Article

Sexual dimorphism and inter-individual variation in the rove beetle, *Creophilus maxillosus* L. (Col: Staphylinidae)

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Abstract
Sexual selection is expected to drive phenotypic differences between conspecific male and females, a widespread phenomenon known as sexual dimorphism. At the same time, individuals may exhibit some degree of intra-sexual variation. We examined the sexual dimorphism and inter-individual variation in different body parts of *Creophilus maxillosus* L. (Col: Staphylinidae), a cosmopolitan rove beetle commonly found on carrion. Male *C. maxillosus* had significantly wider head and pronotum, longer mandibles, and more distant eyes than females. The head width was positively correlated to mandible length, which may reflect stronger adductor muscles and higher bite force in larger individuals. The allometry of traits can be examined by plotting the logarithms of that specific trait against the logarithm of body size and determining the slope (b) of the regression line. Isometry occurs when b=1, i.e. the ratio of given traits to body size remains constant across individuals. Negative allometry occurs when b<1, i.e. larger individuals have relatively smaller traits in relation to body size. Positive allometry occurs when b>1, so that larger individuals have disproportionately larger traits. A positive allometry was found in head width (b=1.32), mandible length (b=2.28), and ocular distance (b=1.49) of males. Our results show that, particularly head size, mandible length and ocular distance are probably under sexual selection in males, while traits such as eye size are isometric to body size. The potential role of these traits in male-male combat as well as female attractiveness has been frequently documented in different insect taxa. The striking similarities in patterns of sexual dimorphism among independently evolved insects indicate that common evolutionary force(s) are probably at work.

Keywords *Creophilus maxillosus*; sexual dimorphism; mandible length; head width; allometry; inter-individual variation.

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1 Introduction

Sexual dimorphism, the phenotypic difference between conspecific males and females, is a common phenomenon in animal kingdom including insects (Fairbairn, 1997). In most cases, this term is used for secondary sexual characters, like the elongated mandibles of stag beetles (Col: Lucanidae), with no direct mechanical role in insemination (Andersson, 1994). Competition for mates, particularly among males, has been suggested to be the major factor underlying the evolution of intra-sexual dimorphism in several animal taxa (Bean and Cook, 2001; Berns, 2013). According to Darwin (1871), males should evolve a variety of weapons as a result of aggressive physical competition over reproductive access to females. Indeed, male fitness in most animal species is more correlated with mating success than female fitness, thus those traits that may contribute to mating success are under more intense sexual selection in males. Consequently, males are expected to exaggerate organs related to rivalry success (Kratochvil and Frynta, 2002; Buzatto et al., 2014). At the same time, males with exaggerated traits may be more attractive to females, a process that underlies inter-sexual dimorphism (Berns, 2013). For example, head width and mouthpart size have been shown to be correlated to reproductive success because those males with larger head and mouthparts are more attractive to females (Judge and Bonanno, 2008; Singh and Singh, 2014). As an alternative or complementary hypothesis for the pattern of sexual dimorphism, secondary sexual characters may be under negative selection in females, as proposed for head and mouthpart size in field crickets (Judge and Bonanno, 2008).

It has been generally accepted that exaggerated male traits in different animal taxa show positive allometry in relation to body size. This means that larger individuals have disproportionately larger secondary sexual characters than small individuals (Kodric- Brown et al., 2006; Bonduriansky, 2007). For example, male mandible length has been shown to be positively allometric to body size in different species of stag beetles (Kawano, 1997). In contrast, nonsexual traits are expected to exhibit negative allometry or isometry, i.e. they should grow relatively smaller than or at the same rate of body size. This rule is of such strength that some authors use the kind of allometry (i.e. positive allometry, isometry or negative allometry) to discriminate between sexual and nonsexual traits (Cuervo and Moller, 2009). On the other hand, sexual and nonsexual traits have been shown to interact with one another. This implies that any variation in one trait may be positively or negatively coupled to variation in another trait. For example, male field crickets with larger head have also larger mandibles (positive correlation) (Judge and Bonanno, 2008), while male horned beetles with longer horns have disproportionately small eyes (negative correlation) (Nijhout and Emlen, 1998).

Beetles (Order Coleoptera) with more than 360000 described species represent about 40% of all known insects (Bouchard et al., 2011). Although, most beetles lack conspicuous sexual dimorphism numerous examples have been documented in almost all major beetle groups and most sexually dimorphic traits have been shown to be positively allometric (Prado, 2013). In beetles, in which adult morphology is fixed once they emerge from pupa, intra-specific variation may results from either innate ontogenetic or developmental program and/or environmental conditions, particularly nutrition (Kawano, 2006). This variation provides ideal systems to explore the correlation among different traits as well as trait allometry. In this study, we examined the sexual dimorphism and inter-individual variation in the cosmopolitan rove beetle, *Creophilus maxillosus* L. (Col: Staphylinidae). Both larvae and adult *C. maxillosus* feed on carrion remains as well as maggots simultaneously existing on dead animals (Newton et al., 2000).

2 Materials and Methods

Creophilus maxillosus has widespread distribution in forests of northern Iran, where it acts on carrion of wild animals such as birds, rats, foxes, and pigs. To analyze sexual dimorphism, we collected 120 individuals (60 males, 60 females) from leaf litters of Nowshahr forests (north Iran) around carrion during May-June 2015. The samples were preserved in ethanol (75%). The head and prothorax of all specimens were detached using a scalpel and preserved separately in jars containing ethanol (75%). Images for each body part were captured using a digital camera (Canon, IXUS 132, Canon Inc. Japan). For all characters, images were magnified at the same scale using a stereomicroscope (Olympus SZ40, Japan) to reduce measurement errors.

For size measurements, the images were processed in ImageJ software. For each specimen, head width at the line drawn between the posterior margins of eyes, right and left mandible length, head length at the midline, right eye length, ocular distance, antennal distance, neck width, pronotum width at the widest point (near the front angles), and pronotum length at midline were measured (Fig. 1). Relative measures of eye diameter to head length, ocular distance to head width, head width to head length, and pronotum width to pronotum length were calculated and relationships between head width and right mandible length as well as that of head width to pronotum width were determined. The allometries of head width, eye diameter, ocular distance, and mandible length were examined by plotting the logarithms of these traits on *y*-axis against logarithm of body size on the *x*-axis. Trait allometry can further be evaluated by calculating the slope (b) of the regression line. Isometry occurs when b=1, i.e. the ratio of given traits to body size remains constant across individuals. Negative allometry occurs when b<1, i.e. larger individuals have relatively smaller traits in relation to body size. Conversely, positive allometry occurs when b>1, so that larger individuals have disproportionately larger traits (Zar, 1999). As the abdomen and neck provide unreliable results of body length due to telescopic property, we used the aggregated length of head and pronotum as an indicator of body size.

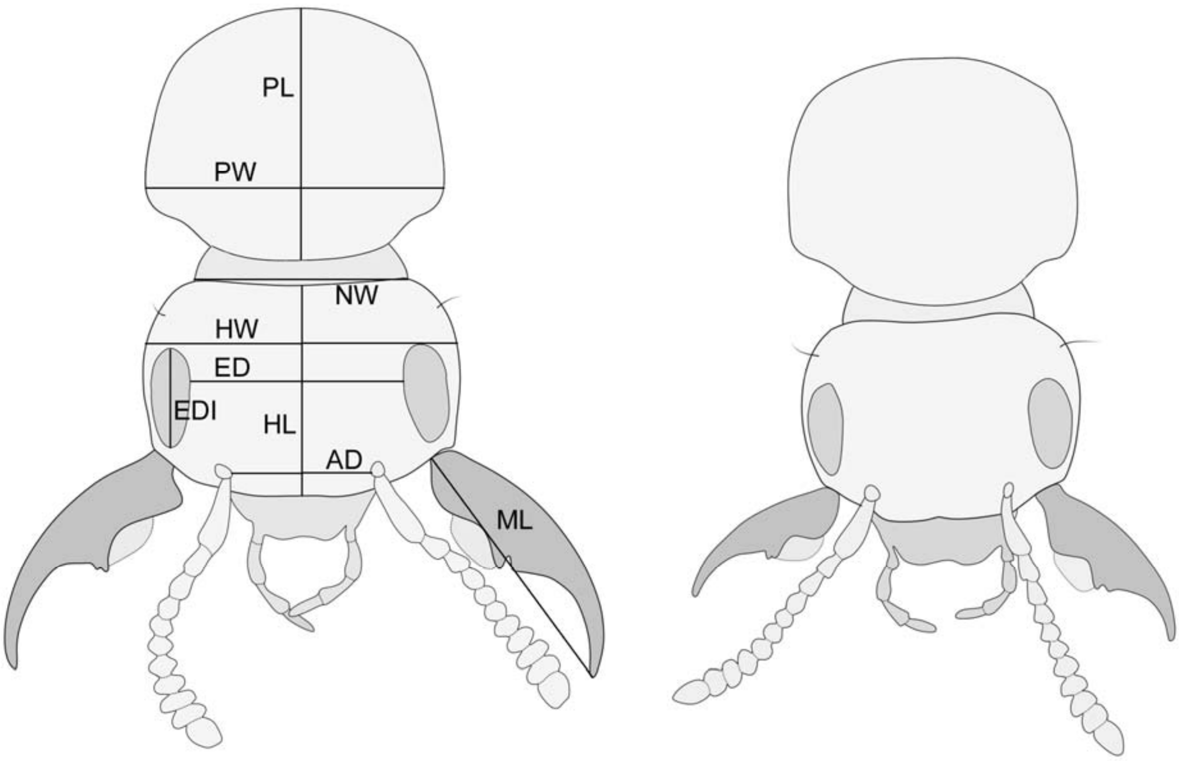

Fig. 1 General habitus of male (left) and female (right) *Creophilus maxillosus* and body parts measured on head and pronotum: HW: head width, HL: head length, OD: ocular distance, AD: antennal distance at base, ED: right eye diameter, ML: mandible length, NW: neck width, PW: pronotum width, PL: pronotum length.

Data were analyzed using SPSS computer software vr. 17.1. Data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov test and Levene’s test for equality of variance, respectively. The average values for all characters were compared by independent t-tests. The average length of right and left mandibles were exposed to one-way analysis of variance (ANOVA).

3 Results

Analysis of size showed that *C. maxillosus* exhibit sexual size dimorphism in nearly all studied characters including head width, head length, mandible length, eye diameter, ocular distance, antennal distance, neck width, pronotum width and pronotum length. Furthermore, the ratios between different parts of the body were statistically different among male and female beetles (Table 1, Fig. 1 & 2). The head of male *C. maxillosus* was averagely 1.4 times wider (t-test: df=118, t=11.56, P<0.01) and 1.36 times longer (t-test: df=118, t=13.38, P<0.01) than females (4.54 vs. 3.23 mm and 3.02 vs. 2.22 mm for head width and length, respectively). The ratio of head width to head length was also higher in male beetles (1.49 vs. 1.45, respectively) (Fig. 2B) (t-test: df=118, t=2.68, P<0.05). Additionally, both right and left mandible of male beetles were about 1.76 times longer than females (3.85 vs. 2.18 mm and 3.97 vs. 2.25 mm for right and left mandible, respectively). Although, no significant difference was found in the length of right and left mandibles of the same individuals, the left mandible in both male and female *C. maxillosus* tended to be slightly longer than right one (Fig. 2C) (one-Way ANOVA: F=81.19, P<0.001). Male *C. maxillosus* showed a significantly wider inter-individual variation in head width and length of mandibles compared to females (Fig. 2A & C).

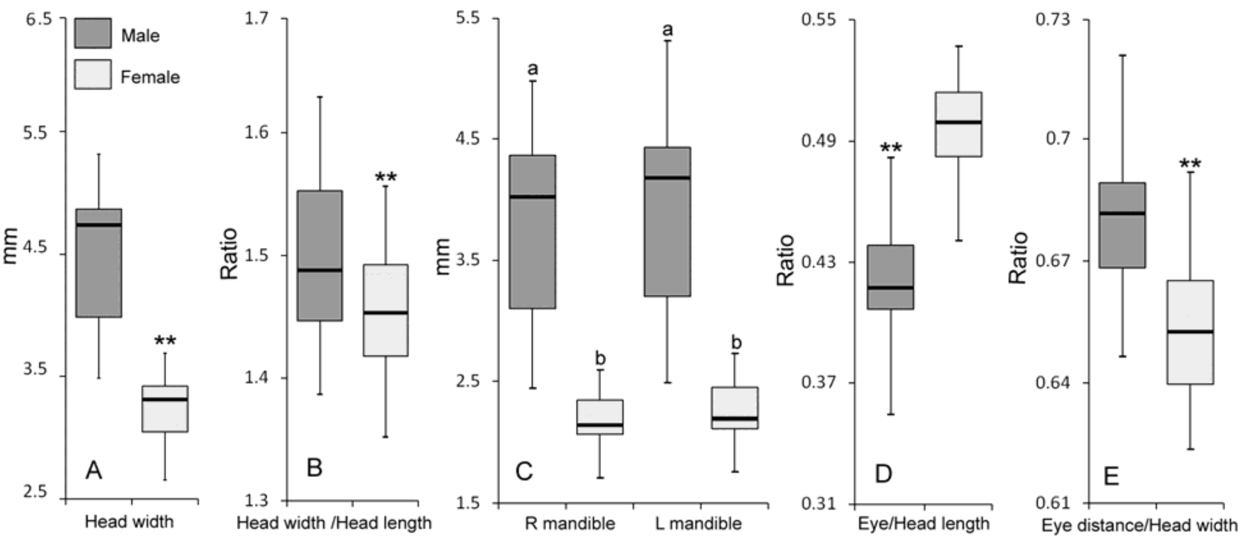

Fig. 2 Sexual dimorphism and inter-individual variation in head width (A), head width to head length ratio (B), right and left mandible length (C), eye diameter to head length ratio (D), and eye distance to head width ratio (E) in male and female *Creophilus maxillosus*, **: different at 0.01 level, different letters show differences at 0.05 level.

Male beetles had significantly larger eyes compared to females (Table 1), however, the ratio between eye diameter and head length was significantly higher in female beetles (Fig. 2D) (t-test: df=118, t=11.58, P<0.01). No significant relationship was found between eye diameter and head size of both male and female beetles (R^2=0.41). The ocular distance was significantly higher in male *C. maxillosus* (3.09 vs. 2.11, respectively) (Table 1). Additionally, male beetles had higher average of ratio between ocular distance to head width (0.68 vs. 0.65, respectively) (t-test: df=118, t=5.61, P<0.01) (Fig. 2E). In contrast, the ratio between antenna distance and head width was significantly higher in female *C. maxillosus* (Table 1).

Table 1 Summary of male and female *Creophilus maxillosus* traits expressed as mean ± SE.

Character	Male (n=60)	Female (n=60)	t	P-value
Eye diameter (mm)	1.24±0.01	1.09±0.01	5.92	P<0.01
Head length (mm)	3.02±0.05	2.22±0.03	13.38	P<0.01
Ocular distance (mm)	3.09±0.07	2.11±0.03	11.61	P<0.01
Antennal distance (mm)	2.09±0.05	1.49±0.02	9.88	P<0.01
Neck width (mm)	2.94±0.06	2.18±0.03	10.40	P<0.01
Pronotum width (mm)	4.41±0.08	3.56±0.05	8.62	P<0.01
Pronotum length (mm)	3.70±0.05	3.11±0.05	7.55	P<0.01
Pronotum width / pronotum length	1.19±0.007	1.14±0.006	4.67	P<0.01
Head width / Pronotum width	1.02±0.006	0.90±0.005	13.74	P<0.01
Head length + Pronotum length	6.72±0.01	5.33±0.07	10.44	P<0.01

The neck width, pronotum width and pronotum length of male *C. maxillosus* was respectively, 1.34, 1.23 and 1.18 times larger than those of females (Table 1). Furthermore, males had a significantly higher ratio of pronotum width to pronotum length (1.19 vs. 1.14) as well as the ratio between head width and pronotum width (1.02 vs. 0.90) (Table 1).

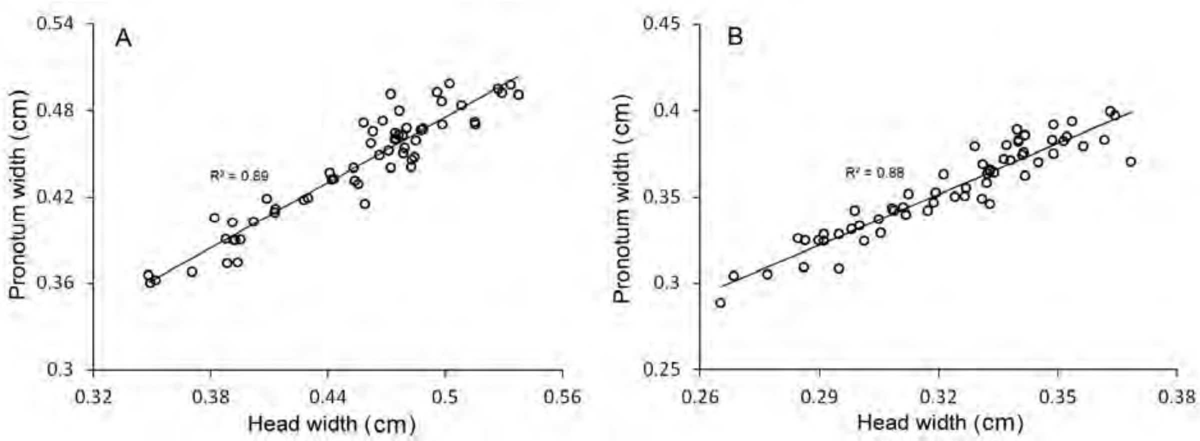

Fig. 3 The relationships between head width and pronotum width in male (A) and female (B) *Creophilus maxillosus*.

A positive linear relationship was found between the width of head and the width of pronotum in both male and female beetles, such that beetles with wider heads had also wider pronotums (Fig. 3). Similarly, the relationship between head width and mandible length was linear in both male and female *C. maxillosus* (Fig. 4).

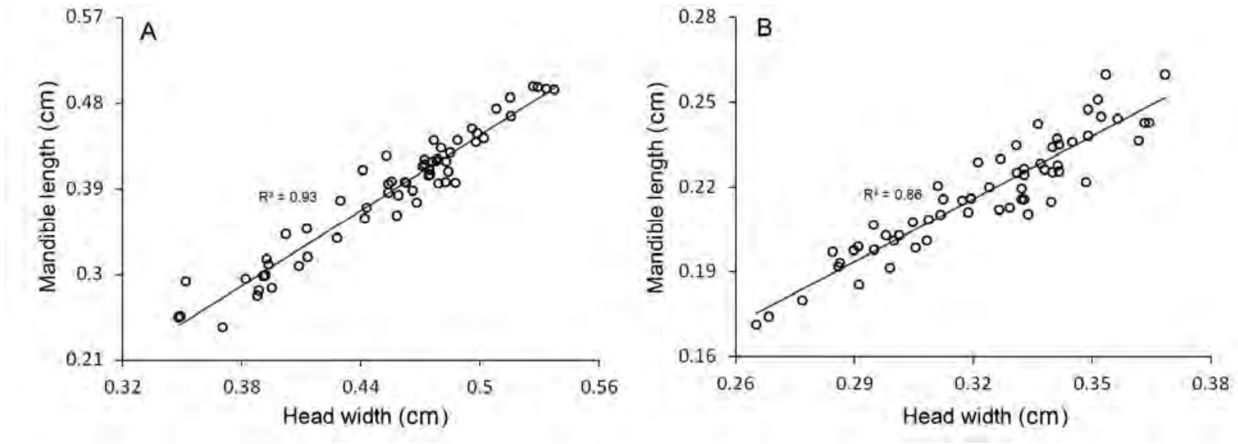

Fig. 4 The relationships between head width and right mandible length in male (A) and female (B) *Creophilus maxillosus*.

We examined the allometries of eye diameter, head width and ocular distance in male and female *C. maxillosus*. The mandible length in both males and females exhibited positive allometry, although, the allometric slope of male mandibles was about twice as large as that of females (2.28 vs. 1.16, respectively) (Fig. 5a). Significant positive allometries were found in head width and ocular distance of male beetles (*b*=1.32 and *b*=1.49, respectively), while these traits were almost isometric relative to body size in the females (*b*=1.05 and *b*=1.06, respectively) (Fig. 5b & d). The eye diameter exhibited negative allometry in both male and female *C. maxillosus*, with the allometric slope being smaller in males (*b*=0.60) than females (*b*=0.77) (Fig. 5c).

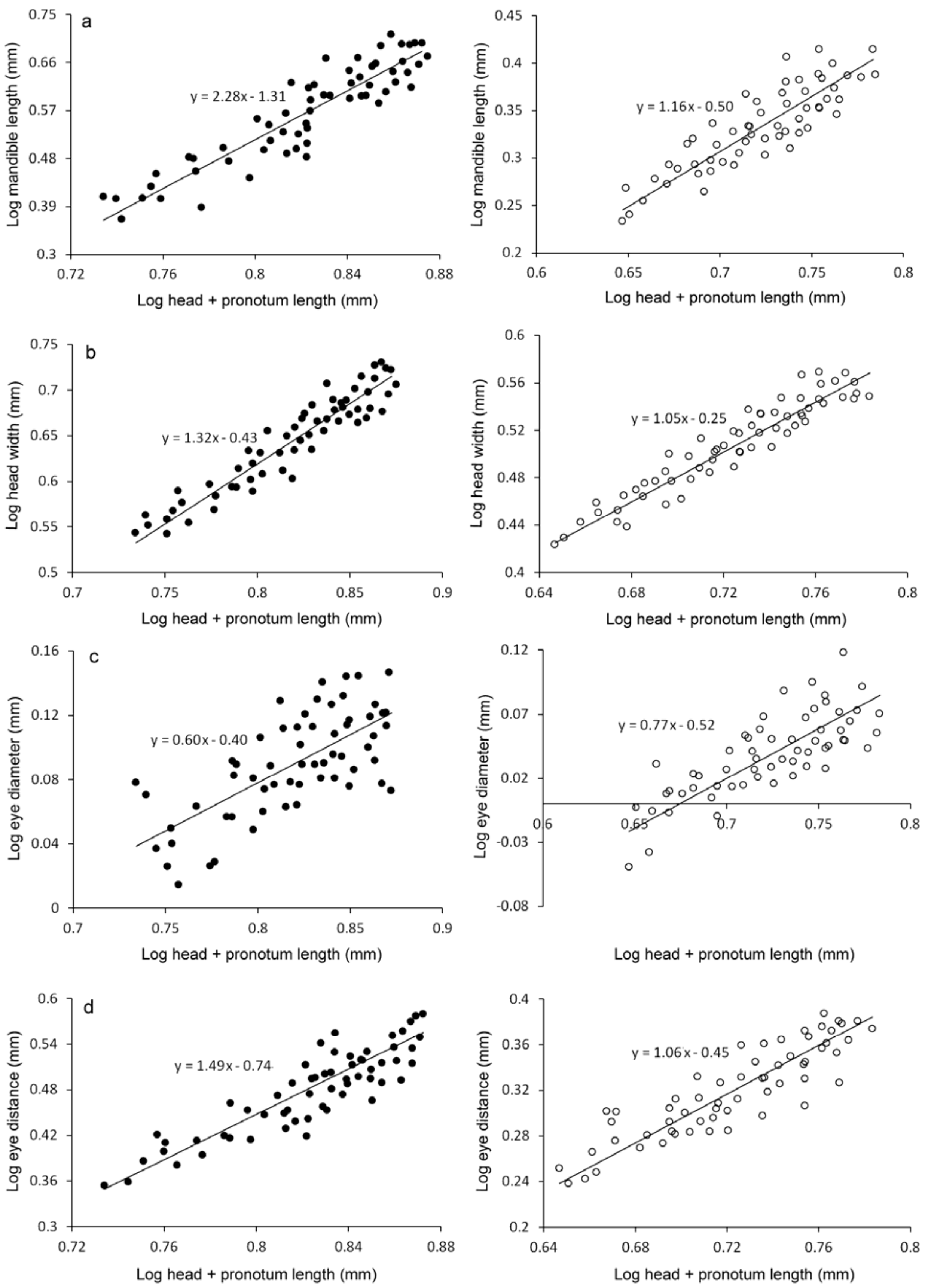

Fig. 5 Log-transformed size of four morphological traits (right mandible length (a), head width (b), right eye diameter (c) and ocular distance (d)) plotted against log-transformed body size in male (closed circles) and female (open circles) *Creophilus maxillosus*, the slope of regression line shows the kind of allometry (b>1), isometry (b=1), or negative allometry (b<1).

4 Discussion

We found strong evidence of sexual dimorphism in the rove beetle, *C. maxillosus*. Particularly, these differences were more evident in head width, pronotum width, and mandible length, where all traits were significantly larger in male beetles. Head size is one of the most important traits used by different arthropods in both male-male combat over access to females and advertisement of body size (Emlen and Nijhout, 2000; Bonduriansky, 2006; Judge and Bonanno, 2008). For example, Judge and Bonanno (2008) showed that male crickets with larger heads and mouthparts won more combats than males with smaller weaponry. Similarly, male *Drosophila* flies with broader heads are more likely to win intra-sexual fights. On the other hand, *Drosophila* females prefer males with broader heads, suggesting that head width is under sexual selection through both female preferences and male–male competition (Boake, 2005; Singh and Singh, 2014). Interestingly, similar patterns of sexual dimorphism have repeatedly evolved in distantly related arthropods, implying that the direction of sexual selection is convergent across taxa (Emlen and Nijhout, 2000; Bonduriansky, 2006). Male *C. maxillosus* had higher ratios of head width/head length (1.49 vs. 1.45) and pronotum width/pronotum length (1.19 vs. 1.14) compared to females. These results may imply that head and pronotum in males tend to grow more in width than length when compared to females (see Fig. 2b). The ratio between head width to pronotum width was also higher in males (1.02 vs. 0.90, respectively) (Table 1), indicating that head width in males is under more forcefully selection than the pronotum.

The mandibles of male *C. maxillosus* were about 1.76 times longer than those of females. The role of mandibles in male-male combat has been well documented in insects (Goldsmith, 1985; Forsyth and Alcock, 1990; Emlen and Nijhout, 2000; Kelly, 2005; Judge and Bonanno, 2008). Particularly in beetles, mandibles are among the most common weapons used in direct combat between males (Emlen and Nijhout, 2000; Goyens et al., 2014). In our field-collected beetles, about 3% of males had broken mandibles, indicating that mandibles are probably involved in male-male combats. A positive linear correlation was found between head width and mandible length, such that beetles with larger head had also longer mandibles. Larger head may provide the enlarged mandibles with stronger adductor muscles, which positively affect the bite force of males. For example, a linear increase in head width of the field cricket, *Gryllus pennsylvanicus* has been shown to results in an exponential increase in adductor muscle volume and therefore increased bite force (Judge and Bonanno, 2008). Similarly, male tree weta, *Hemideina* sp. with larger heads have been shown to possess greater adductor muscle volume and exert greater bite force than females (Field and Deans, 2001). In the stag beetle, *Cyclommatus metallifer* (Col: Lucanidae), males bite three times as forcefully as females. This increase in bite force has been suggested to results from enlargement of the adductor muscle of the mandibles (about 2.5 times as large as females) as well as wider anterior side of the head, which provides elongated input levers (Goyens et al., 2014). A linear correlation was also found between head width and pronotum width.

Although most traits in most organisms has been suggested to be isometric or negatively allometric to body size, a relatively small subset of traits exhibit allometry, most of which are employed either in male-male combat or/and courtship for access to females (Bonduriansky and Day, 2003). Sexual selection has been proposed to favor the evolution of trait allometries in a variety of ways. For example, positive allometry evolves when larger trait size offers a direct advantage in relation to success in sexual combats (e.g. mandible length in stag beetles (Goyens et al., 2014). Alternatively, positive allometry may be favored in traits used as advertisements of body size to the opposite sex (e.g. head width in male field crickets (Judge and Bonanno, 2008). We found strong allometry in head width and ocular distance of male C. maxillosus, while head width and ocular distance of females were isometric to the body size. These results show that head size is under positive sexual selection in males. The increased ocular distance may also favored in males in order to reduce the physical combat of eyes during direct physical combats. The same scenario can explain the smaller eyes of males relative to head size compared to females (Fig. 2D). Although, mandible length of both male and female beetles exhibited positive allometry, the allometric slope of males was about two times as large as females, indicating that mandible size is under strong selection in males. In contrast, the eye diameter was negatively allometric in both male and female *C. maxillosus*, with males tended to have smaller eyes relative to head size (Fig. 2D). This may highlight the eye size as a nonsexual trait in *C. maxillosus*. In line with these results, research in different animal taxa has suggested that traits interact with one another during development, such that variation in one trait may be coupled to variation in another trait. For example, male horned beetles with long horns have disproportionately small compound eyes (Nijhout and Emlen, 1998).

Altogether, results of the current study reveal strong sexual dimorphism in head and pronotum size, mandible length, eye diameter, ocular distances and antennal distance in *C. maxillosus*. All abovementioned traits have been repeatedly argued to play important roles in male-male combat as well as female attractiveness in insects with distant evolutionary origins. The striking similarities in patterns of sexual dimorphism among independently evolved insects indicate that common force(s) are probably at work. Male *C. maxillosus* showed higher variation in head width and mandible length compared to females (see Fig. 2A & C). However, despite this variation, a higher proportion of males tended to have longer mandibles and wider head (Fig. 2A & C). For example, out of 60 male beetles examined for head width and mandible length, 39 specimens had heads wider than the mean calculated head width (4.53 mm) and 40 specimens had mandibles longer than the mean calculated mandible length (3.85 and 3.97 mm for right and left mandible, respectively). The higher frequency of males with larger head and mandibles in the population may reflect the direction of sexual selection. Investment of males in sexually selected traits may occur at the expense of investment in other traits (e.g. testes size) as evidenced, for example, in horned dung beetles and tree wetas (Simmons and Emlen, 2006; Kelly 2008). Therefore, further studies may explore the costs of producing enlarged body parts in male *C. maxillosus*. Additionally, evaluating the efficiency of sexually dimorphic traits in male-male combat over access to mates as well as in female preference may be the subject of future studies.

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