

## Sexual dimorphism in *Apfelbeckia insculpta* (L. Koch, 1867) (Myriapoda: Diplopoda: Callipodida)

Bojan S. Ilić\*, Bojan M. Mitić and Slobodan E. Makarov

University of Belgrade – Faculty of Biology, Institute of Zoology, Studentski Trg 16, 11000 Belgrade, Serbia

\*Corresponding author: bojan.ilic@bio.bg.ac.rs

Received: February 29, 2016; Revised: May 5, 2016; Accepted: May 18, 2016; Published online: July 27, 2016

**Abstract:** *Apfelbeckia insculpta* (L. Koch, 1867) is one of the largest European millipedes and an endemic species of the Balkan Peninsula. We present data on sexual dimorphism in size and body proportions obtained from 179 adult specimens of this species from four caves in Serbia and one in Montenegro using univariate and multivariate morphometric techniques. Sexual dimorphism was apparent and female-biased for all measured characters, except for lengths of the antennae and the 24<sup>th</sup> leg pair (which were larger in males) and lengths of the first, second and fourth leg pairs, which exhibited small differences between sexes. Generally, females had significantly greater body size than males, while males expressed significantly greater values in traits that can be associated with mobility and copulation behavior. Also, we found significant variations in sexual size and body proportions dimorphism among analyzed populations. The influences of fecundity and sexual selection on the adult body plan in *A. insculpta* are discussed.

**Key words:** sexual dimorphism; adult body plan; Balkan Peninsula; evolutionary morphology; millipedes

### INTRODUCTION

Differences between the sexes in body size and body proportions are widespread among many animal groups. As a general pattern, females are the larger sex in most invertebrates [1-3] and in most poikilothermic vertebrates [4,5], while the opposite is prevalent in birds and mammals [6-8].

The emergence and existence of sexual dimorphism is driven by many mechanisms. Several hypotheses addressing these issues are widely accepted and cited in the literature. According to the first, the ‘fecundity advantage hypothesis’, females of larger size have greater fecundity and produce larger or more numerous offspring [9-13]. The second holds that differences between males and females may have evolved as a result of sexual selection acting through intra-sexual competition or mate choice. According to this hypothesis, larger body size in one sex or different body proportions are associated with greater success in feeding, defending territory, or mate acquisition [14,15]. Finally, sexual dimorphism can evolve via a divergence of the morphological traits of males and females due to intersexual competition for resources

[15]. The ‘intersexual niche partitioning’ hypothesis presumes that both sexes phenotypically diverge in a way to reduce competition between them for such things as food or habitat requirements [15,16]. Thus, adult body architecture represents the output of interplay between evolutionary forces acting on both sexes.

Studies of sexual dimorphism are often conducted at the intrapopulation level. However, many organisms exhibit variation in the degree and direction of sexual dimorphism across populations [17-20]. These alterations usually have a genetic basis [19] and can arise as a consequence of mechanisms such as a correlated response of both sexes to sexual selection [21,22] or via a different response of organisms to local environmental conditions [23]. Most studies focusing on intraspecific variation of sexual dimorphism are conducted on a small number of populations or on populations from a small geographical area, and data on widespread species are needed [17]. Still, it is often quite difficult to obtain samples from all or most of the populations across a species’ distributional range. It may be possible to gain a satisfying resolution of the geographical variation of sexual dimorphism by

analyzing isolated populations originating even from a small distributional range. To achieve this, organisms that display low dispersal abilities or inhabit isolated ecosystems (like caves or mountain lakes) would be suitable candidates for such analysis. Millipedes generally display low mobility and show a tendency toward geographical isolation [24,25]. This enhances variability at different levels and can produce interesting intra- or interspecific patterns in the expression of sexual dimorphism.

Millipedes (Myriapoda, Diplopoda) are an arthropod group with more than 12000 described species [26]. Some estimates suggest that this class comprises approximately 80000 species [26,27]. Usually, millipedes are abundant in the leaf-litter of temperate and tropical forests, but members of this group inhabit other terrestrial environments, such as deserts, caves, etc. [24]. Most millipedes are detritivorous organisms with an important role in enhancing the decomposition of decaying plant material and thereby stimulating microbial activity [24,28]. Only one diplopod group (order Callipodida) is presumed to feed on animal material [29], whether it be through predation or through processing remnants of other animals [30].

Although millipedes constitute one of the most diverse groups of terrestrial arthropods [26], we still have only a cursory grasp of the many aspects of their biology. Considering the growing body of data on sexual dimorphism in many animal groups, papers dealing with this topic in millipedes are scarce. Intersexual differences in millipedes comprise differences in the number of leg pairs (observed in Glomerida and Sphaerotheriida), in the number of body segments (in some Polydesmida and Chordeumatida), in the number of post-embryonic stadia (in some Julida), or in the morphology of anterior legs (for example, the first leg pair of males in Julida are almost always modified, while the second pair of legs can be reduced in females of certain representatives of Chordeumatida) [31]. Besides these differences, the males of some species of Julida possess modified mandibles [24, 31], 'inflated' gnathochilarium [24] and glands that open on the coxae of the second leg pairs [24]. Also, some members of the genus *Ammodesmus* Cook, 1896 show differences in morphology and setation of the metaterga between males and females [32].

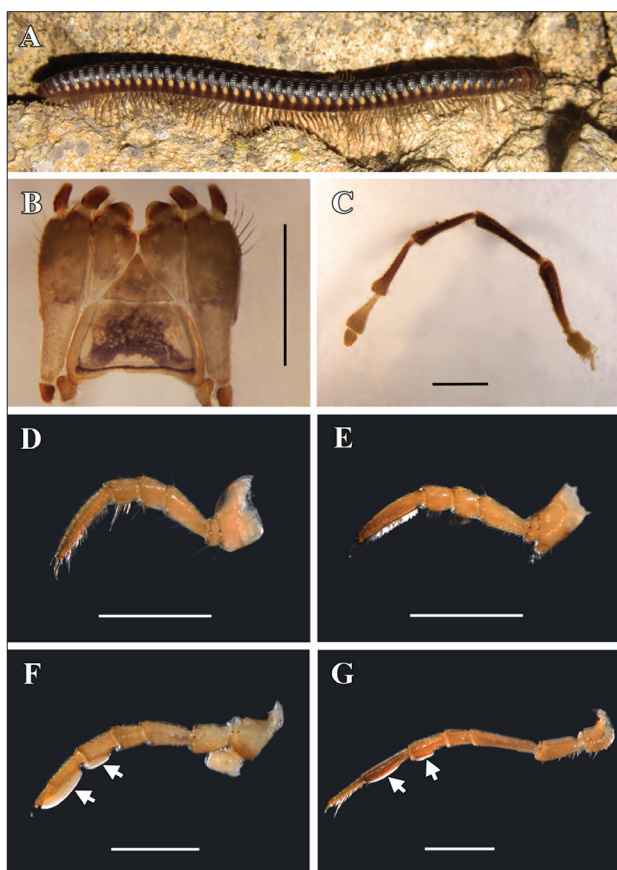
In the present study, the sexual dimorphism of an endemic millipede species was evaluated in five populations distributed in Serbia and Montenegro. The aims of this study were two-fold: i) to analyze sex-related differences in size and body proportion variation at the intra- and interpopulation levels in our focal species, and ii) to try to elucidate the approximate mechanisms underlying these differences.

## MATERIALS AND METHODS

### Study species and sampling sites

*Apfelbeckia* Verhoeff, 1896 is a genus of troglophilic millipedes endemic mostly to caves and other underground habitats of the Balkan Peninsula. This genus is composed of three species: *A. brazzanum* (Attems, 1927), *A. insculpta* (L. Koch, 1867) and *A. synthesis* [33]. The species with the greatest distributional range is *A. insculpta*. It is distributed in Serbia, Montenegro, Bosnia and Herzegovina, Croatia and Albania [33]. *Apfelbeckia insculpta* is one of the largest European millipedes [34]. It is a millipede with an elongated, cylindrical body that is slightly higher than wide. The head is convex in both sexes. Antennae are long and slender. The first two leg pairs are noticeably shorter than the others and consist of six segments, unlike the others. The anterior legs (leg pairs one to seven) are generally shorter and wider than the subsequent legs, which are moderately long. Starting from the fourth leg pair, adhesive pads are present on the tibiae and tarsi of most legs in males. The last body segment (telson) bears a pair of spinnerets. Both sexes have the same color pattern and trunk ornamentation – a dark brown body with a lateral row of yellowish spots located below the openings of the defensive glands and moderately developed crests on the dorsal side of the trunk. The habitus and some morphological features of *A. insculpta* are presented in Fig. 1. Males of *A. insculpta* are distinguished by the presence of gonopods (i.e., a modified eighth leg pair), which are involved in sperm transfer during copulation.

In the spring of 2012, we examined five populations of *A. insculpta*, a total of 179 animals. Four populations were sampled from caves in Serbia: Kovačevića Pećina Cave (44°23'11"N, 19°22'08"E;



**Fig. 1.** *Apfelbeckia insculpta* – habitus (A), gnathochilarium (B), right antenna (C), right leg of the first leg pair (D), right leg of the second leg pair (E), right leg of the fourth leg pair (male; arrows point adhesive pads) (F), right leg of the twenty fourth leg pair (male; arrows point adhesive pads) (G). Scale bars: B=1 mm; C–G=2 mm.

21 males and 18 females), Petnička Pećina Cave (44°14'19"N, 19°56'22"E; 15 individuals of both sexes), Mladenovića Megara Cave (43°51'15"N, 19°44'29"E; 15 males and 19 females), Hadži Prodanova Pećina Cave (43°37'23"N, 20°14'16"E; 21 males and 20 females), and one population was located in a cave in Montenegro: Vilina Pećina Cave (42°48'10"N, 18°54'09"E; 16 males and 18 females). In the following text, populations are indicated by abbreviations of the names of their sampling sites: KP – Kovačevića Pećina Cave, PP – Petnička Pećina Cave, MM – Mladenovića Megara Cave, HPP – Hadži Prodanova Pećina Cave and VP – Vilina Pećina Cave.

We focused on adult animals in all populations. Whether or not individuals were adults was decided by counting the number of podous and apodous segments (apodous segments other than the collum), number

of leg pairs, rows of ocelli and number of ocelli [35]. Adults in our sample had 45–47 podous segments and one apodous penultimate trunk segment, 87–91 pairs of legs, nine rows of ocelli and 40–44 ocelli. The sex of the specimens could be unambiguously determined by the presence of gonopods. All animals were collected by hand, kept in perforated plastic boxes (40 cm × 40 cm) with layer of soil from the sampling site until contents from digestive system were defecated (up to three days), weighed and preserved in 70% ethanol. To avoid coiling, each collected specimen was put in a test tube with an appropriate diameter (Sarstedt, Nümbrecht, Germany) upon return to the laboratory. Also, each sample had a unique registration number.

### Measurements

Animals were weighed with a Scout Pro 123<sup>®</sup> scale (Ohaus Corporation, Pine Brook, NJ, USA). Prior to dissection, all animals were photographed against a ruler accurate to 1 mm. After dissection of the chosen structures (head, right antenna, gnathochilarium (a plate-like structure formed from the first maxillae), trunk, right leg of the first leg pair, right leg of the second leg pair, right leg of the fourth leg pair and right leg of the 24th leg pair), all dissected body parts were photographed using an AxioCam MR camera mounted on a research binocular (Carl Zeiss, Jena, Germany). Scale bars corresponding to the respective magnification were added and all of the measurements were made using Axiovision software (ver. 4.2 for Windows, Carl Zeiss, München, Germany). The definitions of all measurements are given in Table 1.

### Statistical analyses

Univariate and multivariate methods were used to detect and characterize differences in size and body proportions between males and females of *A. insculpta*. First, all data were tested for normality using the Kolmogorov-Smirnov test. To reveal intersexual differences among morphological traits that depict body size (BL and M), one-way analysis of variance (ANOVA) was employed.

For statistical analysis of sex differences in body proportions, we used one-way analysis of covariance (ANCOVA) with multiple covariates. As we were fo-

**Table 1.** The morphological traits examined in both sexes of *A. insculpta*.

Trait	Definition
BL	Body length (from distal margin of the head to the end of the telson)
M	Body mass
HL	Head length (from distal to proximal margin of the head capsule)
HW	Head width (distance between antennae)
GL	Length of the gnathochilarium (from distal to proximal edge of the gnathochilarium)
GW	Width of the gnathochilarium (distance between the two most anterior setae)
AL	Length of antennae (sum of dorsal lengths of each article)
TL	Trunk length (from distal margin of collum to the end of the telson)
TW	Trunk width (maximum distance between left to right side of XV pleurotergite)
TH	Trunk height (maximum distance between ventral to dorsal side of XV pleurotergite)
L1L	Length of the first leg (sum of dorsal lengths of each article)
L2L	Length of the second leg (sum of dorsal lengths of each article)
L4L	Length of the fourth leg (sum of dorsal lengths of each article)
L24L	Length of the twenty-fourth leg (sum of dorsal lengths of each article)

cused on data gathered from different populations, and as our data were represented by a set of linear measurements (i.e., linear distances between defined points of the observed body part) (Table 1), we followed the recommendation of Berner [36] and included several traits as body size proxies. The covariates for HL were BL and HW, while BL and HL were used as body size estimators for HW, GL, GW and AL. Trunk length was controlled for BL and M. The two covariates for the remaining trunk dimensions, as well as for lengths of the first, second, fourth and 24th leg pair, were BL and TL. We tested the interaction between covariates and sex in order to examine the slope heterogeneity of regression lines between the sexes for each population. Insignificance of the interaction ( $P > 0.05$ ) suggested slope homogeneity and we performed ANCOVA.

We then performed principal component analysis (PCA) and canonical variate analysis (CVA) on pooled dataset. Both types of analyses are ordination methods used to simplify descriptions among individuals and/or groups. PCA is used for simplifying descriptions of variation among individuals, while CVA simplifies descriptions of differences between groups [37]. For the effects of sex and population on morphological

variation in *A. insculpta*, we used multivariate analysis of variance (MANOVA) with the individual scores obtained in CVA as the dependent variables, and sex and population as factors.

*Post hoc* analysis consisted of Tukey's test, while the significance level was set at  $\alpha = 0.05$ . When we performed multiple tests, we adjusted the initial statistical significance of 0.05 by Bonferroni correction for each set of analyses independently [38]. All statistical analyses were performed in SPSS (ver. 20 for Windows, IBM, Armonk, NY, USA) or STATISTICA (ver. 7 for Windows, Statsoft, Tulsa, OK, USA).

### Index of sexual dimorphism in body size and body proportions

According to Djordjević et al. [20], we calculated the indices of sexual dimorphism for body size and body proportions for each population of *A. insculpta*. Several formulae for quantifying sexual dimorphism have been proposed in the literature [39-41]. Willemsen and Hailley [41] provided a formula for the sexual dimorphism index (SDI) in the form of  $100 \cdot [(F-M)/M]$ , where F and M are the mean female and mean male values of the measured trait. This formula was chosen because it clearly generates positive values of the index when females are the larger sex and negative values when males are the larger sex. In our study, the mean values of BL and M (from ANOVA) were used for evaluating sexual dimorphism in size, while adjusted means of all other traits (from ANCOVA) were used for evaluating sexual dimorphism in all other measured traits.

## RESULTS

### Sexual dimorphism

The mean absolute (for BL and M) and size-adjusted (for all other traits) morphological measurements are presented in Table 1. Values of BL and M were significantly higher in females than in the males of the five populations (Table 2, Tukey's test, all  $P < 0.001$ ). Both characters remained with significantly higher values after Bonferroni correction (Table 2).

The results of ANCOVA showed that after checking for body size, females had larger dimensions in all

**Table 2.** Results of comparisons between the sexes of *A. insculpta*. Absolute (BL and M) and size-adjusted (all other traits) values are shown in the table. Data are expressed as mean/adjusted-mean±SD; measurements in mm or g. Bolded numbers indicate the sex that has a significantly larger value for the trait even after Bonferroni correction. Superscript letters next to F-values indicate significance level: a for  $P < 0.05$ , b for  $P < 0.005$  (after Bonferroni correction for multiple comparisons of size-related traits), and c for  $P < 0.0008$  (after Bonferroni correction for multiple comparisons of size-adjusted traits).

Locality	Trait	Body size										Body proportions									
		BL	M	HL	HW	GL	GW	AL	TL	TW	TH	L1L	L2L	L4L	L24L						
KP	♂	75.44±2.86	0.61±0.04	3.26±0.13	1.70±0.07	1.33±0.02	1.51±0.03	<b>9.65±0.30</b>	72.25±2.59	3.17±0.12	3.87±0.09	3.20±0.11	3.09±0.10	4.69±0.14	<b>6.23±0.23</b>						
	♀	<b>87.96±2.45</b>	<b>0.91±0.05</b>	<b>3.45±0.11</b>	<b>1.80±0.06</b>	<b>1.42±0.02</b>	<b>1.60±0.02</b>	8.98±0.29	<b>84.17±2.33</b>	<b>3.57±0.13</b>	<b>4.14±0.14</b>	3.55±0.15	3.30±0.11	4.93±0.16	5.97±0.19						
	F	211.65 <sup>a,b</sup>	407.54 <sup>a,b</sup>	24.56 <sup>a,c</sup>	19.41 <sup>a,c</sup>	15.91 <sup>a,c</sup>	16.61 <sup>a,c</sup>	32.01 <sup>a,c</sup>	44.99 <sup>a,c</sup>	37.04 <sup>a,c</sup>	37.26 <sup>a,c</sup>	4.58 <sup>a</sup>	3.91 <sup>a</sup>	6.54 <sup>a</sup>	19.01 <sup>a,c</sup>						
PP	♂	79.88±3.45	0.64±0.04	3.44±0.10	1.82±0.06	1.36±0.03	1.51±0.04	<b>9.85±0.29</b>	76.56±3.65	3.43±0.09	3.91±0.07	3.52±0.11	3.14±0.09	5.02±0.12	<b>6.71±0.14</b>						
	♀	<b>92.88±4.31</b>	<b>1.02±0.05</b>	<b>3.68±0.06</b>	<b>1.91±0.05</b>	<b>1.42±0.04</b>	1.57±0.04	8.99±0.25	<b>88.68±4.02</b>	<b>3.66±0.09</b>	<b>4.47±0.09</b>	3.58±0.13	3.29±0.12	5.09±0.15	6.35±0.20						
	F	83.14 <sup>a,b</sup>	425.28 <sup>a,b</sup>	25.37 <sup>a,c</sup>	18.28 <sup>a,c</sup>	24.32 <sup>a,c</sup>	5.09 <sup>a</sup>	35.66 <sup>a,c</sup>	53.32 <sup>a,c</sup>	20.06 <sup>a,c</sup>	167.08 <sup>a,c</sup>	4.02 <sup>a</sup>	8.22 <sup>a</sup>	5.23 <sup>a</sup>	15.53 <sup>a,c</sup>						
MM	♂	70.99±3.18	0.58±0.04	2.80±0.11	1.66±0.04	1.33±0.02	1.53±0.04	<b>9.13±0.26</b>	68.20±3.33	3.01±0.10	3.70±0.08	3.66±0.09	3.07±0.08	4.54±0.16	<b>6.34±0.22</b>						
	♀	<b>78.14±2.44</b>	<b>0.78±0.07</b>	<b>3.42±0.12</b>	<b>1.79±0.06</b>	1.37±0.02	1.59±0.05	8.19±0.23	<b>74.70±2.78</b>	<b>3.24±0.13</b>	<b>4.05±0.06</b>	<b>4.00±0.10</b>	3.15±0.12	<b>4.71±0.13</b>	6.00±0.17						
	F	55.02 <sup>a,b</sup>	104.27 <sup>a,b</sup>	100.59 <sup>a,c</sup>	29.87 <sup>a,c</sup>	5.85 <sup>a</sup>	4.65 <sup>a</sup>	49.97 <sup>a,c</sup>	43.17 <sup>a,c</sup>	23.99 <sup>a,c</sup>	39.21 <sup>a,c</sup>	26.96 <sup>a,c</sup>	7.66 <sup>a</sup>	16.15 <sup>a,c</sup>	14.32 <sup>a,c</sup>						
HPP	♂	71.20±2.80	0.51±0.03	2.70±0.09	1.51±0.06	1.22±0.02	1.42±0.03	<b>8.84±0.30</b>	68.62±2.45	3.03±0.10	3.52±0.09	3.21±0.12	2.78±0.11	4.06±0.19	<b>6.14±0.14</b>						
	♀	<b>81.26±3.26</b>	<b>0.74±0.06</b>	<b>2.95±0.12</b>	<b>1.62±0.05</b>	<b>1.31±0.03</b>	<b>1.53±0.05</b>	8.23±0.25	<b>78.23±2.88</b>	<b>3.30±0.12</b>	<b>3.99±0.11</b>	3.28±0.10	3.06±0.13	<b>4.72±0.20</b>	5.88±0.20						
	F	112.54 <sup>a,b</sup>	253.54 <sup>a,b</sup>	27.42 <sup>a,c</sup>	18.75 <sup>a,c</sup>	17.13 <sup>a,c</sup>	25.42 <sup>a,c</sup>	28.98 <sup>a,c</sup>	37.35 <sup>a,c</sup>	30.12 <sup>a,c</sup>	84.20 <sup>a,c</sup>	8.58 <sup>a</sup>	7.49 <sup>a</sup>	19.95 <sup>a,c</sup>	14.12 <sup>a,c</sup>						
VP	♂	91.56±3.85	1.16±0.05	4.09±0.10	2.14±0.05	1.61±0.04	1.94±0.05	<b>12.49±0.32</b>	87.52±3.68	4.12±0.09	4.65±0.09	4.64±0.12	4.22±0.14	6.40±0.22	<b>8.86±0.26</b>						
	♀	<b>103.40±4.12</b>	<b>1.71±0.11</b>	<b>4.18±0.09</b>	<b>2.26±0.03</b>	1.67±0.04	<b>2.02±0.04</b>	11.30±0.29	<b>99.06±3.98</b>	<b>4.51±0.09</b>	<b>5.19±0.12</b>	4.72±0.15	4.34±0.12	6.59±0.26	8.17±0.29						
	F	74.43 <sup>a,b</sup>	332.05 <sup>a,b</sup>	16.33 <sup>a,c</sup>	21.02 <sup>a,c</sup>	6.68 <sup>a</sup>	18.95 <sup>a,c</sup>	62.94 <sup>a,c</sup>	88.32 <sup>a,c</sup>	44.12 <sup>a,c</sup>	149.74 <sup>a,c</sup>	9.92 <sup>a</sup>	4.02 <sup>a</sup>	4.30 <sup>a</sup>	35.17 <sup>a,c</sup>						

**Table 3.** Results of principal component analysis. The highest factor loading contribution levels are given in bold face.

Trait	PC1	PC2
	Loadings	
BL	<b>0.94</b>	-0.13
M	<b>0.97</b>	-0.26
HL	<b>0.92</b>	-0.13
HW	<b>0.96</b>	0.03
GL	<b>0.98</b>	-0.01
GW	<b>0.96</b>	0.11
AL	<b>0.86</b>	<b>0.54</b>
TL	<b>0.83</b>	-0.42
TW	<b>0.97</b>	0.01
TH	<b>0.96</b>	0.08
L1L	<b>0.98</b>	0.05
L2L	<b>0.98</b>	0.05
L4L	<b>0.98</b>	0.04
L24L	<b>0.80</b>	<b>0.51</b>
Eigenvalue	11.68	1.09

measured characters except AL and L24L, where the opposite pattern was observed (Table 2, Tukey's test, all  $P < 0.05$ ). Even in situations where Bonferroni correction showed that intersexual differences were nonsignificant, females had consistently greater values of body size-adjusted traits (Table 2).

PCA yielded two significant axes (Eigenvalue greater than 1), which accounted for 94.83% of total variation. The first principal component (PC1) accounted for 89.55% of total variation, with significant loadings of the same sign for all variables (Table 3). Such a pattern across variables indicates that this PC can be interpreted as an estimator of overall body size [42-44]. The second principal component (PC2) accounted for 5.27% of total variation, with positive loadings for nine variables and negative loadings for five variables. It turned out that PC2 correlated strongly with AL and L24L (Table 3). The individual scores of the first two PCs formed clusters in morphospace according to sex and population, and the ordination plot showed clear separations of the sexes within and among populations (Fig. 2).

To examine differences between sexes and populations, we continued with CVA and MANOVA. The CVA resulted in three canonical variates (CVs), which accounted for 98.41% (CV1 - 80.99%; CV2 - 13.11%; CV3 - 4.31%) of the total difference. The values of standardized coefficients of canoni-

**Table 4.** Results of canonical variate analysis. The highest standardized coefficients are given in bold face.

Trait	Canonical variates		
	CV1	CV2	CV3
BL	<b>-1.365</b>	0.302	-0.008
M	-1.094	0.247	-0.013
HL	-0.209	<b>0.683</b>	-0.120
HW	0.099	<b>1.317</b>	<b>1.061</b>
GL	0.361	<b>0.913</b>	<b>1.745</b>
GW	<b>-1.010</b>	<b>-2.231</b>	0.017
AL	-0.565	0.523	-0.585
TL	1.123	0.552	-1.071
TW	-0.085	-0.346	<b>-1.147</b>
TH	<b>0.676</b>	-0.247	-0.445
L1L	<b>0.641</b>	-0.085	-0.105
L2L	0.140	<b>-0.865</b>	0.035
L4L	<b>-0.751</b>	0.070	-0.077
L24L	<b>-0.567</b>	<b>-0.676</b>	0.041

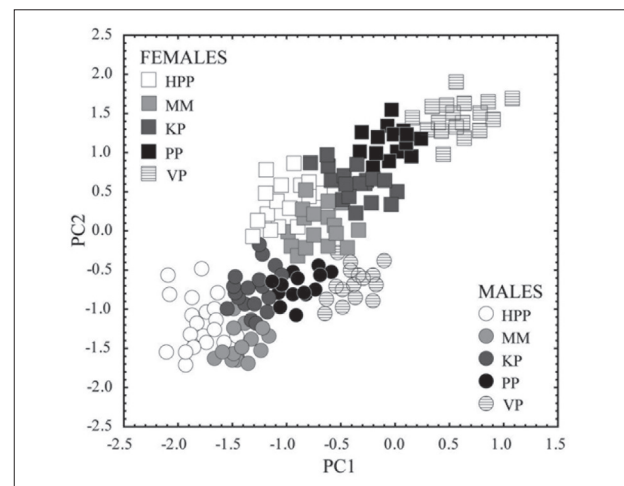
cal variables, which contribute to the discrimination between the first three CVs, are presented in Table 4. Similar to PCA, the position of the individuals in the morphospace defined by the first two CVs revealed distinctions among populations and sexes (Fig. 3). The results of MANOVA showed statistically significant differences between males and females (Wilk's  $\lambda=0.033$ ;  $F=528.20$ ;  $P<0.001$ ), significant differences among populations (Wilk's  $\lambda=0.009$ ;  $F=115.90$ ;  $P<0.001$ ), while the interaction term was also significant (Wilk's  $\lambda=0.071$ ;  $F=17.10$ ;  $P<0.001$ ).

### Variation of sexual dimorphism indices

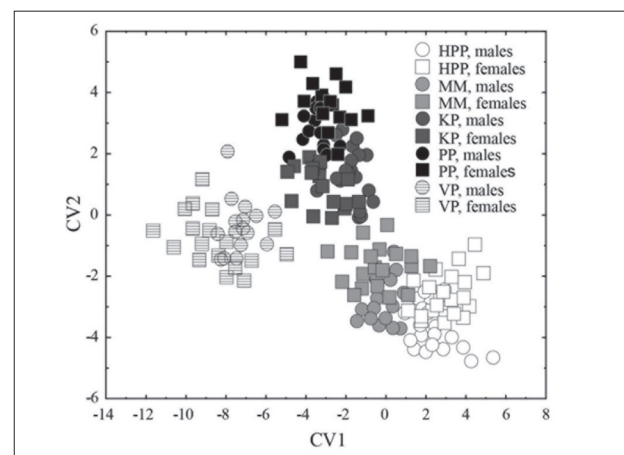
Sexual dimorphism indices showed variation in the degree of sexual dimorphism among populations (Table 5). We found that the divergence in body length and body mass between the sexes was pronounced, while the magnitude of variation of dimorphism indices in other traits had lower values, albeit significant. The direction of SDI was the same across populations.

**Table 5.** Variation of the sexual dimorphism index (SDI) in five populations of *A. insculpta* from Serbia and Montenegro.

Trait	BL	M	HL	HW	GL	GW	AL	TL	TW	TH	L1L	L2L	L4L	L24L	
Locality	Body size			Body proportions											
KP	+16.60	+49.18	+5.83	+4.71	+6.02	+5.96	-6.94	+16.49	+12.62	+6.98	+10.94	+6.80	+5.12	-3.53	
PP	+16.27	+59.37	+6.98	+4.95	+4.41	+3.97	-9.41	+15.83	+6.71	+14.32	+1.70	+4.78	+1.39	-5.36	
MM	+10.07	+34.48	+22.14	+7.83	+3.01	+3.92	-10.30	+9.53	+3.18	+7.24	+9.29	+2.61	+3.74	-5.36	
HPP	+14.13	+45.10	+6.64	+6.62	+7.38	+7.75	-6.90	+14.00	+8.91	+13.35	+2.18	+10.07	+16.26	-4.23	
VP	+12.93	+47.41	+2.20	+3.24	+4.97	+3.59	-9.53	+13.19	+9.47	+11.61	+1.72	+2.84	+2.97	-7.79	



**Fig. 2.** Scatter plot of the first two principal component scores (PC1 and PC2) of both sexes of *A. insculpta* from all studied populations.



**Fig. 3.** Scatter plot of the first two canonical variate scores (CV1 and CV2) of both sexes of *A. insculpta* from all studied populations.

## DISCUSSION

We have demonstrated obvious sexual dimorphism in *A. insculpta*. Both body regions, i.e. head and trunk, are longer and wider in females. Our results thereby

indicate female-biased size dimorphism in this species. Also, females were significantly heavier than males.

As in many other animal taxa with female-biased sexual size dimorphism, we presume that fecundity selection is a major evolutionary force that shapes this condition in our study organism. More specifically, the key target for fecundity selection is maternal body volume, as this trait is one of the constraints that may limit female reproductive output [45]. Our results showed that females of *A. insculpta* have a longer, wider and higher trunk than conspecific males, so it is reasonable to assume that females have a more voluminous body. As previous studies in millipedes showed that female body size correlates positively with the number of eggs [46-48], the 'fecundity advantage hypothesis' is a plausible scenario for this pattern of sexual dimorphism in *A. insculpta*. A similar explanation of greater values for some trunk dimensions and body mass was proposed for the polydesmid species *Cladethosoma clarum* (Chamberlin, 1920) [49].

Head shape is usually dimorphic in representatives of the order Callipodida [50]. For example, males of *Apfelbeckia brazzanum* (Attems, 1927) have concave heads, while females of this species have convex heads [33]. Our study species, *A. insculpta*, does not express such a pattern (both sexes have convex heads), but head dimensions were statistically different between males and females. The relative dimensions of the head and gnathochilarium were larger in females. This can be explained by the importance of high feeding efficiency. It is known that in some animal groups feeding frequency or prey size correlate with variations in head size [51]. Being the larger sex, it is logical to assume that females need more energy for maintenance. Also, females need enough energy to invest in egg production. Since it was observed that females spend most of the time feeding (B.S. Ilić, pers. observ.), it is no mere chance that these structures were larger in this sex. Our results thus suggest that fecundity selection is the underlying mechanism that generates and maintains sexual dimorphism in these characters.

Males, like the males of some other millipedes, have adhesive pads on their legs [33]. The first leg pair with adhesive pads is located on the fifth pleurotergite (fourth leg pair). Counted as a secondary sexual character, adhesive pads help the male to hold

the female during mating [24,52]. Relative measures showed that the fourth leg pair is larger in females in two populations (MM and HPP), while intersexual differences in this trait were statistically nonsignificant in the other three analyzed populations. Besides the fourth leg pair, the first two leg pairs were generally monomorphic in *A. insculpta*. Considering the obtained results, we are unable to find a meaningful biological explanation for such a pattern and presume that significant differences between males and females in some populations represent a statistical artifact.

The relative dimensions of the 'walking' leg (L24L) were greater in males. In the scenario where males scramble rather than fight for females, as in millipedes [49,53-55], it can be presumed that the selection favors increased activity and mobility in males [56]. Manton [57] found that leg length influences locomotor capacity in Diplopoda because longer legs can make longer strides, which results in an overall increase in speed. Also, a small body size in males can be advantageous in habitats such as cave systems where food is often limited [56,58,59], i.e., males require less food and have more time for the pursuit of females. The smaller body size combined with longer legs and lower weight provide further proof that evolutionary forces favored agility in *A. insculpta* males. This conclusion also follows from data on the patterns of sexual dimorphism in other millipedes [49].

Relative dimensions of the antennae (AL) were also greater in males. Millipedes generally live in habitats where the levels of light are quite low (leaf-litter or underground habitats) and despite the fact that many of them possess eyes, they must rely on other sensory structures. Millipede antennae carry arrays of sensory structures, including gustatory and olfactory receptors [24]. It is clear that these multimodal sensory organs play an important role in millipede mating, since a male cannot mate successfully if his antennae are removed [60,61]. The active use of antennae during copulation, i.e., the process when the male taps his antennae on the head or body of a female, was observed in certain representatives of the genera *Centrobolus* Cook, 1897 (order Spirobolida) [54] and *Parafontaria* Verhoeff, 1936 (order Polydesmida) [55]. The greater dimensions of these organs in the males of *A. insculpta* can be attributed to a pronounced need to search for mates, as well as to their probable role during copula-

tion. Unfortunately, we found no mating pairs during our field research and the copulation behavior of our study species remains unknown, so we cannot confirm the role of antennae during copulation.

However, the abovementioned pattern of intersexual differences can shed light on the possible mechanisms of sexual selection operating in *A. insculpta*. Darwin [62] provided two modes of sexual selection: contest competition for mates and mate choice. Contest competition (also known as intrasexual selection) involves fights or contests of the members of one sex (usually males, so this mode of sexual selection is also called male-male competition) for access to mates, while mate choice (or intersexual selection) can be described as the competition to attract members of the other sex (typically females are the choosy sex and this mode of sexual selection will lead to the evolution of traits that make males more attractive to females than their rivals) [63]. Competition over mates takes many forms and most kinds of sexual differences are thought to have emerged through one or more mechanisms of competition among males – sperm competition, endurance rivalry, scramble competition, contests, coercion, infanticide or attractiveness competition (arising from mate choice) [63,64]. Among these mechanisms, scramble competition is the most probable mechanism that operates among *A. insculpta* males. This mode of competition arises when males gain a mating advantage by the prompt localization of potential mates and favors well-developed sensory and locomotor organs [63,64]. This being the case, competition between males may indeed enforce larger dimensions of the male antennae, and this pattern is probably coupled with a greater mobility in individuals of the male sex and/or their copulation behavior.

The observed pattern of sexual dimorphism in *A. insculpta* not only correlates with foraging ecology or mate search and acquisition, but can also be the result of reproductive interactions between the sexes. Behavioral observations during the copulation of *Alloporus uncinatus* Attems, 1914 (order Spirostreptida) demonstrated that a larger male can kill his mate [53]. As male behavior may pose a threat to the physical well-being of the female during copulation, evolutionary forces may favor physical prowess in the female; thus, females became larger than males. The larger body size in *A. insculpta* may represent one of the

“enduring” mating strategies of females (*sensu* Cooper and Telford [54]).

In the light of the obtained sexual dimorphism indices, we can say that the divergence in body size is pronounced in *A. insculpta*, while indices of sexual dimorphism in body proportions had lower values, albeit also significant. The directions of SDI were the same at all localities (Table 5), indicating that both sexes undergo a similar degree of evolutionary pressure throughout the distributional range of this species. Estimates of sexual dimorphism showed that the magnitude of sexual dimorphism in *A. insculpta* varies among populations (Table 5). Differences in genetic correlations between the sexes, allometry or phylogenetic effects can be drivers for interpopulation variability in sexual dimorphism [23,65-67]. On the other hand, such variation can be the result of intersexual differences in body-size plasticity [23,67] or microevolutionary changes among populations [68,69]. Finally, population history and differences in behavior can cause the variation in the degree of sexual dimorphism among populations of the same species [17,70]. Our study was based on field-collected animals and we cannot easily draw conclusions about the underlying mechanisms of the observed variation in sexual dimorphism. However, significant interactions between sex and population effects suggested that the degree to which both sexes respond to local environmental factors may influence the variation in magnitude of sexual dimorphism. This means that the observed sexual size and body proportion variation among populations is probably due to phenotypic plasticity in response to the local environmental conditions [23]. However, because of the lack of data on *A. insculpta* biology (and other millipedes as well), we cannot exclude the possibility that the variation in degree of sexual dimorphism among the different populations observed in our study may be due to the effect(s) of other factor(s).

In conclusion, we have demonstrated sexual dimorphism in size and body proportions in *A. insculpta*. Also, our results show that the direction of both aspects of sexual dimorphism is the same across the analyzed populations, while the degree of dimorphism varied between them. *A. insculpta* females exhibit a morphology that may be associated with increased fecundity in this sex, while the male body may be shaped by



intrasexual selection. This study provides initial data on sexual dimorphism in a callipodidan millipede. We focused on general body size and body proportions and included some measurements that have not been recorded in previous studies related millipede body size [71]. Millipedes are an underrepresented animal group in studies of this type and much comprehensive data, especially on population structure, behavior, mating strategies or utilization of environmental resources, are lacking, so many factors that may influence the degree and direction of sexual dimorphism are unknown. Such studies will shed new light on millipede biology and more help us to broaden our knowledge about patterns of sexual dimorphism.

**Acknowledgments:** The authors thank three anonymous reviewers for their constructive and helpful comments on an earlier version of the manuscript. Funding for this study was provided by the Serbian Ministry of Education, Science and Technological Development (Grant No. 173038).

**Authors' contribution:** BSI, BMM and SEM: study conception, study design, sample collection, drafting and writing of the manuscript; BSI: acquisition, analysis and interpretation of data.

**Conflict of interest disclosure:** The authors declare no conflict of interest.

## REFERENCES

- Teder T, Tammaru T. Sexual size dimorphism within species increases with body size in insects. *Oikos*. 2005;108(2):321-34.
- Foellmer MW, Moya-Laraño J. Sexual size dimorphism in spiders: patterns and processes. In: Fairbairn DJ, Blanckenhorn WU, Székely T, editors. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford: Oxford University Press; 2007. p. 71-81.
- Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, Fox C. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annu Rev Entomol*. 2010;55:227-45.
- Shine R. Sexual size dimorphism in snakes revisited. *Copeia*. 1994;1994:326-46.
- Monnet JM, Cherry MI. Sexual dimorphism in anurans. *Proc Biol Sci*. 2002;269(1507):2301-7.
- Lindenfors P, Gittleman JL, Jones KE. Sexual size dimorphism in mammals. In: Fairbairn DJ, Blanckenhorn WU, Székely T, editors. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford: Oxford University Press; 2007. p. 16-26.
- Székely T, Lislevand T, Figuerola J. Sexual size dimorphism in birds. In: Fairbairn DJ, Blanckenhorn WU, Székely T, editors. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford: Oxford University Press; 2007. p. 27-37.
- Webb TJ, Freckleton RP. Only half right: species with female-biased sexual size dimorphism consistently break Rensch's rule. *PLoS One*. 2007;2(9):e897.
- Honek A. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*. 1993;66(3):483-92.
- Preziosi RF, Fairbairn DJ, Roff DA, Brennan JM. Body size and fecundity in the water strider *Aquarius remigis*: a test of Darwin's fecundity advantage hypothesis. *Oecologia*. 1996;108(3):424-31.
- Fox CW, Czesak ME. Evolutionary ecology of progeny size in arthropods. *Annu Rev Entomol*. 2000;45:341-69.
- Davidowitz G. Population and environmental effects on the size-fecundity relationship in a common grasshopper across an aridity gradient. *J Orthoptera Res*. 2008;17(2):265-71.
- Salavert V, Zamora-Muñoz C, Ruiz-Rodríguez M, Soler JJ. Female-biased size dimorphism in a diapausing caddisfly, *Mesophylax aspersus*: effect of fecundity and natural and sexual selection. *Ecol Entomol*. 2011;36(3):389-95.
- Navarro J, Kaliontzopoulou A, Gonzalez-Solis J. Sexual dimorphism in bill morphology and feeding ecology in Cory's shearwater (*Calonectris diomedea*). *Zoology*. 2009;112(2):128-38.
- Berns CM. The evolution of sexual dimorphism: understanding mechanisms of sexual shape differences. In: Moriyama H, editor. *Sexual dimorphism*. Rijeka: InTech; 2013. p. 1-16.
- Pekár S, Martišová M, Bilde T. Intersexual trophic niche partitioning in an ant-eating spider (Araneae: Zodariidae). *PLoS One*. 2011;6(1):e14603.
- Roitberg ES. Variation in sexual size dimorphism within a widespread lizard species. In: Fairbairn DJ, Blanckenhorn WU, Székely T, editors. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Oxford: Oxford University Press; 2007. p. 143-53.
- Stillwell RC, Morse GE, Fox CW. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *Am Nat*. 2007;170(3):358-69.
- Lengkeek W, Didden K, Côté IM, van der Zee EM, Snoek RC, Reynolds JD. Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (Blenniidae). *Can J Zool*. 2008;86(10):1173-78.
- Djordjević S, Djurakić M, Golubović A, Ajtić R, Tomović Lj, Bonnet X. Sexual size and body shape dimorphism of *Testudo hermanni* in central and eastern Serbia. *Amphibia-Reptilia*. 2011;32:445-58.
- Fairbairn DJ. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu Rev Ecol Syst*. 1997;28:659-87.
- Székely T, Freckleton RP, Reynolds JD. Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *PNAS*. 2004;101(3):12224-7.
- Fairbairn DJ. Allometry for sexual size dimorphism: testing two hypotheses for Rensch's rule in the water strider *Aquarius remigis*. *Am Nat*. 2005;166(S4):S69-S84.
- Hopkin SP, Read HJ. *The biology of millipedes*. Oxford: Oxford University Press; 1992.
- Wojcieszek JM, Simmons LW. Divergence in genital morphology may contribute to mechanical reproductive isolation in a millipede. *Ecol Evol*. 2013;3(2):334-43.

26. Sierwald P, Bond JE. Current status of the myriapod class Diplopoda (millipedes): Taxonomic diversity and phylogeny. *Annu Rev Entomol.* 2007;52:401-20.
27. Hoffman RL. Classification of the Diplopoda. Genève: Museum d'Histoire Naturelle; 1979.
28. Blower JG. Milipedes. Keys and notes for the identification of the species. 35. The Linnean Society of London and the Estuarine and Brackish-Water Sciences Association. London: E.J. Brill/Dr. W. Backhuys; 1985.
29. Hoffman RL, Payne JA. Diplopods as carnivores. *Ecology.* 1969;50:1096-8.
30. Stoev P, Sierwald P, Billey, A. An annotated world catalogue of the millipede order Callipodida (Arthropoda: Diplopoda). *Zootaxa.* 2008;1706:1-50.
31. Minelli A, Michlik P. Diplopoda - Reproduction. In: Minelli A, editor. *The Myriapoda. Vol. 2, Treatise on zoology - Anatomy, taxonomy, biology.* Leiden, Boston: Brill; 2015. p. 237-65.
32. VandenSpiegel D, Golovatch SI. A new millipede of the family Ammodesmidae found in central Africa (Diplopoda, Polydesmida). *Zookeys.* 2015;483:1-7.
33. Stoev P, Enghoff H. A revision of the millipede tribe Apfelbeckiini Verhoeff, 1900 (Diplopoda: Callipodida: Schizopetalidae). *Steenstrupia.* 2008;29(1):47-66.
34. Frederiksen SB, Petersen G, Enghoff H. How many species are there of *Pachyiulus*? A contribution to the taxonomy of Europe's largest millipedes (Diplopoda: Julida: Julidae). *J Nat Hist.* 2012;46(9-10):599-611.
35. Ilić BS, Tomić VT, Lučić LR, Mitić BM. Anamorphic development of *Apfelbeckia insculpta* (L. Koch, 1867) (Diplopoda: Callipodida: Schizopetalidae). *Arch Biol Sci.* 2016;68(2):445-50.
36. Berner D. Size correction in biology: how reliable are approaches based on (common) principal component analysis? *Oecologia.* 2011;166:961-71.
37. Zelditch ML, Swiderski DL, Sheets HD, Fink WL. Geometric morphometrics for biologists: a primer. San Diego: Elsevier Academic Press; 2004.
38. Rice WR. Analyzing tables of statistical tests. *Evolution.* 1989;43(1):223-5.
39. Rossolimo OL, Pavlinov IY. Sex-related differences in development, size, and proportions of the skull of martens *Martes martes* (Mammalia, Mustelidae). *Bull Moscow Soc Nat Biol Ser.* 1974;79:23-35. Russian.
40. Lovich JE, Gibbons JW. A review of techniques for quantifying sexual size dimorphism. *Growth Develop Aging.* 1992;56:269-81.
41. Willemsen RE, Hailey A. Sexual dimorphism of body size and shell shape in European tortoises. *J Zool.* 2003;260(4):353-65.
42. Jolicoeur P. The multivariate generalization of the allometry equation. *Biometrics.* 1963;19:497-99.
43. Son MH, Hughes RN. Sexual dimorphism of *Nucella lapillus* (Gastropoda: Muricidae) in North Wales, UK. *J Moll Stud.* 2000;66:489-98.
44. Shariffi M, Farsat H, Vaissi S. Sexual size dimorphism in *Neurergus kaiseri* (Caudata: Salamandridae) in southwestern Zagros Mountains, Iran. *Amphib Reptile Conserv.* 2012;6(4):1-8.
45. Du W-G, Lü D. An experimental test of body volume constraint on female reproductive output. *J Exp Zool.* 2010;313A:123-8.
46. Heath J, Bocock KL, Mountford MD. The life history of the millipede *Glomeris marginata* (Villers) in north-west England. *Symp Zool Soc Lond.* 1974;32:433-62.
47. Baker GH. The post-embryonic development and life history of the millipede, *Ommatoiulus moreletii* (Diplopoda: Julidae), introduced in south-eastern Australia. *J Zool.* 1978;186:209-28.
48. Bhakat S, Bhakat A, Mukhopadhyaya MC. The reproductive biology and post-embryonic development of *Streptogonopus phipsoni* (Diplopoda: Polydesmoidea). *Pedobiologia.* 1989;33(1):37-47.
49. Rowe M. Copulation, mating system and sexual dimorphism in an Australian millipede, *Cladethosoma clarum*. *Aust J Zool.* 2010;58(2):127-32.
50. Enghoff H, Golovatch S, Short M, Stoev P, Wesener T. Diplopoda – Taxonomic overview. In: Minelli A, editor. *The Myriapoda. Vol. 2. Treatise on zoology – Anatomy, taxonomy, biology.* Leiden, Boston: Brill; 2015. p. 363-453.
51. Krause MA, Burghardt GM, Gillingham JC. Body size plasticity and local variation of relative head and body size sexual dimorphism in garter snakes (*Thamnophis sirtalis*). *J Zool.* 2003;261(4):399-407.
52. Chung K-H, Moon M-J. Microstructure of the adhesive pad in the millipede *Orthomorphella pekuensis* (Polydesmida: Paradoxosomatidae). *Entomol Resarch.* 2008;38(3):216-20.
53. Telford SR, Dangerfield JM. Mating tactics in the tropical millipede *Alloporus uncinatus* (Diplopoda: Spirostreptidae). *Behavior.* 1993;124(1):45-56.
54. Cooper MI, Telford S. Copulatory sequences and sexual struggles in millipedes. *J Insect Behav.* 2000;13(2):217-30.
55. Tanabe T, Sota T. Complex copulatory behavior and the proximate effect of genital and body size differences on mechanical reproductive isolation in the millipede genus *Parafontaria*. *Am Nat.* 2008;171(5):692-99.
56. Yasuda H, Dixon AFG. Sexual dimorphism in the two spot ladybird beetle *Adalia bipunctata*: developmental mechanism and its consequences for mating. *Ecol Entomol.* 2002;27(4):493-98.
57. Manton SM. The evolution of arthropodan locomotory mechanisms. Part 11. Habits, morphology and evolution of the Uniramia (Onychophora, Myriapoda, Hexapoda) and comparisons with the Arachnida, together with a functional review of uniramian musculature. *Zool J Linn Soc.* 1973;53:257-375.
58. Blanckenhorn WU, Preziosi RF, Fairbairn DJ. Time and energy constraints and the evolution of sexual size dimorphism: to eat or to mate? *Evol Ecol.* 1995;9(4):369-81.
59. Blanckenhorn WU. Behavioral causes and consequences of sexual size dimorphism. *Ethology.* 2005;111(11):977-1016.
60. Haacker U. Patterns of communication in courtship and mating behavior of millipedes (Diplopoda). *Symp Zool Soc Lond.* 1974;32:317-28.
61. Carey CJ, Bull CM. Recognition of mates in the Portuguese millipede *Ommatoiulus moreletii*. *Aust J Zool.* 1986;34(6):837-42.
62. Darwin C. The descent of man and selection in relation to sex. London: John Murray; 1871.
63. Savalli UM. Sexual selection. In: Fox CW, Roff DA, Fairbairn DJ, editors. *Evolutionary ecology. Concepts and case studies.* Oxford: Oxford University Press; 2001. p. 207-21.
64. Andersson M, Iwasa Y. Sexual selection. *TREE.* 1996;11(2):53-8.

65. Butler MA, Losos JB. Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards. *Ecol Monogr.* 2002;72(4):541-59.
66. Rutherford PL. Proximate mechanisms that contribute to female-biased sexual size dimorphism in an anguid lizard. *Can J Zool.* 2004;82(5):817-22.
67. Stillwell RC, Fox CW. Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity. *Oikos.* 2009;118(5):703-12.
68. King RB. Body size variation among island and mainland snake populations. *Herpetologica.* 1989;45(1):84-8.
69. Bronikowski AM. Experimental evidence for the adaptive evolution of growth rate in the garter snake *Thamnophis elegans*. *Evolution.* 2000;54(5):1760-7.
70. Badyaev AV, Hill GE. The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. *Evolution.* 2000;54(5):1784-94.
71. Enghoff H. The size of a millipede. In: Meyer E, Thaler K, Schedl W, editors. *Advances in Myriapodology. Proceedings of the 8th International Congress of Myriapodology, Innsbruck, 15-20 July 1990.* Ber nat-med Verein Innsbruck, 10. Innsbruck: Universitätsverlag Wagner; 1992. p. S47-S56.