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Sex-Specific Morphological Responses to Urbanisation Modulate Geographic Clines in *Pterostichusmelanarius* (Coleoptera, Carabidae)

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Abstract

Urbanisation and macroclimatic geographic gradients exert distinct selective pressures on the morphology of widespread insect species. However, the combined effects of these factors on sexual dimorphism remain under-explored. This study investigated variations in body size and shape, and sexual dimorphism, in the ground beetle *Pterostichusmelanarius* (Illiger, 1798) across a three-level urbanisation sites (rural, suburban, and urban). Linear morphological traits were extracted and analysed for 4616 specimens collected across nine regions spanning Europe and Russia. We used a Random Forest machine learning approach to establish morphological trajectories for sex prediction, alongside Principal Component Analysis to separate general size from size-independent shape. We observed persistent female-biased dimorphism in both size and shape, with scaling occurring isometrically between sexes across populations. Overall body size increased significantly as populations transitioned from rural to urban habitats. However, this size increase was severely dampened in males within the most urbanised areas, resulting in wider sexual size dimorphism. Furthermore, human-altered habitats caused a widespread shift in population shape towards wider, larger-headed phenotypes, a transformation driven disproportionately by females. These statistically significant differences suggest that, while broad geographic clines establish baseline morphology, localised urbanisation levels drive rapid, sex-specific phenotypic adaptations. Ultimately, females exhibit greater morphological plasticity in response to urbanisation pressures.

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1. Introduction

Urbanisation and macroclimatic gradients are widely recognised as important drivers of phenotypic variation across a diverse range of plant and animal populations [1,2]. As rural landscapes are progressively transformed into human-dominated environments, associated changes in temperature regimes, habitat structure, pollution load, and resource availability impose complex selective pressures on local populations [3–5]. Although broad geographic clines in morphology have long been documented across latitudinal and climatic gradients [6], the extent to which these large-scale patterns are modified by local anthropogenic conditions remains partially understood [7]. Urban environments can generate highly heterogeneous microhabitats that differ substantially from surrounding rural systems, potentially altering or overriding regional climatic signals [8,9]. Consequently, populations inhabiting urban landscapes may exhibit phenotypic trajectories that differ from those expected under rural environmental gradients alone [7].

Insects are particularly suitable organisms for studying these processes because of their short generation times, strong physiological dependence on environmental conditions, and high sensitivity to microclimatic variation [10–12]. Among insects, ground beetles (Coleoptera: Carabidae) are widely used as indicators of environmental change due to their ecological diversity, habitat fidelity, and measurable morphological responses to disturbance [13–15]. Previous studies have shown that both climatic gradients and urbanisation can influence body size, structural proportions, developmental stability, and dispersal-related traits in carabids [16–19]. However, these responses are often inconsistent among species, populations, and traits, suggesting that local anthropogenic pressures interact with broader environmental backgrounds in complex and context-dependent ways [20,21].

One potentially sensitive response to environmental change is the modification of sexual dimorphism. In many insect taxa, females are larger than males, reflecting differences in reproductive investment, energetic allocation, and life-history strategy [22,23]. Female body size is commonly associated with fecundity and abdominal capacity, whereas smaller male size may facilitate mobility and mate-searching behaviour. Because females generally require greater energetic investment to achieve larger body size, they may also be more sensitive to environmental stressors such as habitat degradation, pollution, or resource limitation [24]. Anthropogenic environments may therefore alter both Sexual Size Dimorphism (SSD) and Sexual Shape Dimorphism (SShD) through sex-specific morphological responses [25,26].

Recent evidence further suggests that morphological responses to urbanisation are frequently non-linear and trait-specific rather than uniformly directional [27,28]. Urban habitats may simultaneously impose thermal instability, habitat fragmentation, and chemical contamination while also providing novel resource subsidies and altered trophic interactions [29,30]. These contrasting pressures can produce divergent responses among taxa and even between sexes within the same species [31]. In some cases, urban habitat-specific pressures may constrain growth and reduce body size [32], whereas in others, increased resource availability and anthropogenic subsidies may offset these constraints and promote larger body size [33,34]. Similarly, the magnitude and direction of SSD may vary across anthropogenic gradients depending on local environmental conditions and sex-specific sensitivities [35,36]. Current evidence from ground beetles suggests that female-biased dimorphism is often maintained across environmental gradients, although

the degree of dimorphism may shift substantially depending on habitat context and trait examined [18,20,37].

An additional challenge in interpreting morphological responses is distinguishing generalised size reduction from changes in proportional body architecture [38]. Eco-morphological studies focus primarily on absolute body size, making it difficult to separate isometric variation from allometric restructuring of morphology [39]. This distinction is important because isometric reductions may reflect broad energetic limitations, whereas allometric shifts can indicate selective pressures acting on specific functional traits [8,27,32]. Despite increasing interest in urban evolutionary ecology, relatively few studies have simultaneously examined size-independent shape variation, sexual dimorphism, and macroclimatic background conditions within a unified analytical framework [40].

The widespread ground beetle *Pterostichusmelanarius* (Illiger, 1798) represents an appropriate model species for addressing these questions. This ecologically flexible and predominantly flightless species occupies a broad distribution spanning Western Europe to the continental interior of Eurasia, exposing populations to substantial variation in climate, habitat structure, and anthropogenic disturbance. At the same time, the limited dispersal capacity associated with their brachypterous nature ensures that local populations closely reflect the specific environmental conditions under which they developed [21]. Previous studies have documented geographic variation in body morphology and sexual dimorphism in *P. melanarius* associated with climatic gradients and urbanisation [41–43]. However, most existing studies remain geographically restricted to individual cities or regional levels [43,44], limiting the ability to disentangle local urban effects from broader macroclimatic influences.

In this study, we investigate sex-specific morphological variation in *P. melanarius* across a broad continental transect spanning Western Europe and Russia. Using a standardised three-level urbanisation scheme comprising rural and suburban habitats and urban cores, we quantify variation in both absolute body size and size-independent body proportions. Specifically, we test whether urbanisation is associated with consistent changes in sexual dimorphism and whether these responses are primarily isometric or allometric in nature. We hypothesise that: (1) urban populations are expected to exhibit morphological divergence from rural populations, reflecting the combined influence of habitat-specific pressures and altered resource conditions; (2) morphological responses will be more pronounced in females, driven by their greater energetic demands and higher environmental sensitivity; and (3) morphological shifts among levels may follow non-linear trajectories, with suburban populations potentially exhibiting distinct trait configurations relative to both rural and highly urbanised habitats.

By integrating macroclimatic and anthropogenic perspectives within a large-scale morphometric framework, this study aims to improve understanding of how urbanisation shapes phenotypic variation and sexual dimorphism in widespread insect species.

2. Methods

2.1. Study Design and Sampling

Sampling was conducted across nine regions spanning a broad geographic range, including Tatarstan, Sverdlovsk, Kemerovo, Udmurtia, Mariy El, the Cis-Urals, Belarus (53.529° N, 28.045° E), Kirov, and Turin (NW Italy) (Figure 1). To assess the influence of anthropogenic disturbance on morphology, study sites were assigned to a standardised three-level urbanisation scheme representing increasing human impact. Rural sites consisted primarily of agricultural landscapes and managed countryside with relatively low infrastructural development. Suburban sites represented transitional habitats with intermediate levels of urbanisation, including residential areas and urban ecotones. Urban sites

were located within heavily modified city centres characterised by dense built infrastructure and intensive human activity.

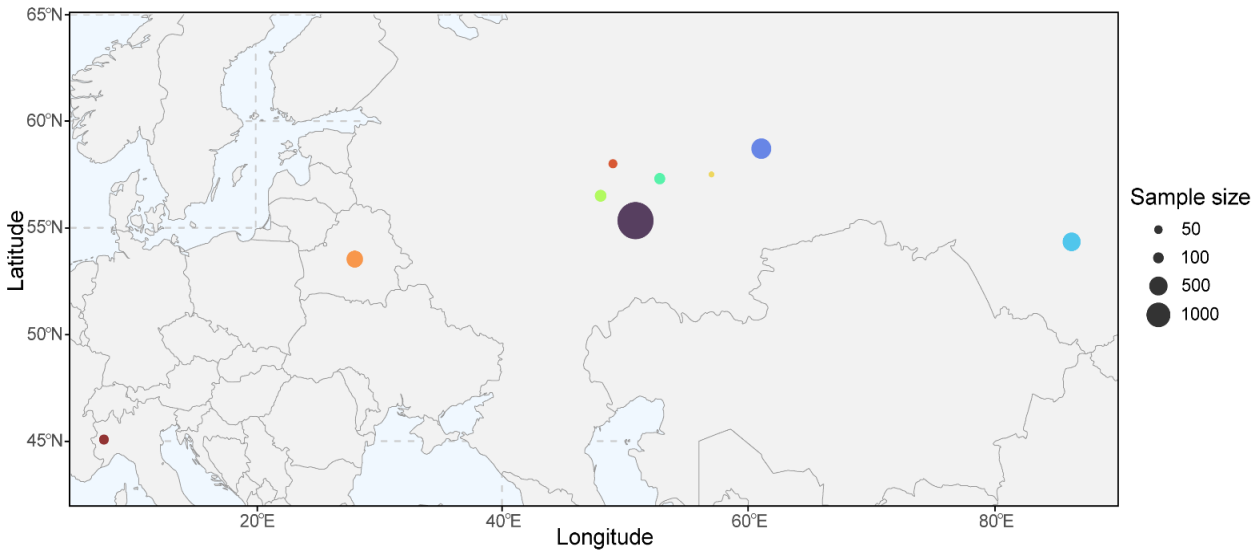


Figure 1. Map of the study area and regional sample sizes. Sampling locations spanning from Western Europe to Central Russia. Coordinates represent the mean latitude and longitude for each region's rural-to-urban site network. Circle areas are scaled to represent the total sample size at each region, with key size thresholds (50, 100, 500, 1000) indicated in the legend. Coloursdenote distinct datasets across nine sampled regions: Tatarstan, Sverdlovsk, Kemerovo, Udmurtia, Mariy El, Cis-Urals, Belarus, Kirov, and Turin.

Specimens were originally collected from ten habitat types, including meadow, birch, elm, oak, pine, lime, willow, shrub, lawn, and spruce habitats. However, because habitat type was strongly autocorrelated with geographic region, this variable was excluded from subsequent analyses to reduce potential confounding effects.

Fieldwork was conducted annually from 2018 to 2024, spanning early April through October. Beetles were collected using pitfall traps exclusively. Each trap consisted of a plastic receptacle (height: 8.5 cm; top diameter: 7.0 cm; base diameter: 4.5 cm) installed flush with the soil surface. Traps were baited with a 6% acidity red vinegar solution containing a small amount of dish soap to reduce surface tension. Protective lids were used to prevent flooding, minimise evaporation, and exclude vertebrates.

Ten traps were deployed at each study location and monitored at 15-day intervals. Collected specimens were counted, preserved in 70% ethanol and transported to the laboratory for processing.

2.2. Morphometric Data and Dataset Preparation

Adult beetles were identified to the species level following Hůrka [45]. All *P. melanarius* specimens were sexed and arranged in a straightened position on cotton pads. To ensure consistency and minimise observer bias, all specimens were photographed by a single operator at the Laboratory of Biomonitoring (Institute of Problems in Ecology and Mineral Wealth, Tatarstan Academy of Sciences) using a standardised protocol. Prior to measurement, specimens were blotted on filter paper and air-dried.

Digital images of the specimens were analysed to extract linear morphological traits using ImageJ [46]. Images were stored as JPEGs and captured at a resolution of 2940 × 3621 pixels, yielding a calibrated measurement resolution of 127.21 pixels/mm. We selected six linear traits to capture overall morphological variation, specifically targeting structures

critical for survival and reproductive success (Figure 2). Elytra and pronotum dimensions (**EL**, **EW**, **PL**, **PW**) are standard proxies for structural body size, which correlates with fecundity, survivability, and locomotor performance [21]. Head dimensions, including head length (**HL**) and interocular distance (**DE**), capture variation related to sensory capability and foraging capacity [47–49]. Because these functional traits often covary, we analysed them jointly using a multivariate approach to separate general size variation from size-independent shape differences.

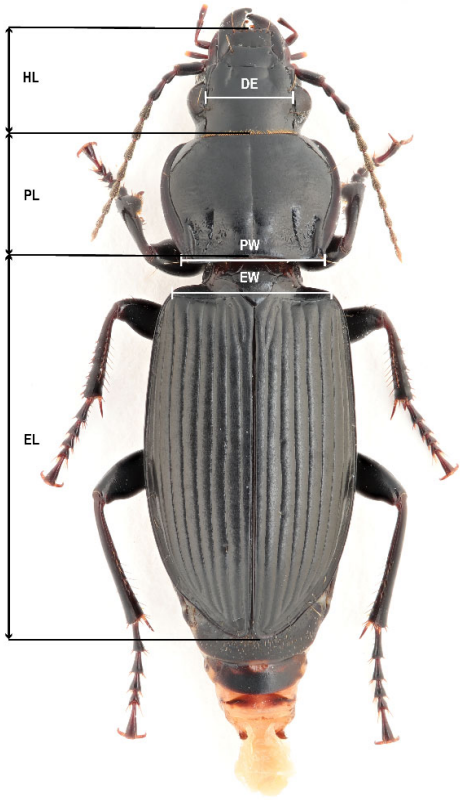


Figure 2. Measurement scheme for *Pterostichusmelanarius* (Illiger, 1798). **EL**, elytra length (posterior scutellum to elytral terminus); **EW**, elytra width (between anterior-distal corners); **PL**, pronotum length (along central furrow); **PW**, pronotum width (between posterior corners); **HL**, head length (labrum to occiput/postgena juncture); **DE**, interocular distance (between inner margins, dorsal view).

2.3. Statistical Analysis

We analysed a total of $n = 4616$ specimens of the carabid beetle *P. melanarius*. Specimens were differentiated by sex (male/female).

Preliminary analysis revealed that allometric relationships between traits (e.g., relative pronotum and elytra dimensions) shift along the urbanisation level (see Table S1). To explicitly separate isometric scaling (overall body size) from these size-independent shape variations, we performed a Principal Component Analysis (PCA) on the log-transformed, centred, and scaled values of the six linear traits using the "prcomp" function (centred and scaled).

The first principal component (PC1) accounted for 44.8% of the total morphological variance. Because all six traits exhibited negative loadings on this axis (ranging from −0.24 to −0.49), PC1 scores were multiplied by −1 (Table 1). As the algebraic sign of eigenvectors is mathematically arbitrary [50], this simple inversion ensures that positive PC1 values serve as an intuitive proxy for larger overall body size.

Table 1. Principal Component Analysis (PCA) loadings and variance explained for six linear morphometric traits. PC1 loadings have been inverted to reflect positive correlation with overall body size.

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Elytra length	0.346	0.571	0.133	0.635	−0.279	0.234
Elytra width	0.243	−0.602	−0.601	0.417	−0.133	0.161
Pronotum length	0.49	0.195	−0.221	0.069	0.681	−0.452
Pronotum width	0.466	0.218	−0.3	−0.616	−0.096	0.508
Head length	0.352	−0.432	0.651	0.065	0.333	0.388
Eye distance	0.49	−0.197	0.244	−0.186	−0.566	−0.554
Standard deviation	1.639	1.12	0.884	0.709	0.644	0.601
Proportion of variance	44.80%	20.90%	13%	8.40%	6.90%	6%
Cumulative proportion	44.80%	65.70%	78.70%	87.10%	94%	100%

The second principal component (PC2) explained an additional 20.9% of the variance (cumulative variance = 65.7%) and represented a size-independent shape axis. Specifically, PC2 captured a morphological trade-off between elongated forms with robust pronota (positive loadings for elytra length and pronotum dimensions) and forms with wider elytra and larger heads (negative loadings for elytra width, head length, and eye distance). Exact variable loadings for all principal components are reported in Table 1. To visualise morphological patterns, individual scores were extracted and plotted along the PC1 and PC2 axes, categorised by urbanisation levels (Figure S1) and sex (Figure S2). These extracted PC1 (size) and PC2 (shape) scores were subsequently used as response variables in the mixed-effects models.

Prior to downstream regression modelling, extreme morphological outliers were filtered from the dataset to prevent high-leverage observations from artificially influencing model fit. To account for differences in variance between principal components, individuals with PC1 or PC2 scores exceeding 3.5 standard deviations from the population mean ($Z > \pm 3.5$) were excluded (n = 57; 1.2% of the dataset). Although Tukey's IQR-based fences are commonly used to identify outliers, the extreme-fence method ($3 \times$ IQR) proved too conservative for this dataset and would have retained observations that were visually and biologically anomalous. In contrast, the selected Z-score threshold closely matches the observed distribution, allowing the removal of severe anomalies, including potential digitisation errors or damaged specimens, while preserving rural phenotypic variation. This approach ensures that model assumptions regarding leverage and residual normality are met without discarding biologically meaningful extremes.

2.3.1. Population-Level Scaling of Body Size and Shape Between Sexes

To assess the scaling of sexual size dimorphism (SSD) and sexual shape dimorphism (SShD) across different environments, we analysed the relationship between male and female morphological traits at the population level. In this context, we defined 11 population groups as unique combinations of geographic region and urbanisation levels, with the latter quantified using a 3-point index (rural = 1, suburban = 2, urban = 3). Individual-level data were aggregated by calculating the weighted mean principal component scores for overall body size (PC1) and body shape (PC2) for each sex within these specific region-by-intensity groups. To account for the uncertainty in morphological sexing, the probability scores generated by the preliminary Random Forest classification model were applied as observation weights during this aggregation. Group means were only calculated for populations containing a minimum of five individuals per sex to prevent skewing from low sample sizes.

We evaluated the allometric relationship between male and female trait means using Reduced Major Axis (RMA) regression (a Type II regression model). RMA was specifically chosen over standard Ordinary Least Squares (OLS) regression because both the independent (female mean scores) and dependent (male mean scores) variables are biological traits subject to natural biological variance and measurement error.

RMA regressions were conducted separately for the size (PC1) and shape (PC2) components using the *lmodel2* package [51]. Because principal component scores are mean-centred, interval-scale data that include negative values, the axis ranging method was strictly defined as "interval" for both variables. The statistical significance of the models, along with the 95% confidence intervals for the slopes and intercepts, was computed using permutation tests with 1000 iterations.

In this framework, an RMA slope whose 95% confidence interval includes 1.0 indicates isometric scaling (i.e., the magnitude of sexual dimorphism remains proportionally constant across regions and urbanisation levels). Conversely, a slope significantly differing from 1.0 indicates allometric scaling, suggesting that one sex exhibits a disproportionate morphological shift across the environmental sites relative to the other. Furthermore, regression intercepts were evaluated to assess baseline sexual dimorphism independently of the scaling slopes.

2.3.2. Estimating Individual Level Responses Towards Urbanisation Levels

To evaluate how urbanisation impacts linear morphology, we fitted a Generalized Additive Mixed Model (GAMM) using the *mgcv* package [52,53]. Body size (PC1) and body shape (PC2) scores were analysed as separate response variables. Both models included urbanisation sites (three levels: rural, suburban, urban), sex, and their interaction as fixed parametric effects. Urbanisation level was treated as a factor due to the uneven distribution of sites across regions (Table 2), which prevented the model from assuming a continuous linear effect.

Table 2. Distribution of *Pterostichusmelanarius* samples across nine regions and three levels of urbanisation (rural, suburban, urban). The number of females is indicated in brackets.

Urbanisation/Region	Tatarstan	Sverdlovsk	Kemerovo	Udmurtia	Mariy El	Cis-Urals	Belarus	Kirov	Turin
Rural	2541(1100)	621(300)	0	110(50)	130(62)	27(12)	371(179)	0	0
Suburban	129(78)	0	308(154)	0	0	0	0	57(25)	77(38)
Urban	75(35)	0	170(70)	0	0	0	0	0	0

Region (9 levels) was incorporated as a random effect to account for broad-scale spatial variation and to improve model fit when assessing the interaction between sex and the urbanisation level. Utilising region as a random effect effectively captured phenotypic variation across the study area while preventing the over-interpolation of localised effects in areas where sampling was discontinuous.

While our primary GAMM model successfully captured broad spatial variation using regions, we performed a complementary exploratory analysis to investigate continuous geographic clines. For this, we fitted an additional GAMM incorporating a two-dimensional thin-plate regression spline of geographic coordinates. This spatial term allowed us to visualise and quantify smooth phenotypic transitions across the study area independent of discrete regional boundaries (Supplementary Material; Figures S3 and S4).

To account for missing sex specificity, individual observations were weighted by probability scores generated from our Random Forest sex-prediction model. All models assumed a Gaussian error distribution with an identity link and were fitted using Restricted Maximum Likelihood (REML).

Model fit and underlying assumptions were evaluated using diagnostic tools within the *mgcv* and *gratia* packages [54]. Specifically, we utilised the "gam.check()" function to visually inspect residual diagnostics (including QQ-plots and residuals vs. fitted values) and to verify adequate basis dimensions for the models. Because sample sizes across the three urbanisation levels were unevenly distributed among regions, with only Tatarstan spanning the complete rural–urban transition and Kemerovo spanning the suburban–urban transition (Table 2), we explicitly evaluated collinearity between the fixed parametric terms (sex × urbanisation site) and the regional random effect using the concurvity function in the mgcv package (Table S2). Although the theoretical worst-case concurvity reaches 1.000 due to the single-level regions in the unpenalised design matrix, treating region as a penalised random intercept estimated via REML shrinks regional deviations toward zero when within-region level replication is sparse, allowing the parametric urbanisation terms to be anchored by regions containing multiple anthropogenic levels. Consequently, both the expected concurvity (Estimate = 0.493; pairwise estimate = 0.391) and the actual concurvity evaluated at the fitted model parameters (observed = 0.414 for PC1 size and 0.182 for PC2 shape; Table S2) remained well below the critical threshold of 0.80, confirming that the urbanisation effects are statistically separable from regional baselines. The significance of the fixed effects was evaluated using approximate *p*-values derived from the model summaries. All data visualisations were generated using the *ggplot2* package [55], with marginal model predictions and 95% confidence intervals extracted using the *ggeffects* package [56].

3. Results

3.1. Scaling of Sexual Dimorphism Along the Regions and Urbanisation Level

To evaluate the allometric scaling of SSD and SShD, we quantified the relationship between male and female trait means across 11 population groups (defined by unique combinations of geographic region and urbanisation level) using Reduced Major Axis (RMA) regression. Male and female trait values were strongly and positively correlated across populations for both body size (PC1: r = 0.98, $p < 0.001$) and body shape (PC2: r = 0.92, $p < 0.001$), indicating highly concordant morphological responses between the sexes among the environmental levels (Figures 3 and 4).

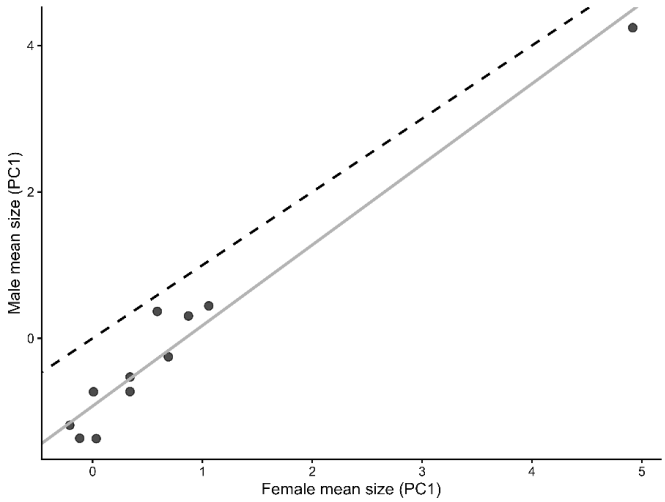


Figure 3. Allometric scaling of body size (PC1) between sexes. The relationship between male and female mean body size across 11 population groups defined by geographic region and urbanisation level. The solid grey line represents the fitted Reduced Major Axis (RMA) regression, while the dashed black line indicates a 1:1 isometric relationship (slope = 1). The RMA slope (1.101) does not

significantly differ from isometry. The downward shift of the RMA line relative to the 1:1 line reflects a significantly negative intercept (−0.926), indicating consistent, female-biased sexual size dimorphism across population groups.

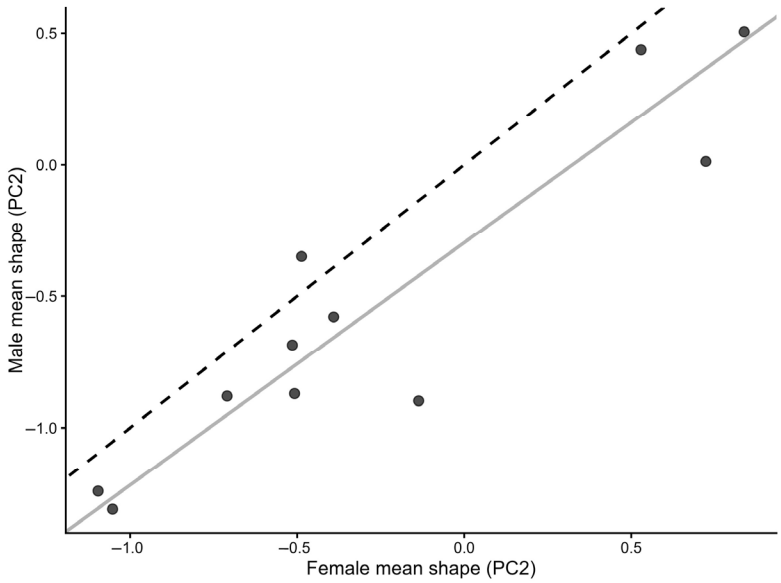


Figure 4. Allometric scaling of body shape (PC2) between sexes. The relationship between male and female mean body shape across 11 population groups defined by geographic region and urbanisation level. The solid grey line represents the fitted Reduced Major Axis (RMA) regression, while the dashed black line indicates a 1:1 isometric relationship (slope = 1). Similar to body size, body shape exhibits isometric scaling between the sexes (RMA slope = 0.920), with a significant negative intercept (−0.298) demonstrating a stable baseline level of sexual shape dimorphism across population groups.

The RMA regression for body size yielded a slope of 1.101 (95% CI: 0.944–1.284), which does not significantly deviate from unity (Figure 3). This indicates isometric scaling of body size between males and females across populations. In contrast, the intercept was significantly negative (−0.926; 95% CI: −1.067 to −0.804), demonstrating a consistent baseline difference between the sexes. Specifically, males exhibited lower mean PC1 values than females across all population groups, indicating persistent female-biased SSD independent of overall size variation among population groups (Figure 3).

A comparable pattern was observed for body shape (Figure 4). The RMA slope was 0.920 (95% CI: 0.663–1.272), again not significantly different from 1.0 (Figure 4), supporting isometric scaling of shape variation between the sexes. The intercept was significantly negative (−0.298; 95% CI: −0.363 to −0.208), indicating a consistent offset between male and female shape values across populations. As with body size, this reflects a stable baseline level of SShD.

Taken together, these results indicate that both body size and body shape scale isometrically between sexes across population groups, as the estimated slopes did not differ significantly from unity (Figures 3 and 4). Despite variation in overall morphology among populations, the magnitude of differences between males and females remained consistent. The significantly negative intercepts in both models further demonstrate persistent baseline sexual dimorphism, with females consistently exhibiting higher PC scores than males.

3.2. Body Size and Shape Responses to Urbanisation Levels

To evaluate how urbanisation impacts linear morphology at the individual level, we fitted GAMMs using the extracted PC1 (size) and PC2 (shape) scores as response variables,

with females in rural habitats set as the reference baseline (Table 3). For overall body size (PC1 model, deviance explained = 33.5%), there was a strong baseline SSD; in rural habitats, males were consistently smaller than females (Estimate = −0.888, t = −20.24, $p < 0.001$; Table 3). Because females are the reference sex, the main effects of urbanisation represent the morphological shift in females relative to the rural baseline. Female body size increased significantly from rural to suburban (Estimate = 0.367± 0.098, t = 3.74, $p < 0.001$) and urban habitats (Estimate = 0.589± 0.111, t = 4.70, $p < 0.001$). The interaction between sex and the suburban intensity was not significant (Estimate = 0.109 ± 0.111, $p = 0.325$), indicating that males and females experienced a comparable size increase in suburban habitats. However, there was a significant negative interaction between male sex and urban habitats (Estimate = −0.378± 0.150, t = −2.53, $p = 0.012$). Consequently, while females continued to increase in size in urban habitats (0.589 PC1 units relative to rural females), the corresponding size increase in males was substantially dampened (net male shift from rural to urban = 0.589–0.378 = 0.211 PC1 units), resulting in widening of female-biased SSD in the most urbanised habitats (Figure 5).

Table 3. Generalized Additive Mixed Model (GAMM) results for body size (PC1) and body shape (PC2). The models test the interactive effects of sex and urbanisation level on linear morphology, with region included as a random smooth term. The reference baselines for the categorical parametric terms are female for sex and rural for urbanisation level. Significant *p*-values (<0.05) are bolded. Note: For the size (PC1) model, adjusted R-squared = 0.334, deviance explained = 33.5%. For the shape (PC2) model, adjusted R-squared = 0.498, deviance explained = 50.0%. "edf" stands for estimated degrees of freedom for the smooth term.

Term	Size (PC1) Estimate ± SE	t-Value	*p*-Value	Shape (PC2) Estimate ± SE	t-Value	*p*-Value
Parametric terms (fixed effects)						
Intercept (female, rural)	0.767 ± 0.520	1.48	0.14	0.097 ± 0.227	0.43	0.67
Sex [male]	−0.888 ± 0.044	−20.24	**<0.001**	−0.481 ± 0.028	−17.48	**<0.001**
Anthropogenic [suburban]	0.367 ± 0.098	3.74	**<0.001**	−0.994 ± 0.061	−16.20	**<0.001**
Anthropogenic [urban]	0.589 ± 0.125	4.7	**<0.001**	−1.110 ± 0.078	−14.14	**<0.001**
Sex [male] × suburban	0.109 ± 0.111	0.99	0.325	0.179 ± 0.069	2.58	**0.01**
Sex [male] × urban	−0.378 ± 0.150	−2.53	**0.012**	0.324 ± 0.094	3.46	**<0.001**
Smooth terms (random effects)	Edf	F−value	*p*−value	edf	F−value	*p*−value
s(region)	7.95	196.6	**<0.001**	7.9	280	**<0.001**

In contrast, the model for body shape (PC2) explained 50.0% of the deviance and revealed pronounced sex-specific responses to urbanisation levels (Table 3). At the rural baseline, males exhibited significantly lower PC2 scores than females (Estimate = −0.481 ± 0.028, t = −17.48, $p < 0.001$), reflecting proportionally wider elytra and larger heads relative to the more elongated female form. Along the urbanisation levels, baseline (female) PC2 scores decreased from rural to suburban (Estimate = −0.994± 0.061, t = −16.20, $p < 0.001$) and urban habitat(Estimate = −1.110± 0.078, t = −14.14, $p < 0.001$), indicating a strong shift toward wider, larger-headed phenotypes in human-altered habitats (Figure 6).

Crucially, the interaction terms between male sex and both suburban (Estimate = 0.179± 0.069, t = 2.58, $p = 0.010$) and urban intensity (Estimate = 0.324± 0.094, t = 3.46, $p < 0.001$) were statistically significant and positive (Table 3). Because the baseline environmental shift (the main effect of females) is strongly negative, adding these positive interaction terms attenuates the negative slope in males, yielding a smaller net shape shift for males (−0.994 + 0.179 = −0.815 PC2 units in suburban; −1.110 + 0.324 = −0.786 PC2 units in urban habitats). Thus, although both sexes shift toward wider, more robust phenotypes in urbanised environments, females undergo a significantly steeper reduction in PC2 units than males, causing their shape trajectories to converge slightly in urban habitats (Figure 6).

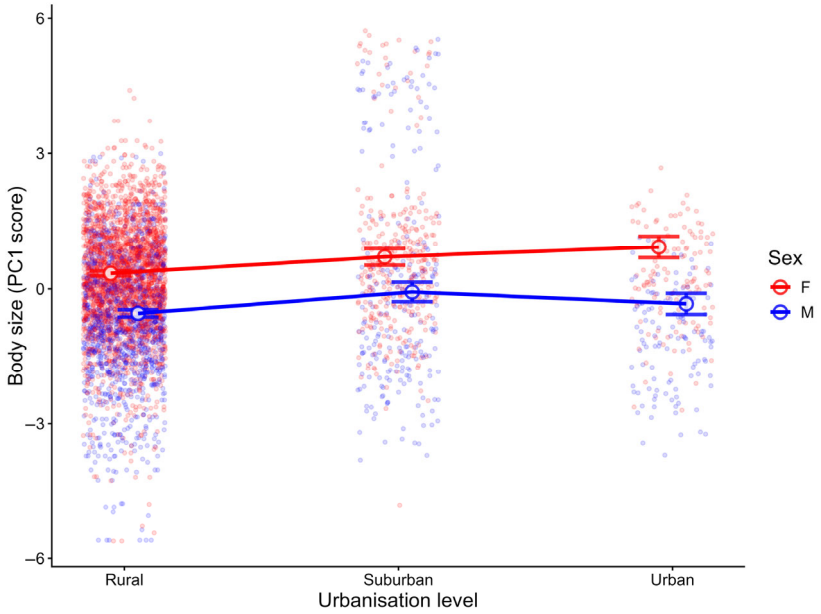


Figure 5. Response of overall body size (PC1) to urbanisation level. Predicted marginal means and 95% confidence intervals from the Generalized Additive Mixed Model (GAMM) for female (red) and male (blue) body size across rural, suburban, and urban habitats. Points represent jittered raw individual PC1scores. HigherPC1 scores correspond to larger overall structural body size.

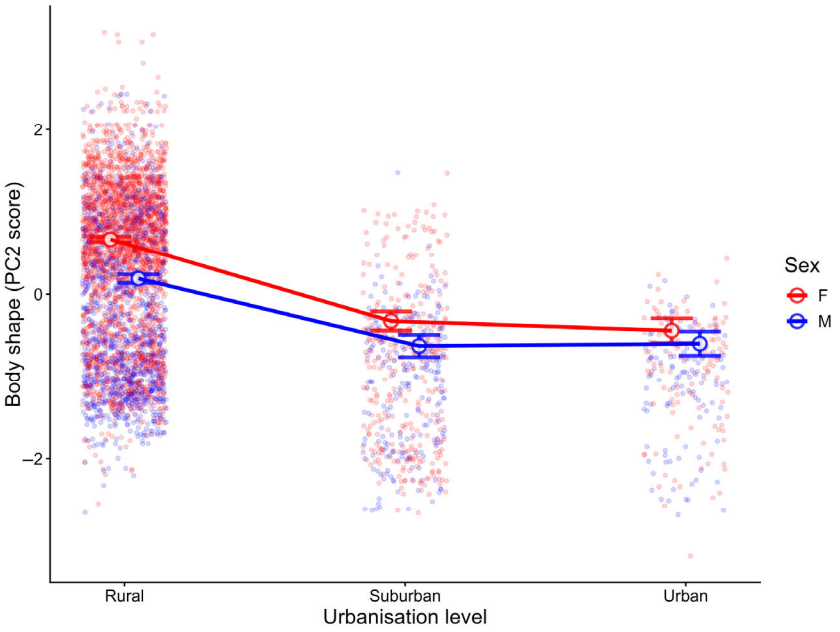


Figure 6. Response of body shape (PC2) to urbanisation levels. Predicted marginal means and 95% confidence intervals from the GAMM for female (red) and male (blue) body shape across rural, suburban, and urban habitats. Points represent jittered raw individual PC2scores. Lower PC2 scores correspond to proportionally wider elytra and larger heads relative to body length.

The region random effect was highly significant in both the size and shape models ($p < 0.001$; Table 3), confirming the importance of accounting for broad spatial baselines. Concurvity diagnostics confirmed that after accounting for REML penalisation, observed concurvity between the fixed parametric terms (sex × urbanisation level) and region was low to moderate (0.414 for the PC1 size model and 0.182 for the PC2 shape model; pairwise estimate = 0.391; Table S2), demonstrating that local anthropogenic effects were not con-

founded with regional baseline differences. Body size (PC1) exhibited a broad geographic cline, generally decreasing from the western portion of the study range (Europe) toward the east (Russia) (Figure S3). This suggests that macro-climatic sites exert independent selective pressures on overall body size, operating simultaneously alongside local urbanisation levels. In contrast, baseline body shape (PC2) showed a highly non-linear spatial response characterised by a distinct phenotypic peak in the central portion of the study range (Figure S4).

4. Discussion

Our results show that urbanisation exerts a profound influence on the morphology and sexual dimorphism of *P. melanarius* across broad macroclimatic gradients. Although large-scale geographic variation in body size and shape was evident across the continental transect, local anthropogenic conditions substantially modified these baseline patterns. Urban and suburban populations consistently diverged from their natural counterparts, indicating that urban environments act as potent selective filters capable of reshaping phenotypic trajectories, even within widespread and ecologically flexible species [2,15,41].

4.1. Urbanisation Promotes Increased Body Size and Widens Sexual Size Dimorphism

Consistent with our first hypothesis, urban and suburban populations exhibited morphological divergence from natural populations, generally characterised by an increase in overall body size along the urbanisation gradient. This pattern indicates that the metabolic costs associated with urban habitat-specific pressures may be offset by enhanced resource availability in human-modified environments [57,58]. Features such as suburban gardens, irrigated green spaces, compost sites, and synanthropic prey communities have the potential to provide stable food subsidies, which could facilitate greater larval biomass accumulation during development [33]. Comparable size increases have been documented in other urban arthropod taxa, where anthropogenic subsidies appear to compensate for elevated habitat-specific pressures [9,33].

As predicted by our second hypothesis, the increase in body size was strongly sex-specific [18]. Females showed greater morphological plasticity than males, especially in urban areas where SSD increased significantly [59]. This finding agrees with broader entomological theories suggesting that environmental variation in SSD is mainly driven by sex-specific phenotypic plasticity [59]. In addition, because females of *P. melanarius* are generally larger than males, they are likely to be more sensitive to environmental quality and resource availability. Similar patterns have been reported across taxa, where the larger sex tends to exhibit greater size plasticity [60]. Since female fecundity in insects is closely associated with body size and represents an important evolutionary driver of sex-specific life-history strategies, females are likely to benefit more directly from urban resource subsidies [61].

Conversely, male growth trajectories appeared to be constrained in highly urbanised conditions. This substantiates our third hypothesis regarding non-linear responses by showing that, while male and female growth scaled similarly in suburban habitats, their trajectories diverged sharply in urban cores. This divergence likely reflects sex-specific energetic trade-offs [20]. While females prioritise somatic growth to maximise reproductive output, males may allocate proportionally more energy towards locomotion and survival in heavily fragmented urban environments. Consequently, urbanisation alters overall body size and actively amplifies female-biased SSD [42,43]. Achieving and maintaining such exaggerated female-biased SSD strongly implies a corresponding sex difference in larval development time, as insects rarely attain a substantially larger final size without a proportionally prolonged development period for the larger sex [61].

Crucially, our findings challenge classical evolutionary expectations derived from Rensch's rule, which posits that in species with female-biased SSD, an overall increase in body size should correspond with a reduction in dimorphism [6,22]. While these classical models often assume uniform scaling, recent syntheses highlight that sex-specific plasticity and local developmental constraints frequently override these broad allometric expectations [62].This suggests that localised ecological conditions and sex-specific resource allocation strategies can disrupt or supersede broader evolutionary scaling laws, particularly in rapidly changing anthropogenic environments [63].

4.2. Sex-Specific Restructuring of Body Shape

Beyond sheer size, our PCA-based analysis revealed that these morphological responses were not purely isometric. Urban populations underwent substantial allometric restructuring, characterised by wider bodies and proportionally larger heads [39]. These structural shifts likely represent functional adaptations rather than generalised growth byproducts. In predatory carabids, head size and pronotum architecture are functionally coupled with mandibular musculature and bite force [31]. Urban trophic networks frequently feature altered prey communities, often dominated by mechanically resistant taxa such as gastropods and heavily sclerotised invertebrates. Proportionally larger heads may therefore optimise prey handling efficiency and feeding performance under these specific urban trophic conditions (see [63] for parallel adaptations in Orthoptera).

The pronounced, female-driven shift towards a more robust body architecture provides further support for our hypothesis regarding sex-specific ecological pressures. Due to the high energy demands of egg production, females are likely to experience stronger selective pressure to exploit calorie-dense urban prey efficiently [64]. As a result, female morphology appears to be more sensitive to local environmental filters, which is consistent with their greater capacity for phenotypic plasticity [59]. This heightened sensitivity may drive substantial modifications in SShD across the urban gradient [38].

Furthermore, the predominantly brachypterous nature of *P. melanarius* may mechanically reinforce these observed trait shifts [21]. Because this species relies on walking locomotion rather than flight, urban habitat fragmentation may strongly select for individuals with superior locomotor performance and endurance [13]. Increased body size and a broader biomechanical structure may enhance movement efficiency across discontinuous and highly modified urban substrates, such as asphalt and compacted soils (e.g., [64]). In this context, morphological enlargement and robust restructuring likely represent an adaptive phenotypic response to reduced habitat connectivity and increased spatial isolation [27].

4.3. Urbanisation Modulates Geographic Clines

While macroclimatic gradients remained significant predictors of morphological variation, our data indicate that urbanisation acts as a powerful secondary selective layer that fundamentally reshapes these geographic clines [65]. Current evidence suggests that urbanisation modifies or amplifies latitudinal temperature-size patterns rather than simply cancelling them [66]. Therefore, rather than universally overriding large-scale latitudinal patterns, urbanisation gradients impose strong, highly localised environmental filters, including elevated temperatures, habitat fragmentation, and altered disturbance regimes [67]. These localised conditions tend to intensify existing temperature-size relationships rather than erase them, modulating baseline trait expressions in complex ways that are highly dependent on the species involved [68].

The consistent emergence of similar urban phenotypes across geographically distant regions in our study supports the concept of "global urban signatures." Under this framework,

cities generate convergent selective pressures that favour specific trait syndromes, including enhanced mobility, thermal tolerance, and morphological robustness, thereby promoting phenotypic homogenisation among local populations [1,4,57,68–70]. However, it is crucial to recognise that this urban filtering is highly context-dependent. For example, studies on ground beetles demonstrate that while urban habitats often select against forest specialists in favour of mobile generalists [71], specific body size responses remain highly variable; some species exhibit marked size reductions, whereas others display idiosyncratic size shifts without a consistent cline override [15,19,58,72]. Therefore, while broad geographic clines establish the baseline trait parameters, shared urban characteristics drive parallel, context-dependent evolutionary and plastic responses. This introduces a dominant local layer of phenotypic variation that interacts with the prevailing regional macroclimate, with ultimate morphological outcomes depending on species-specific traits, body-size classes, and habitat specialisations [4,7].

5. Conclusions

Sexual dimorphism is rightly considered a driving force of evolution, as the differing responses of males and females to environmental factors lead to complex—yet often adaptive—rearrangements within populations. Despite numerous studies examining both the phenomenon itself and the dynamics of sexual dimorphism across various animal taxa, there is still no clear consensus regarding its underlying, intrinsic sources. This is partly due to a lack of systematic approaches in such research—specifically, a shortage of standardised model organisms, consistent methodologies, and, most importantly, large-scale datasets. It appears that to derive generalised conclusions, it is necessary to conduct broad-scope studies that incorporate multiple species within a single genus, analyse a range of traits, and employ advanced statistical methods.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18110712/s1. Figure S1: Principal Component Analysis (PCA) biplot visualising the morphospace of populations across the urbanisation gradient (rural, suburban, urban). The scatterplot visualises individual scores for the first two principal components derived from six linear morphometric traits. PC1 (44.8% of variance) serves as a proxy for overall body size, while PC2 (20.9% of variance) captures size-independent shape variation. Points are coloured by level of urbanisation, with corresponding ellipses encompassing 95% of the data distribution for each category. While there is extensive morphological overlap among environments, they differ notably in dispersion: urban populations form a tightly constrained cluster indicating reduced morphological variation, whereas suburban populations exhibit the widest dispersion along the body size axis (PC1), and rural populations show broad variation across the shape axis (PC2). Figure S2: Principal Component Analysis (PCA) biplot visualising sexual dimorphism in morphospace based on six linear morphometric traits. Individual scores are plotted along PC1 (overall body size) and PC2 (size-independent body shape). Points represent individual specimens, colour-coded by sex, with ellipses denoting the 95% confidence level for each group's multivariate distribution. The rightward shift of the female distribution along PC1 reflects the observed female-biased sexual size dimorphism. Additionally, females exhibit slightly broader dispersion along the shape axis (PC2), whereas males show greater variation along the size axis (PC1). Figure S3: Partial effect of the two-dimensional spatial smooth s(X = longitude, Y = latitude) on *Pterostichusmelanarius* body size (PC1). Warmer colours (red) indicate larger overall body size, while cooler colours (blue) indicate smaller body size. Grey areas represent regions excluded from interpolation due to distance from sampling coordinates (black points). Figure S4: Partial effect of the two-dimensional spatial smooth s (X = longitude, Y = latitude) on *Pterostichusmelanarius* body shape (PC2). Warmer colours(red) indicate higher values of the shape component, while cooler colours(blue) indicate lower values. Grey areas represent regions excluded from interpolation due to distance from sampling coordinates (black points). Figure S5: Diagnostic evaluation of the body size (PC1) Generalized Additive Mixed Model (GAMM) with Gaussian error

distribution with an identity link. Four panels display: (top left) a Quantile-Quantile (Q-Q) plot of deviance residuals indicating an approximately normal distribution; (top right) residuals vs. the linear predictor, demonstrating homoscedasticity (equal variance) across predicted values; (bottom left) a histogram of deviance residuals confirming a symmetric, normal distribution; and (bottom right) observed vs. fitted values. The vertical clustering of data points reflects the discrete categorical nature of the fixed effects (sex, urbanisation gradient) and random spatial effect (region). Figure S6: Diagnostic evaluation of the body shape (PC2) Generalized Additive Mixed Model (GAMM) with Gaussian error distribution and an identity link. Four panels display: (top left) a Quantile-Quantile (Q-Q) plot of deviance residuals indicating a generally normal distribution, with only minor deviations at the extreme tails typical of large ecological datasets; (top right) residuals vs. the linear predictor, demonstrating homoscedasticity (equal variance) across predicted values; (bottom left) a histogram of deviance residuals confirming a symmetric, normal distribution; and (bottom right) observed vs. fitted values. The vertical clustering of data points reflects the discrete categorical nature of the fixed effects (sex, urbanisation gradient) and random spatial effect (region); Table S1: Summary of Generalized Additive Mixed Models (GAMMs) fitted with a Gaussian identity link evaluating the allometric scaling of five morphological traits along an anthropogenic gradient ranging from rural to urban sites. Models test the interactive effects of body size (proxy: log-transformed elytra length), sex, and urbanisation gradient (used as a factor) on trait dimensions. Significant interaction terms (log(elytra length) × urbanisation gradient) indicate changes in the allometric slopes for specific traits, notably revealing positive allometric shifts for pronotum length and negative shifts for pronotum and elytra width at higher (urban) anthropogenic.

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Data Availability Statement: Datasets used in the present study can be found in the Figshare.com data repository https://doi.org/10.6084/m9.figshare.32238144. R scripts used in RStudio are available at the GitHub repository URL https://github.com/DomecStoces/Morphology_melanarius.git (accessed on 15 November 2026).

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