test in *Ceratophyus rossii*.

Trait	Female			Male			F	p
	Linear	Non-linear	F-test	Linear	Non-linear	F-test		
	R <sup>2</sup>	R <sup>2</sup>	F	R <sup>2</sup>	R <sup>2</sup>	F		
HHL1	0.59	–	–	0.81	0.87	12.16	< 0.001	
HHL2	0.27*	0.78	–	0.82	0.84	2.13	0.126	
PHL1				0.89	0.92	12.21	< 0.001	
PHL2				0.84	0.87	5.16	0.009	

Note: For P < 0.05, the non-linear fit model was accepted. Empty values (–) meant that no sigmoidal fit curve was found, except for female HHL2, where the segmented linear regression (\*) has not allowed a comparison with the sigmoidal model by running the Extra Sum of Squares F-test.

**Table 2.** Parameters of regression fit for each trait of *Ceratophyus rossii*. For linear regressions slope and intercept were shown; for non-linear regressions the four parameters were shown.

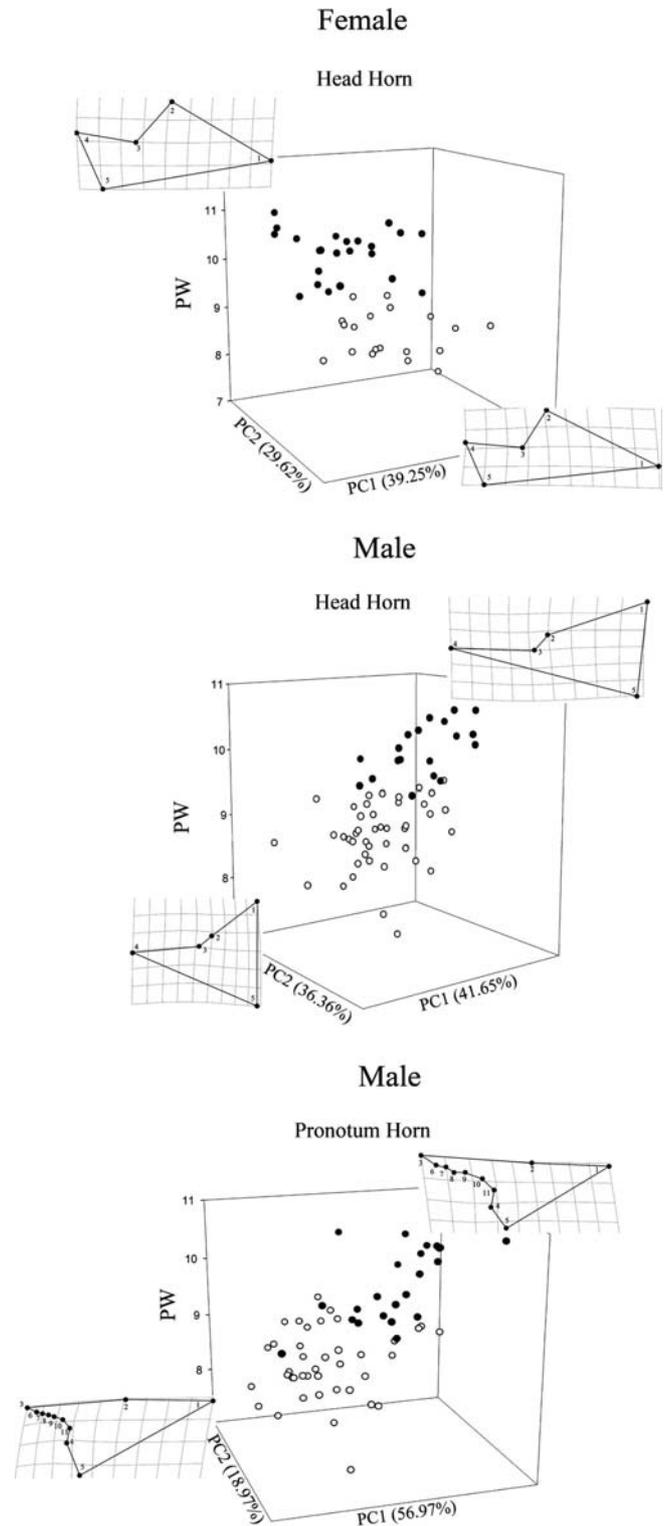
Trait	Parameter	Female	Male
HHL1	a	–	0.37
	b	1.51	42.44
	c	–	0.97
	y <sub>0</sub>	–1.56	0.19
HHL2	a	0.11	–
	b	<b>140.31</b>	3.35
	c	0.99	–
	y <sub>0</sub>	0.45	–2.88
PHL1	a		0.50
	b		33.13
	c		0.97
	y <sub>0</sub>		0.32
PHL2	a		0.47
	b		27.98
	c		0.97
	y <sub>0</sub>		0.37

Note: Non-linear regression parameters were a (trait size range), b (slope), c (body size at the point of inflection of the curve) and y<sub>0</sub> (minimum trait size). Bold value meant a not significant parameter.

**Table 3.** Values resulting from AICc comparison between linear and non-linear fit model in *Ceratophyus rossii*. SSR indicates the sum of square of residuals and AICc the corrected Akaike’s information criterion. The lowest AICc values for each model for each trait is indicated in bold.

Trait	Female				Male			
	Linear		Non-linear		Linear		Non-linear	
	SSR	AICc	SSR	AICc	SSR	AICc	SSR	AICc
HHL1	0,1486	–236,423	–	–	0,1462	–381,746	0,1036	<b>–402,8</b>
HHL2	0,0412	–290,302	0,0319	<b>–300,012</b>	0,2074	<b>–359,716</b>	0,1935	–359,071
PHL1					0,1196	–394,398	0,0846	<b>–415,564</b>
PHL2					0,1201	–394,135	0,1022	<b>–403,657</b>

Note: Empty values (–) meant that no sigmoidal fit curve was found and the SSR and AICc values cannot be calculated.



**Fig. 3.** Three-dimensional scatter plots of Principal Components 1 and 2 from shape variable PCA vs. body size (PW) for each structure analyzed by means of geometric morphometrics in *Ceratophyus rossii*. The percentage of variance explained by each principal component is shown in bracket. In each plot, the smaller size class was represented with white points, and the bigger size class with black points. Deformation grids representing the shape of each structure at the minimum (bottom) and maximum (top) value of the body size are shown on the corners.

Their distribution appears to be bimodal. HHL1 measures were normally distributed (see Fig. 2).

Plots of log-log measures of pronotum width (as a proxy for body size) versus horn length are shown in Fig. 2.

The presence of segmented regressions, tested by running Segreg (Oosterbaan, 1994) software, was suggested only for female HHL2. However, before the break-point (whose value on the x-axis was 0.98) there is no significant relationship between HHL2 and body size, and after which there is a linear regression. As the coefficient of determination of the segmented regression was low (0.27) and a parameter of the regression line (the intercept) was not significant, we also tried to fit a non-linear regression to these data. HHL2 allometry showed a strong increase of the fit with the sigmoidal regression, even if the parameter  $b$  was not significant. Female HHL1 scaled linearly with pronotum width (Fig. 2; Table 1; Table 2).

In males, a sigmoidal regression model led to a modest, but significant, increase in the fit for horn allometries; the extra-sum of squares F-test (Table 1) revealed that the four parameter non-linear regression was a more appropriate model of growth to describe male horn development than a linear reaction norm for all traits except HHL2, where linear seemed more preferable. Parameters of each regression equation are shown in Table 2. The Gompertz equation fitted to the same data produced a sigmoidal curve almost identical to that calculated with the four-parameter sigmoidal equation. Results of AICc comparisons between linear and non-linear models are shown in Table 3. The two analytical approaches (AICc and  $r^2$  comparisons) give the same basic results.

### Geometric morphometrics

Taking the point of inflection of the sigmoid curve as a threshold (Table 2), we divided the sample into two groups of small and large specimens.

Values of the first two principal component scores obtained from the PCA of head and pronotal horn shape variables obtained from the GPA were plotted on an axis system together with pronotum width (PW) measures. Overall, PC1 and PC2 described more than 80% of total head and pronotal horn shape variation (Fig. 3).

The first principal component showed a stronger correlation between both horn shapes and body size. The head horn of the smallest male was shorter, wider and with a more linear back edge; the largest male, instead, bore a more pointed head horn, with a curve on the back edge, forming a hump at landmark 2. Shape variations in the pronotal horn were the most evident: smaller specimens had a shorter horn with the dorsal edge inclined downwards and

the ventral edge drawing an almost flat curve; larger specimens had a longer horn with a straight dorsal edge and a ventral edge drawing a wavy line with peaks at semi-landmarks 6 and 8. Also, the line between landmarks 4 and 5 was longer at lower body size, while it was shortened by the downward movement of landmark 4 at a larger size. Comparing the head horn shape of minimum and maximum female sizes, it could be seen that, with the increase of body size, there was an elongation of the frontal line of the horn (the distance between landmarks 4 and 5) and a backwards movement of landmark 2.

Multivariate regressions between body size and head and pronotal horns, as described by partial warps, show the correlation between body size and horns, giving statistically significant results both for Wilks' Lambda and Generalized Goodall F-tests, as shown in Table 4.

### Trade-off between head and pronotal horns

We did not find a negative correlation between head and pronotal horn length residuals. The linear regression equation had a low  $r^2$  value ( $r^2 = 0.35$ ) and only one significant parameter, the positive slope ( $b = 0.48$ ;  $P < 0.001$ ).

## DISCUSSION

This paper describes for the first time the horn size and shape patterns of a beetle, *C. rossii*, belonging to the Geotrupidae, a family that has received little attention from researchers with regard to the study of the horn expression. The results obtained for male (head and pronotal) and female (head) horns show rather peculiar and complex patterns, which enrich the landscape of horn expression models described to date in other scarab beetles.

The main findings of this study can be summarized as follows:

1) In males, both horn and pronotum measures have a log-normal distribution (left skewed in the no log-transformed dataset), suggesting that males of *C. rossii* are not dimorphic. However, all the horn-body size scaling relationships seem better described by a non-linear (sigmoidal) but continuous allometry, with the unique exception of the length of the posterior part of the head horn (Fig. 2, Table 1). Moreover, significant horn shape changes along with variation in body size suggest that bigger animals are not simply the enlarged versions of the smaller ones (Fig. 3, Table 4).

2) Females show an allometric pattern that was difficult to interpret, suggesting intrasexual dimorphism. Measures of head horn and pronotum did not follow a normal distribution but appeared bimodally distributed. The bimodal distribution observed is unlikely to have arisen due to sampling error: this pattern is in fact reflected also by: a) the non lin-

**Table 4.** Values resulting from multivariate regression analysis in *Ceratophyus rossii*. Test values (Wilks'  $\lambda$ ,  $F_s$  and  $F$ ), numbers of degrees of freedom ( $df1$  and  $df2$ ) and p values were reported.

Sex	Trait	Wilks' $\lambda$				Generalized Goodall F-test			% var. explained
		Wilks' $\lambda$	$F_s$	$df1, df2$	$p$	$F$	$df1, df2$	$p$	
Female	Head horn	0.56	4.59	6, 35	0.002	6.60	6, 240	< 0.001	15%
Male	Head horn	0.48	9.94	6, 56	< 0.001	10.99	6, 366	< 0.001	16%
	Pronotal horn	0.39	3.68	18, 44	< 0.001	27.14	18, 1098	< 0.001	31%

Note: In the last column is shown the percentage of shape variation explained by body size variance.

ear/segmented allometry of the head horn (HHL2, the dorsal measure better describing the real dimensional variation of the horn, following crests and troughs) (Fig. 2); b) the significant horn shape change along with body size variation (Fig. 3, Table 4).

3) In males, no evidence supporting the occurrence of a developmental trade-off between head and pronotal horn was found. There was instead a weak significant positive correlation between head and pronotal horn length residuals, suggesting that males that express a relatively long head horn also developed a relatively long horn on the pronotum.

These findings have been compared with the patterns of male and female horn expressions found in other horned beetles, in particular with the best-studied *Onthophagus* species, which have virtually monopolized the scientific literature on this topic. Due to the lack of information available on almost all aspects of the biology of *C. rossii*, most of the evaluations we can make here have an exploratory nature, and must be considered as a track to drive more focused studies on the reproductive behavior, ecology and life history of this species.

1) Shape changes along with body size increase and sigmoidal allometric relationships between horn length and body size are frequently found in other Scarabaeinae and Dynastinae beetles (i.e. in *Onthophagus* (Emlen, 2001; Emlen et al., 2005; Macagno et al., 2009; Pizzo et al., 2012); *Xylotrupes* (Rowland, 2003) and *Calchosoma* (Kawano, 1995)): when these patterns are accompanied by non-linear allometry and by a clear bimodality in horn length frequency distributions, they can reveal a threshold-dependent mechanism of horn expression, with a developmental switch point separating major and minor morphs within males. However, in *Ceratophyus rossii* males, even if larger males seem to be not simply expanded versions of the smallest ones, no such bimodality in horn length or in pronotum width frequency distributions was found. Moreover, the very weak difference in the goodness of the fit between linear and non-linear allometry, although in favor of the sigmoid, suggests a not well defined boundary between a linear and a non-linear model of growth: this kind of deviations from simple linearity in static allometries, without other clues suggesting a threshold-dependent mechanism of horn expression, can be interpreted as the results of a competition for limited resources within the pupae (Nijhout and Wheeler, 1996; Knell et al., 2004) and changes in the nature of selection on body parts with increasing body size (Pomfret and Knell, 2006; Kojima et al., 2014). Moreover, our frequency distribution of horn and body size showed that intermediate phenotypes are selectively favored: this occurs when the developmental system imposes constraints on maximum body size in smaller males and minimum body size in larger males and horn length changes rapidly over a narrow range of body sizes (Rowland, 2003).

Hongo (2007), studying a population of the Dynastinae *Trypoxylus dichotomus septentrionalis*, observed a non-bimodal distribution for the measures of horns and body size and a non-linear model of growth for the horn. He attributed the non-linear but continuous allometry to rather low male-male competition, as a result of a low density of individuals in the populations studied, and the non bimodal but skewed

distribution (in this case slightly biased in favor of large males) to the fact that larger males gained more reproductive success than minor males. As *C. rossii* is a rare and localized member of the winter beetle fauna with a precarious conservation status, we hypothesize that population density and, consequently, male-male competition may be quite low; however, more focused analyses should be conducted to understand whether the left skewed distribution found in *C. rossii* might indicate a greater reproductive success for the minor forms in a context of low male-male competition (Buzatto et al., 2012).

2) Since males are typically the more adorned sex, the majority of studies have focused solely on secondary sexual trait evolution in males (Kijimoto, 2012). The evolution of such traits in females has generated much less interest and has only rarely been explored. To our knowledge, there are no studies on female horn allometries in beetle species not belonging to the genus *Onthophagus*, so it is not possible to place our results on *Ceratophyus* in a wider range of comparisons. Emlen et al. (2005) mapped the presence/absence of horns in females, major and minor males onto a phylogeny of the genus *Onthophagus*, to explore how horn development has changed over time. In all cases but one, when females bear horns, they had the same basic shape as corresponding horns expressed in males. The single exception involved the head horn of *O. sagittarius* females, which was qualitatively different from the corresponding males horn as for *C. rossii*). In all that cases, and in *O. sagittarius* too, female horns showed linear allometry (Emlen et al., 2005; Moczek, 2009). So, the non-linear allometry found in the female horn of *C. rossii* seems to be an important exception to this general rule. Unfortunately, at the present there are no detailed observations on the reproductive behavior, female competition and parental care for the *C. rossii* female which may help to understand the reasons for this dimorphism in females. We only know, from a study on the congeneric *C. hoffmanseggii* (Klemperer, 1984), that females bury dung to form a series of underground brood masses, and that females help males in resisting intruders in the entrance to their burrows by a head-to-head pushing contest. As male-male competition played a role in shaping the pattern of horn expression, thus similar selective forces should be able to shape female horn expression pattern; for example, Watson and Simmons (2010) found that *O. sagittarius* females with longer horns produced more brood balls, gaining greater resource acquisition and achieving higher fitness, a rare example of sexual selection via female contest competition acting on female secondary sexual traits. This kind of force should be a good candidate to produce dimorphism in females even in a threshold dependent manner. However, experimental studies on the reproductive behaviour of *C. rossii* are needed to evidence if the size of the cephalic horn might be subject to sexual selection.

3) Developmental tradeoffs have been described by phenotypic correlations in a certain number of species, especially between traits that grow in close proximity or at the same time (Kawano, 1995, 1997; Klingenberg and Nijhout, 1998; Nijhout and Emlen, 1998; Moczek and Nijhout, 2004). Experimental studies on *Onthophagus* species have also suggested that, if there is an overlap between growth periods, even distant structures, such as other horns

and copulatory organs, can engage in a developmental tradeoff (Moczek and Nijhout, 2004; Parzer et al., 2008; Pizzo et al., 2012). In *Ceratophyus rossii* no evidence supporting the occurrence of a tradeoff between head and pronotal horn development was found: there was instead a weakly significant, positive correlation between head and pronotal horn length residuals. A very similar scenario was described by Moczek et al. (2004) for the developmental relationships between head and pronotal horns in *Onthophagus watanabei*: male *O. watanabei* develop a pair of long curved horns on the head which scale in a largely linear fashion with body size and a thoracic horn which develop in a strongly body size threshold-dependent fashion: no evidence of trade off between the two horn type was found, but a positive correlation. The authors suggested that the lack of trade off between head and pronotal horns could be explained by the horns' location in different body regions and the lack of overlap in timing of horn development; they reported, in support of their hypothesis, that large *Onthophagus nigriventris* initiate the growth of their single thoracic horn at least 24 h earlier than *O. taurus* initiate the growth of their paired head horns. Besides, the positive correlation might arise if some aspect of the regulation of horn growth is shared between horn types and affects different horns in the same fashion.

The uniqueness and distinctiveness of the patterns found in *C. rossii* suggest us the importance of focusing efforts on widening the study of all possible horn expression patterns going beyond those species currently considered as models for the whole horned beetle group. The results obtained from *C. rossii* are also important in relation to the peculiar ecological status of rare, endangered stenoendemic species; we cannot predict how long there is left to investigate the model of growth evolved in such species characterized by rarity and extinction risk.

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#### REFERENCES

- Agoglietta R, Moreno CE, Zunino M, Bonsignori G, Dellacasa M (2012) Cumulative annual dung beetle diversity in Mediterranean seasonal environments. *Ecol Res* 27: 387–395
- Akaike H (1973) Information theory and an extension of the maximum likelihood principle. Budapest: Proc. 2nd Inter. Symposium on Information Theory 267–281
- Arrow GJ (1951) Horned beetles: A study of the fantastic in nature. W Junk, The Hague
- Audisio P (2003) Sandy shores and their animals. In: S Ruffo, editor. Sand dunes and beaches: environments between land and sea. Udine: Edizioni del Museo Friulano di Storia Naturale: 59–112
- Bargagli G (1987) Materiali per la fauna entomologica dell'Isola di Sardegna. *Coleotteri*. *Boll Soc Entomol Ital* 4: 279–290
- Bisby FA, Roskov YR, Orrell TM, Nicolson D, Paglinawan LE, Bailly N, Kirk PM, Bourgoin T, Baillargeon G, Ouvrard D (2011) Species 2000 & ITIS Catalogue of Life: 2011 Annual Checklist
- Bookstein FL (1991) Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge
- Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach. New York: Springer, p 488
- Buzatto BA, Tomkins JL, Simmons LW (2012) Maternal effects on male weaponry: female dung beetles produce major sons with longer horns when they perceive higher population density. *BMC Evol Biol* 12: 118
- Carpaneto GM (1976) Ritrovamento di *Ceratophyus rossii* Jekel nel rifugio faunistico del W.W.F. Lago di Burano (Coleoptera: Geotrupidae). *Boll Ass Rom Entomol* 30: 27–29
- Cartwright OL (1966) A new species of *Ceratophyus* found in California (Coleoptera: Scarabaeidae). *California Dept of Food and Agriculture Occasional Papers in Entomology* 9: 1–7
- Cunha RL, Verdú JR, Lobo JM, Zardoya R (2011) Ancient origin of endemic Iberian earth-boring dung beetles (Geotrupidae). *Mol Phylogenet Evol* 59: 578–586
- Dellacasa M (2004) Scarabaeoidea “Laparosticta” di Corsica. *Atti della Società toscana di Scienze naturali, Memorie Serie B (Supplemento)* 110: 1–361
- Eberhard WG (1978) Fighting behavior of male *Golofa porter* beetles (Scarabaeidae: Dynastinae). *Psyche* 83: 292–298
- Eberhard WG, Gutiérrez EE (1991) Male dimorphisms in beetles and earwigs and the question of developmental constraints. *Evolution* 45: 18–28
- Emlen DJ (1994) Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc R Soc Lond (Biol)* 256: 131–136
- Emlen DJ (1997) Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behav Ecol Sociobiol* 41: 335–341
- Emlen DJ (2000) Integrating development with evolution: A case study with beetle horns. *Bioscience* 50: 403–418
- Emlen DJ (2001) Costs and the diversification of exaggerated animal structures. *Science* 291: 1534–1536
- Emlen DJ (2008) The evolution of animal weapons. *Annu Rev Ecol Syst* 39: 387–413
- Emlen DJ, Nijhout HF (1999) Hormonal control of male horn length dimorphism in the horned beetle *Onthophagus taurus*. *J Insect Physiol* 45: 45–53
- Emlen DJ, Nijhout HF (2000) The development and evolution of exaggerated morphologies in insects. *Annu Rev Entomol* 45: 661–708
- Emlen DJ, Nijhout HF (2001) Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae): A second critical period of sensitivity to juvenile hormone. *J Insect Physiol* 47: 1045–1054
- Emlen DJ, Marangelo J, Ball B, Cunningham CW (2005) Diversity in the weapons of sexual selection: horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). *Evolution* 59: 1060–1084
- Emlen DJ, Corley Lavine L, Ewen-Campen B (2007) On the origin and evolutionary diversification of beetle horns. *PNAS* 104: 8661–8668
- Goodall C (1991) Procrustes methods in the statistical analysis of shape. *Roy Statist Soc, London* 53: 285–339
- Gouws EJ, Gasaton KJ, Chown SL (2011) Intraspecific Body Size Frequency Distributions of Insects. *PLoS ONE* 6: e16606. doi:10.1371/journal.pone.0016606
- Grimaldi D, Engel M (2005) *Evolution of the Insects*, Cambridge University Press, New York
- Hongo Y (2003) Appraising behaviour during male-male interaction in the Japanese horned beetle *Trypoxylus dichotomus septentrionalis* (kono). *Behaviour* 140: 501–517
- Hunt J, Simmons LW (2001) Status-dependent selection in the dimorphic beetle *Onthophagus taurus* *Proc R Soc Lond (Biol)* 268: 2409–2414
- Iguchi Y (2001) Differences in the frequency of fights between minor

- and major males in the horned beetle *Trypoxylus dichotomus septentrionalis* (Coleoptera: Scarabaeidae). *Entomol Rev Jpn* 56: 11–14
- Iguchi Y (2003) Male mandible trimorphism in the stag beetle *Dorcus rectus* (Coleoptera: Lacanidae). *Eur J Entomol* 110: 159–163
- Kawano K (1995) Horn and wing allometry and male dimorphism in giant rhinoceros beetles (Coleoptera: Scarabaeidae) of tropical Asia and America. *Ann Entomol Soc Am* 88: 92–99
- Kawano K (1997) Cost of evolving exaggerated mandibles in stag beetles (Coleoptera: Lucanidae). *Ann Entomol Soc Am* 90: 453–461
- Kijimoto T, Costello J, Tang Z, Moczek AP, Andrews J (2009) EST and microarray analysis of horn development in *Onthophagus* beetles. *BMC Genomics* 10: 504
- Kijimoto T, Pespeni M, Beckers O, Moczek AP (2012) Beetle horns and horned beetles: emerging models in developmental evolution and ecology. *Wiley Interdisciplinary Reviews in Developmental Biology* 81: 405–418
- Klemperer HG (1984) Nest construction, fighting, and larval behaviour in a geotrupine dung beetle, *Ceratophyus hoffmannseggii* (Coleoptera: Scarabaeidae). *J Zool* 204: 119–127
- Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour* 11: 353–357
- Klingenberg CP, Nijhout HF (1998) Competition among growing organs and developmental control of morphological asymmetry. *Proc R Soc Lond (Biol)* 265: 1135–1139
- Knell RJ (2009) On the analysis of non-linear allometries. *Ecol Entomol* 34: 1–11
- Kojima W, Sugiura S, Makihara H, Ishikawa Y, Takanashi T (2014) Rhinoceros Beetles Suffer Male-Biased Predation by Mammalian and Avian Predators. *Zool Sci* 31: 109–115
- Krell FT (2007) Catalogue of fossil Scarabaeoidea (Coleoptera: Polyphaga) of the Mesozoic and Tertiary. Version 2007. Denver Museum of Nature and Science Technical Report 2007 8: 1–79
- L.R. 6 Aprile 2000, n. 56 “Norme per la conservazione e la tutela degli habitat naturali e seminaturali, della flora e della fauna selvatiche. - Modifiche alla Legge Regionale 23 gennaio 1998, n. 7.” *Bollettino Ufficiale della Regione Toscana*. 17
- Macagno ALM, Pizzo A, Roggero A, Rolando A, Palestini C (2009) Horn polyphenism and related head shape variation in a single-horned dung beetle: *Onthophagus (Palaeonthophagus) fracticornis* (Preyssl, 1790) (Coleoptera: Scarabaeidae). *J Zool Syst Evol Res* 47: 96–102
- Macagno ALM, Pizzo A, Parzer HF, Palestini C, Rolando A, Moczek AP (2011) Shape – but Not Size – Codivergence between Male and Female Copulatory Structures in *Onthophagus* Beetles. *PLOS ONE* 6(12): e28893 DOI: 10.1371/journal.pone.0028893
- May RM (1981) Models for single populations. In: “Theoretical ecology”, Ed by RM May, Blackwell Scientific, Oxford, pp 5–29
- Moczek AP (1998) Horn polyphenism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behav Ecol* 9: 636–641
- Moczek AP (2006) A matter of measurements: challenges and approaches in the comparative analysis of static allometries. *Am Nat* 167: 606–611
- Moczek AP (2009) Developmental plasticity and the origins of diversity: a case study on horned beetles. In: “Phenotypic plasticity in insects: mechanisms and consequences” Ed by N Ananthakrishnan, D Whitman, Science Publishers, Plymouth, pp 81–134
- Moczek AP (2011) Evolution and development: *Onthophagus* beetles and the evolutionary developmental genetics of innovation, allometry, and plasticity. In: “Dung Beetle Ecology and Evolution” Ed by LW Simmons, J Ridsdill-Smith, Wiley-Blackwell, pp 126–151
- Moczek AP, Emlen DJ (1999) Proximate determination of male horn dimorphism in the beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *J Evol Biol* 12: 27–37
- Moczek AP, Emlen DJ (2000) Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: Do alternative reproductive tactics favour alternative phenotypes? *Anim Behav* 59: 459–466
- Moczek AP, Nijhout HF (2003) Rapid evolution of a polyphonic threshold. *Evol Dev* 5: 259–268
- Moczek AP, Nijhout HF (2004) Trade-offs during the development of primary and secondary sexual traits in a horned beetle. *Am Nat* 163: 184–191
- Moczek AP, Rose DJ (2009) Differential recruitment of limb patterning genes during development and diversification of beetle horns. *PNAS* 106: 8992–8997
- Moczek AP, Brühl CB, Krell FTK (2004) Linear and threshold dependent expression of secondary sexual traits in the same individual: insights from a horned beetle. *Biol J Linnean Soc* 83: 473–480
- Moczek AP, Cruickshank TE, Shelby A (2006) When ontogeny reveals what phylogeny hides: Gain and loss of horns during development and evolution of horned beetle. *Evolution* 60: 2329–2341
- Motulski HJ, Christopoulos A (2003) Fitting models to biological data using linear and nonlinear regression: A practical guide to curve fitting. GraphPad Software, Inc; San Diego, CA
- Munguia-Steyer R, Buzatto BA, Machado G (2012) Male dimorphism of a neotropical arachnid: harem size, sneaker opportunities, and gonadal investment. *Behavioral Ecology* 23: 827–835
- Nijhout HF (2003) The control of body size in insects. *Dev Biol* 261: 1–9
- Nijhout HF, Emlen DJ (1998) Competition among body parts in the development and evolution of insect morphology. *PNAS* 95: 3685–3689
- Nijhout HF, German RZ (2012) Developmental causes of allometry: new models and implications for phenotypic plasticity and evolution. *Integr Comp Biol* 52: 43–52
- Nijhout HF, Grunert LW (2002) Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. *PNAS* 99(24): 15446–15450. doi:10.1073/pnas.242548399
- Nijhout HF, Wheeler DE (1996) Growth models of complex allometries in holometabolous insects. *Am Nat* 148: 40–56
- Nistri A, Vanni S, Bartolozzi L, Bambi S (1991) Contributo della conoscenza dell'entomofauna del Parco Naturale della Maremma. II. Coleoptera: Scarabaeidae (Scarabeinae e Coprine) e Geotrupidae. *Atti del Museo Civico di Storia Naturale di Grosseto* 14: 5–13
- Oosterbaan RJ (1994) Frequency and regression analysis. In: “Drainage Principles and Applications” Ed by HP Ritzema, Wageningen, the Netherlands: ILRI. pp 175–224
- Painting CJ, Holwell GI (2013) Exaggerated Trait Allometry, Compensation and Trade-Offs in the New Zealand Giraffe Weevil (*Lasiorynchus barbicornis*). *Plos One* DOI: 10.1371/journal.pone.0082467
- Palestrini C, Rolando A, Laiolo P (2000) Allometric relationship and character evolution in *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Can J Zool* 78: 1199–1206
- Parzer HF, Moczek AP (2008) Rapid antagonistic coevolution between primary and secondary sexual characters in horned beetles. *Evolution* 62: 2423–2428
- Pizzo A, Roggero A, Palestini C, Del Pero M, Cervella P, Rolando A (2006a) Genetic and morphological differentiation patterns between sister species: the case of *Onthophagus taurus* and *O. illyricus* (Coleoptera: Scarabaeidae). *Biol J Linnean Soc* 89: 197–211
- Pizzo A, Mercurio D, Roggero A, Palestini C, Rolando A (2006b) Male differentiation patterns in two polyphenic sister species of the genus *Onthophagus* (Coleoptera: Scarabaeidae): a geometrical morphometric approach. *J Zool Syst Evol Res* 44: 54–

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- Pizzo A, Macagno ALM, Dusini S, Palestini C (2012) Trade-off between horns and other functional traits in two *Onthophagus* species (Coleoptera: Scarabaeidae). *Zoomorphology* 131: 57–68
- Rasmussen JL (1994) The influence of horn and body size on the reproductive behavior of the horned rainbow scarab beetle *Phanaeus difformis* (Coleoptera: Scarabaeidae). *J Insect Behav* 7: 67–82
- Rohlf FJ (2009) *TpsRegr*, version 1.37. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook (<http://life.bio.sunysb.edu/morph/>)
- Rohlf FJ (2010) *TpsDig*, digitize landmarks and outlines. Version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook (<http://life.bio.sunysb.edu/morph/>)
- Rowland JM (2003) Male horn dimorphism, phylogeny and systematics of rhinoceros beetles of the genus *Xylotrupes* (Coleoptera: Scarabaeidae). *Aust J Zool* 51: 213–258
- Shingleton AW, Frankino WA, Flatt T, Nijhout HF, Emlen DJ (2007) Size and Shape: The developmental regulation of static allometry in insects. *BioEssays* 29: 536–548
- Spiess AN, Neumeyer N (2010) An evaluation of R2 as an inadequate measure for nonlinear models in pharmacological and biochemical research: a Monte Carlo approach. *BMC Pharmacology* 10: 6
- Sugiura S, Yamaura Y, Makihara H (2007) Sexual and male horn dimorphism in *Copris ochus* (Coleoptera: Scarabaeidae). *Zool Sci* 24: 1082–1085
- Tomkins JL, Kotiaho JS, LeBas NR (2006) Major Differences in Minor Allometries: A Reply to Moczek. *Am Nat* Vol 167, pp 612–618
- Watson NL, Simmons LW (2010) Reproductive competition promotes the evolution of female weaponry. *Proc R Soc B* 277: 2035–2040
- Zunino M (1973) Il genere *Ceratophyus* Fish. (Coleoptera: Scarabaeoidea). *Boll Mus di Zool Univ Torino* 2: 9–40

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