








Original Article

Morphology of mandibles of clown beetles (Coleoptera: Histeridae): hidden diversity, sexual dimorphism, and implications for prey range

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ABSTRACT

Clown beetles (Histeridae) are stable components of carrion communities, but their prey range is poorly recognised. An indirect way to deduce species' diet is to study the functional morphology of their mandibles. The use of a multimodal approach in the study of *Margarinotus brunneus*, *Margarinotus striola succicola*, *Saprinus semistriatus*, and *Saprinus planiusculus* revealed a hidden morphological diversity in their mandibles. Geometric morphometric analysis showed inter- and sex-specific differences in their shape. Synchrotron X-ray microtomography revealed variability in musculature between genera and the presence of a joint-like structure at the basal margins in the *Saprinus* species. Scanning electron microscopy showed variation in the surface of inner margins. Traditional morphometrics revealed differences in mandibular arc according to beetle species, sex, and left and right body sides. All species retained a roughly 17° asymmetry, with the right mandible always having the higher arc. We conclude that *Saprinus* species are adapted for cutting and puncturing soft-bodied prey, whereas *Margarinotus* beetles also feed on hard-bodied prey. Future studies should consider our findings for better planning of prey-choice experiments. Emphasis should be placed on recognising whether subtle differences in mandible shape involve differences in diets. This will be critical to determine the role of species in carrion ecosystems.

Keywords: asymmetry; carrion; geometric morphometrics; *Margarinotus*; *Saprinus*; SEM; synchrotron X-ray microtomography

INTRODUCTION

Histeridae, commonly known as clown beetles, are a large family with over 4800 described species worldwide (Newton 2023). Adults are small, usually not exceeding 10 mm in length. Their bodies are heavily sclerotized, rounded, shiny, and darkly coloured, typically black. Clown beetles are an ecologically diverse insect group with many species occurring in ephemeral resources rich in decomposing organic matter such as excrements, compost heaps, or carrion, where they find food and reproduce

(Mazur 1981, Summerlin *et al.* 1984, Hanski 1987, Matuszewski *et al.* 2010, Kovarik and Caterino 2016). In most species, both adults and larvae are predatory feeders (Hanski 1987). Previous studies showed that in necrophilous communities, histerids are one of the main groups of predators and may even outnumber rove beetles (Staphylinidae) (e.g. De Faria *et al.* 2018, Singh and Bala 2019, Jarmusz *et al.* 2020).

Necrophilous clown beetles are a frequent object of forensically oriented experiments on insect succession on carrion (e.g.

Bajerlein et al. 2011, Prado e Castro et al. 2013, Szelec et al. 2018, Feddern et al. 2019, Martín-Vega et al. 2019, Correa et al. 2020, Jarmusz et al. 2020). As a result, their residency patterns, particularly for species from central Europe, have been well recognised. The knowledge of their appearance time, length of their presence periods, and the number of breaks within the presence periods on carrion have enabled the determination of their usefulness for the succession-based estimation of the time elapsed since death (postmortem interval, PMI) (Matuszewski et al. 2010).

Although our knowledge of the succession of histerids on carrion has been remarkably increased recently, many essential aspects of their basic biology, such as growth rate or feeding habits, are still largely unknown. In the literature, only very general information is usually given, e.g. that necrophilous clown beetles feed on immature stages of Diptera, i.e. their eggs and larvae (e.g. Nuorteva 1970, Byrd and Castner 2009). Thus, we can currently better answer whether a given clown beetle species can be used for forensic purposes than giving an exact answer to the extent of its prey range.

Studies of residency patterns of insects visiting domestic pig carrion in western Poland suggest that the diet of histerid beetles can be more differentiated than previously thought (Bajerlein et al. 2011). It was observed that *Margarinotus brunneus* (Fabricius, 1775) and *Margarinotus striola succicola* (Thomson, 1862) were present on carrion for a much longer time compared to *Saprinus semistriatus* (Scriba, 1790). At that time, their abundances were low and relatively constant, whereas a clear peak in abundance of *S. semistriatus* was found. This raises questions about the causes of observed differences in their residencies on carrion. A hypothesis was presented that different patterns of carrion colonization in *Margarinotus* and *Saprinus* can result from differences in the spectrum of their prey. *Margarinotus brunneus* and *M. striola succicola* can feed on prey present for almost the entire decomposition period. In contrast, *S. semistriatus* is hypothesized to be able to prey only on larvae of blow flies (Calliphoridae) that occur on the carcass for a limited time during the active decay.

The decomposition of organic matter is fundamental to ecosystem function because it contributes to energy flow and nutrient recycling, and the presence of predatory histerids has the potential to alter this process. Patterns and rates of decomposition are strongly affected by insect-feeding activity, particularly maggots of blow flies (Calliphoridae). In high-density predatory beetles, the number of necrophagous flies on carrion may be reduced, slowing the duration of the decomposition process (Nuorteva 1970, Pechal et al. 2014). Therefore, knowledge of the feeding habits of necrophilous predators is required to determine their exact role in carrion communities and understand how the carrion ecosystems function.

Studies of feeding requirements in small arthropod predators can be challenging for several reasons. Firstly, such observations can be difficult, if not even impossible, to conduct under natural conditions since many of them exhibit a concealed lifestyle. This is true for necrophilous histerids, which spend most of their time in the soil or on the soil surface beneath the carrion. In turn, the results of the prey-choice experiments conducted under laboratory conditions can be challenging to interpret because it is not possible to recreate natural ecosystems in the laboratory. Thus,

the selected prey does not necessarily have to be preferred under natural conditions. Moreover, the sharp distinction within carrion feeding guilds into necrophagous and predatory feeders is not always consistent with the natural feeding habits of species. The position of many species in their food webs is more complex. Some of them are more or less omnivorous, and even cannibalism can be expected (Hanski 1987). An indirect way to deduce species' food resources is to study the functional morphology of their mouthparts. As insect feeding habits are reflected in the morphology of their mandibles, these can be used as predictors of dietary adaptations (e.g. Hörnschemeyer et al. 2013, Karolyi et al. 2016, Sasakawa 2016, Klunk et al. 2023).

In this study, we aimed to analyse the functional morphology of mandibles in four species of clown beetles of forensic interest: *Margarinotus brunneus*, *Margarinotus striola succicola*, *Saprinus semistriatus*, and *Saprinus planiusculus* Motschulsky, 1849, to deduce their diet. The comparative external morphology of mouthparts of histerid beetles has only rarely been described and is usually only available for taxa that needed taxonomic revision (e.g. Lackner 2010, Lackner and Leschen 2017). These studies have revealed asymmetry in the mandibles of many histerid species. Nevertheless, these analyses were largely taxonomically oriented and did not involve functional interpretation (e.g. Lackner 2010, Caterino and Tishechkin 2013). Considering differences in residency patterns on carrion between clown beetle species, the relationships between the morphology of their mouthparts and their diet must be better understood. We have tested the following hypotheses:

- (i) Differences in patterns of residencies on carrion between *Margarinotus* and *Saprinus* species may be related to differences in their diet, which should be reflected in the functional morphology of their mandibles.
- (ii) Within the genera, there are no differences in mandible shape between *S. semistriatus* and *S. planiusculus* and between *M. brunneus* and *M. striola succicola*; therefore, a similar diet will be expected.
- (iii) The mandibles of both species of *Saprinus* are adapted to feeding on soft-bodied prey, such as fly larvae. In contrast, the mandibles of the *Margarinotus* species are adapted to feeding on more differentiated prey, including hard-bodied invertebrates.
- (iv) The development of the abductor and adductor mandible muscles is related to beetle feeding mode. Mandibles adapted to crushing have well-developed muscles. In contrast, mandibles adapted to puncturing soft-bodied larvae have weaker developed muscles. The abductor and adductor mandibular muscles work antagonistically during mandible movement. The abductor muscles open the mandibles, whereas the better-developed adductor muscles close the mandibles.

To test these hypotheses, we have used a multimodal approach to study the functional morphology of mandibles, which included the application of geometric morphometrics (GM), synchrotron X-ray microtomography (SR-μCT), scanning electron microscopy (SEM), and linear morphometrics. Geometric morphometrics was used to study intra- and interspecific

differences in mandibular shape. Linear morphometrics was employed to analyse the mandibular arc explicitly. SR- μ CT was applied to compare the anatomy of the beetles' head capsules, particularly emphasizing the structure of the adductor and abductor mandibular muscles. SEM allowed us to analyse the external surface morphology of mandibles to find possible changes resulting from their feeding action.

MATERIALS AND METHODS

Species of clown beetles (Histeridae)

Individuals of *Margarinotus brunneus* (Fabricius, 1775), *Margarinotus striola succicola* (Thomson, 1862), *Saprinus planiusculus* Motschulsky, 1849, and *Saprinus semistriatus* (Scriba, 1790) were analysed. These histerids are stable components of carrion communities in Central Europe (e.g. Bajerlein *et al.* 2011, Feddern *et al.* 2019, Jarmusz *et al.* 2020). Beetles were collected during forensically oriented experiments on insect succession and decomposition of the carrion of the domestic pig (*Sus scrofa domestica* Linnaeus, 1758) in open and forest habitats of the Biedrusko military range near Poznań (western Poland) (Matuszewski *et al.* 2010, 2016, Matuszewski and Szafalowicz 2013). We analysed beetles collected from carcasses and not beetles that had been artificially reared because the morphology of their mandibles reflects the actual life history of a given individual. Histerids were gathered using two ground pitfall traps located next to the decomposing carrion and manually from the carrion surface and the soil beneath the carrion in the spring, summer, and autumn of 2006 and 2007, and during the spring and summer of 2011 and 2012. Collected individuals were preserved in a 75% solution of ethylene alcohol. Details on the experimental design can be found in Matuszewski *et al.* (2010, 2016). Taxonomic identification of beetles was made using the key for identification by Mazur (1981). Genitalia of all specimens were dissected to ensure accurate determination of sex (Mazur 1981). Beetle individuals are deposited at the Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland.

Analysis of beetle body size

Since the body size of predators is key when considering their feeding habits, differences in body length according to beetle species and sex were studied. Body length was measured along the body midline as a distance between the anterior margin of the pronotum and the apex of the elytra and expressed in mm. Differences in body length were evaluated with two-way analysis of variance (two-way ANOVA). The Bonferroni post hoc test was used to reveal differences in multipair comparisons. In all analyses, a $P < .05$ level of significance was accepted. The analyses were carried out in the R environment (v.4.2.2; R Core Team 2022), additionally using the readxl (v.1.3.1; Wickham and Bryan 2019) and dplyr (v.1.1.2; Wickham *et al.* 2023) packages.

Images of mandibles

Mandible shape was studied according to beetle species, sex, and body side (left, right) using a two-dimensional geometric morphometric approach. A data set consisting of 2080 mandible

images, saved as TIFF files, was used. For each of the 16 groups of studied mandibles (four species*two sexes*two body sides) (Fig. 1), 130 images were taken with a scale bar and digitized. Dried individuals were put on a paper towel slightly moistened with water to prevent their heads and mandibles from being lost during detachment. Firstly, the head was removed from the body, and then both mandibles were removed from the head capsule using a dissecting needle. The detached mandibles were cleaned with 75% ethylene alcohol and put on the stereomicroscope stage plane with a brush. For each mandible, images of the dorsal side were taken using the Olympus SZ61 stereomicroscope fitted with a camera and Cell A software (Olympus Corporation, Tokyo, Japan).

Digitizing landmarks and semilandmarks

Both landmarks and semilandmarks were digitized (Fig. 2). The landmarks' positions were easily identified (i.e. the basal point of the inner margin, the mandibular apex, and the cavity within the outer margin next to the ventral condyle). The first curve of semilandmarks was traced along the inner mandibular margin, starting from the point marked with the landmark localized at the basal point of the inner margin and ending at the mandibular apex, marked with the second landmark. The second curve was traced along the outer mandibular margin; it started at the mandibular apex and ended at the point marked with the third landmark localized in the cavity next to the ventral condyle (Fig. 2). Twenty-two and thirty-five semilandmarks were plotted at regular intervals on the inner and outer mandible margins, respectively, using tpsDig2 software (Rohlf 2017). To minimize measurement error, all images were taken and digitized by the same person (D.B.).

Geometric morphometric analysis

To estimate measurement error, two images of the dorsal side of the right mandibles for 20 male individuals of *S. planiusculus* were taken. Next, two observers (D.B. and A.M.K.) digitized each set of images. To quantify the measurement error at two levels, i.e. imaging and digitizing, the analysis of variance (Procrustes ANOVA) was used (Klingenberg *et al.* 2002).

Raw coordinates of landmarks and semilandmarks were transformed into shape coordinates using a generalised Procrustes analysis (GPA). This method mathematically quantifies shape by removing translation and rotation and scaling the studied object to a unit centroid size (Zelditch *et al.* 2012). Centroid size obtained from landmarks serves as a measure of the size of the structure. It is calculated as the square root of the summed square distances of each landmark from the centroid of a landmark configuration, i.e. the average position of all landmarks (Zelditch *et al.* 2004). To test the presence of allometry, multivariate regression with a permutation test (10 000 randomized rounds) was performed between Procrustes coordinates and centroid size. Size effects are crucial to shape variation; therefore, they should be tested in biological studies (Outomuro and Johansson 2017). In case of a significant correlation between the shape and size of the mandible, further geometric morphometric analysis is usually performed on the multivariate regression residuals. This approach excludes the size of the mandible as a component explaining the shape variation in the mandible.

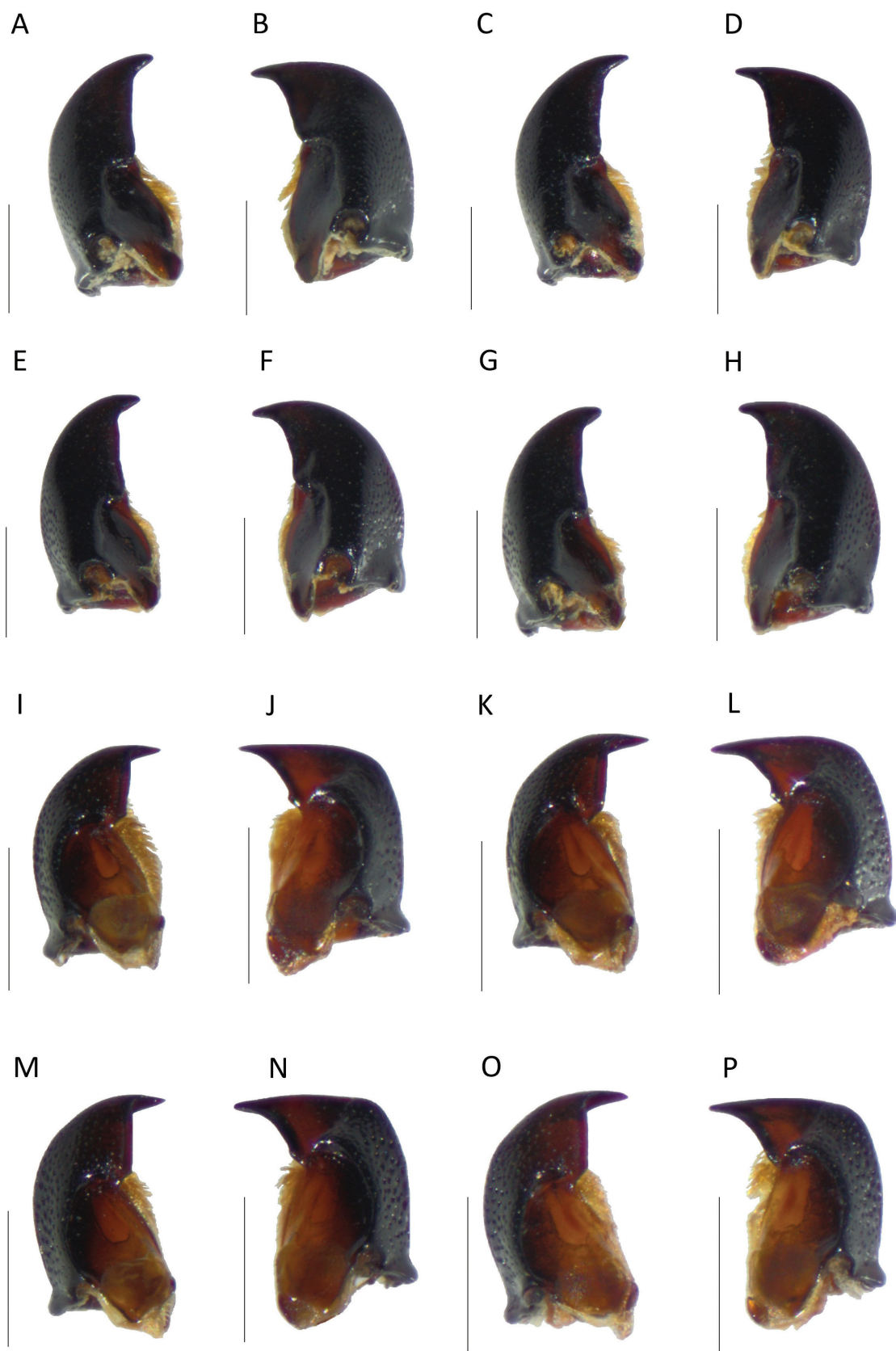


Figure 1. Mandibles of studied clown beetles (Histeridae). A, B, left and right mandibles of females of *Margarinotus brunneus*. C, D, left and right mandibles of males of *M. brunneus*. E, F, left and right mandibles of females of *Margarinotus striola succicola*. G, H, left and right mandibles of males of *M. striola succicola*. I, J, left and right mandibles of females of *Saprinus planiusculus*. K, L, left and right mandibles of males of *S. planiusculus*. M, N, left and right mandibles of females in *Saprinus semistriatus*. O, P, left and right mandibles of males of *S. semistriatus*. Scale bars: 500 μ m.

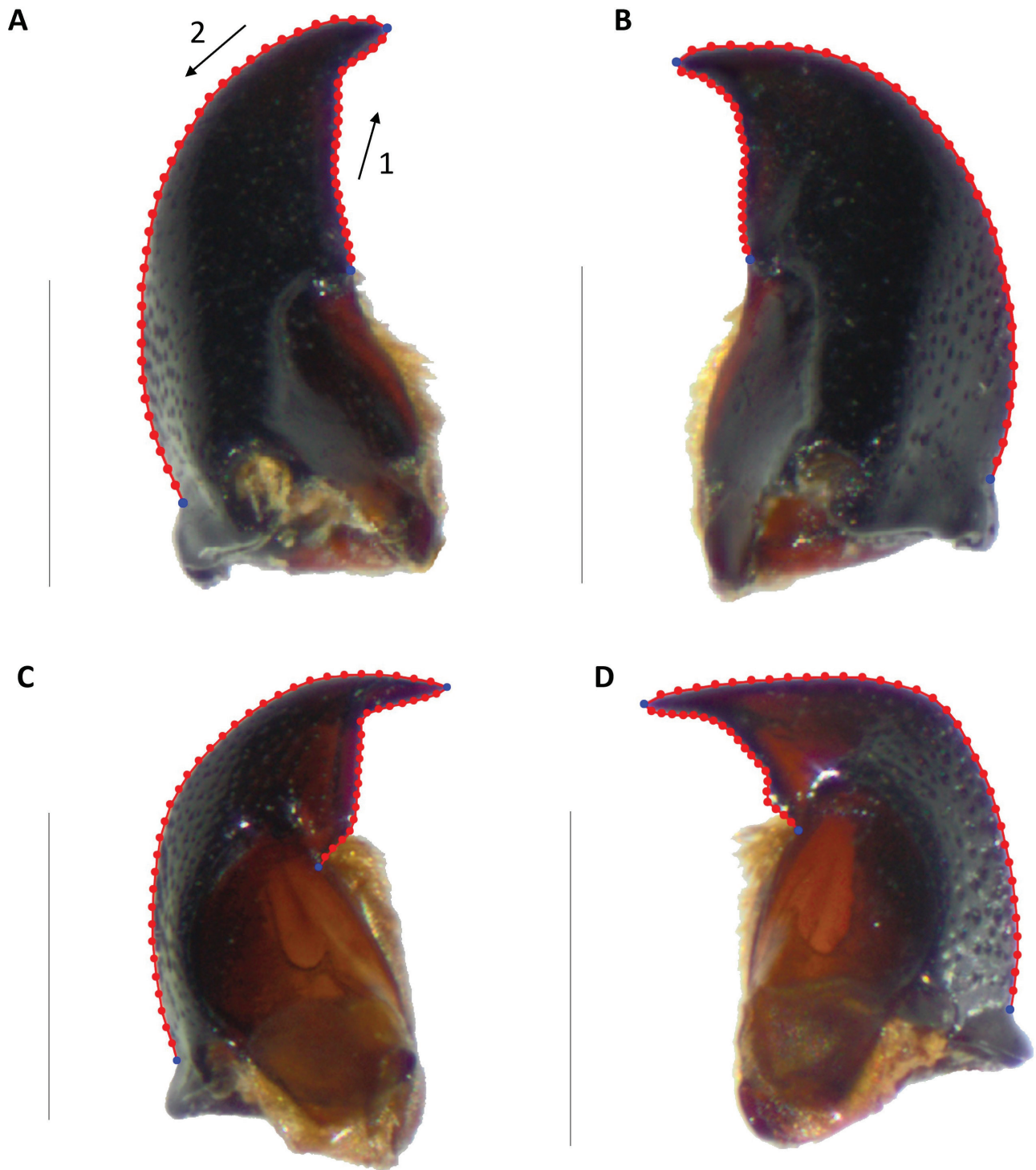


Figure 2. Description of curves used in the geometric morphometric analyses of mandibles in clown beetles (Histeridae). Due to the similarity in the shape of mandibles within both species of *Saprinus* and both species of *Margarinotus*, the description of curves has been presented for the left (A) and right (B) mandibles of males of *Margarinotus striola* and left (C) and right (D) mandibles of males of *Saprinus planiusculus*. The inner curve was resampled in 22 semilandmarks, and the outer curve was resampled in 35 semilandmarks. Red points represent semilandmarks, and blue points represent landmarks. The black arrows, and 1-2 show the tracing order of the inner and outer curves. Scale bars: 500 μm .

Differences in mandible shape according to body side (left and right mandibles) and beetle sex were studied using the Procrustes analysis of variance (Procrustes ANOVA). Next, the studied mandible samples were subjected to a principal component

analysis (PCA) to analyse the overall variation patterns in mandible shape among all species. Next, the between-species shape differences were tested using canonical variate analysis (CVA) followed by a permutation test as performed in the multivariate

regression of shape on size. The CVA was performed separately for the left and right mandibles. Geometric morphometric analyses were performed using MorphoJ (v.1.06d) (Klingenberg 2011).

Mandibular arc

The centre of each mandible arc was found using standard geometric principles (Fig. 3). A detailed description of the methodology and line tracing of the mandible showing this method is presented in the Figure 3 caption. Measurements were made using ImageJ (v.1.53a; Rasband 2020) and the macro by Olivier Burri (GIST) (Burri 2016). The same images that were taken for GM analyses were used. All arc measurements were taken by a single person (D.B.). The arc of mandibles expressed as a mean value of mandibular angle was compared according to beetle species, sex, and body side (left and right). Analysis of variance of aligned rank transformed data (ART ANOVA) was performed due to heteroscedasticity of error variance and heterogeneity of variance. The Bonferroni post hoc test was used to reveal differences in multipair comparisons. The statistical analysis was carried out in the R environment (v.4.2.2; R Core Team 2022) with the use of the ARTool package (v.0.11.1; Elkin et al. 2021), the readxl package (v.1.3.1; Wickham and Bryan 2019), the dplyr package (v.1.1.2; Wickham et al. 2023), and emmeans (v.1.8.6; Lenth et al. 2023).

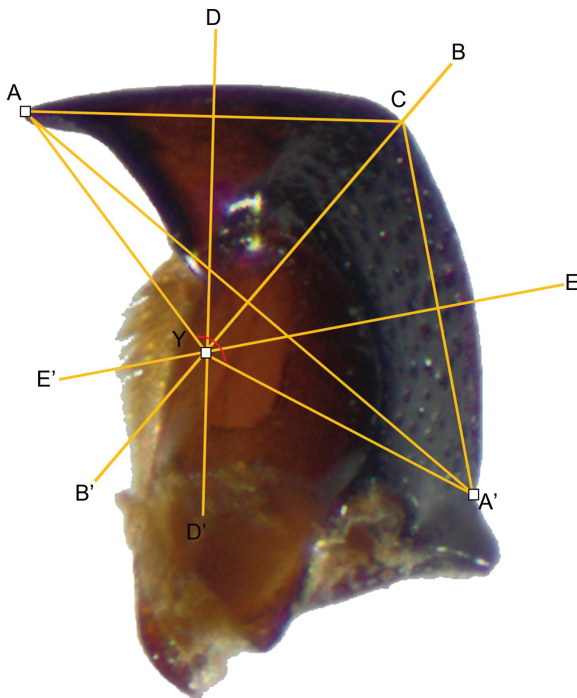


Figure 3. Measurements of mandibular arc in clown beetles (Histeridae). Method description: Initially, a chord AA' was drawn from the apex to the cavity next to the ventral condyle. Then the chord AA' was bisected by the perpendicular BB'. Next, chords AC and CA' were drawn, and the C point was determined by the bisection of the chord BB' with the outer mandible curve. Two perpendiculars, DD' and EE', were drawn to bisect AC and CA'. The extended perpendiculars DD' and EE' have met in point Y. The mandibular arc is taken from the angle subtended between radii YA and YA' (marked in red). Scale bar: 500 μ m.

For 326 pairs of left and right mandibles per beetle specimen, we calculated differences in their arcs. We studied their relation within and between species using linear models in standard and Generalized Least Squares (GLS) frameworks (Pinheiro and Bates 2000, 2023). In analyses, a $P < .05$ level of significance was used to accept or reject models.

Scanning electron microscopy (SEM) imaging

The SEM analysis covered mandibles with various degrees of use, i.e. without visible changes, with more or less worn parts, and with visible breaks. Beetle mandibles were mounted on stubs with double-sided sticky tape, coated with gold, and observed with a Zeiss Evo 40 Scanning Electron Microscope (Carl Zeiss SMT Ltd., Cambridge, UK) at the Faculty of Biology, Adam Mickiewicz University, Poznań.

Synchrotron X-ray microtomography

SR- μ CT of ethanol-preserved clown beetle heads was performed at the high throughput station of the Imaging Cluster at KIT Light Source. We employed a parallel polychromatic X-ray beam produced by a 1.5 Tesla bending magnet that was spectrally filtered by 0.5 mm aluminium. The resulting spectrum had a peak at about 15 keV, with a full width at half maximum bandwidth of about 10 keV. A fast indirect detector system was employed, consisting of a 12 μ m LSO:Tb scintillator (Cecilia et al. 2011) and a diffraction limited optical microscope (Optique Peter) (Douissard et al. 2012) coupled with a 12 bit pco.dimax high speed camera with 2016 \times 2016 pixels. For each scan, we took 200 dark field images, 200 flat field images, and 3000 equiangularly spaced radiographic projections in a range of 180° with 70 frames per second. The optical magnification was set to 10 \times , resulting in an effective pixel size of 1.22 μ m. We used the control system concert (Vogelgesang et al. 2016) for automated data acquisition. Online and final data processing included flat field correction and phase retrieval of the projections based on the transport of intensity equation (Paganin et al. 2002). The 3D tomographic reconstructions were done with tof (Faragó et al. 2022) and yielded phase and absorption contrast data sets. These were blended and converted into 8-bit volumes.

Post-processing and analysis of SR- μ CT data

Tomographic volumes were cropped to the region of interest. The head capsule, mandibles, mandibular muscles, and apodemes were pre-segmented in Amira 2022.2. The labels served as input for smart interpolation with Biomedisa (biomedisa.info) (Lösel et al. 2020). The results were imported back into Amira, and minor errors were corrected. The segmented head capsules were manually closed at the neck and between the clypeus and the level of the posterior mandibular articulations. The volumes of the final labels were calculated with the Amira's 'MaterialStatistics' module. They were converted into polygon meshes, exported as OBJ files and reassembled and smoothed in CINEMA 4D R20.

The movement axes of the mandibles were determined by positioning a cylinder through the two articulations of the dicondylic mandible with the head capsule. Subsequently, the orientation of the heads was adjusted so that the axes of rotation were perpendicular to the viewer, and the inward tilt angle was determined. The angular motion range of the mandibles

was specified by moving them along their rotation axes and identifying the points of contact between the mandible and the head capsule. For a male *S. semistriatus*, CINEMA 4D R20 was used to animate the hypothetical movement of the mandibles along the determined axes (Supporting information, Movie S1).

RESULTS

Body size of beetles

Species varied statistically significantly in body length ($P < .001$) (Fig. 4A; Supporting information, Table S1). *Margarinotus brunneus* was the largest (5.76 ± 0.44 mm; mean \pm SD), *M. striola* was slightly smaller (5.23 ± 0.38 mm), and both *S. semistriatus* (4.55 ± 0.37 mm) and *S. planiusculus* (4.55 ± 0.32 mm) were the smallest among the studied beetles. No statistically significant differences in body length between the two species of *Saprinus* were found. Sexual dimorphism in body size was found for all species, with females being significantly larger than males ($P < .001$) (Fig. 4A; Supporting information, Tables S1–S2). The interaction of effects was significant ($P = .001$) (Supporting information, Table S1).

Geometric morphometric analysis

The mean squares for the two error levels (imaging and digitizing) were smaller than for an individual (Supporting information, Table S3). This meant that the measurement error was smaller than the smallest level of biological variation. Therefore, imaging or digitizing errors did not affect the obtained results.

Allometry was revealed in mandibles of all species $P < .05$ (Supporting information, Table S4), therefore, all further geometric morphometric analyses (i.e. Procrustes ANOVA, PCA, and CVA) were performed on the multivariate regression residuals to remove the allometry effect.

The Procrustes ANOVA revealed no statistically significant differences in mandible size between left and right mandibles within an individual (Table 1). Statistically significant differences were observed in mandible shape between the left and right mandibles within an individual (Table 1).

Figure 5 shows that the same level of variation in the shape of the mandible characterized all groups. PC1 and the second PC2 axes explained 67.89% and 16.09% of the variation in shape, respectively (Fig. 5). Strikingly, PC1 separated the left from the right mandibles, and in more detail, PC1 further explained a great amount of the variation in right mandible shape between sexes of *M. striola succicola*. Changes in PC1 were largely related to the outer mandibular margin. This axis of variation went from narrow mandibles with relatively broad apices (negative scores) to more angular mandibles with narrower and longer apices (positive scores). Changes in PC2 were mainly related to the inner mandibular margin. This axis of variation went from mandibles with short and rounded apices (negative scores) to narrower mandibles with long and pointed apices (positive scores) (Fig. 5).

The CVA emphasized shape differences between all studied groups, which were statistically significant in all pairwise tests (Supporting information, Table S5). For left mandibles, CV1 and CV2 explained 72.98% and 14.08% of the total variance in shape, respectively (Fig. 6). CV1 represented the shape from

broader mandibles with longer and more pointed apices (negative values) to narrower mandibles with shorter and less pointed apices (positive values). In turn, individuals with negative values of CV2 were characterized by broader mandibles with longer apices than those with positive values of CV2 (Fig. 6).

For right mandibles, CV1 and CV2 explained 75.02% and 15.27% of the total variance in shape, respectively (Fig. 7). Negative values of CV1 showed the shape of the mandible with strongly convex outer margins and longer and pointed apices. In turn, positive values of CV1 represented narrower mandibles with shorter and broader apices. Individuals with positive values of CV2 were characterized by more convex mandibles than those with negative values of CV2 (Fig. 7).

Differences in mandible shape between sexes were similar in both species of *Margarinotus* (Fig. 8A, B). The inner margin of the left mandibles was more indented in males than in females. Consequently, left mandibles in males were narrower. The apices of the left and right mandibles were distinctly pointed in males. In both species of *Saprinus*, males had distinctly more indented inner margins of left mandibles when compared to left mandibles in females (Fig. 8C, D). On the other hand, females had narrower and pointed apices. Less distinct sexual differences were related to the shape of outer mandibular margins.

Mandibular muscles

Mandibles are articulated to the head capsule by the anterior (dorsal) and the posterior (ventral) condyles, and their movement is controlled by the abductor (opener) and adductor (closer) muscles (van de Kamp *et al.* 2022). The adductor muscle (M1) consisted of two muscle bundles (M1a and M1b), with M1a located dorsally and M1b ventrally (Fig. 9). Both bundles of the adductor muscle inserted into the inner margin of the mandibular base via an apodeme that was composed of a large distal sclerotized part and a small proximal flexible ligament at the insertion area. The M1a bundle originated from the lateral sides of the head and the posterodorsal parts of temples, whereas the M1b bundle connected with the lateral sides of the head and the posteroventral parts of temples (Fig. 9). The abductor muscle (M2) was located between M1a and M1b and inserted into the outer margin of the mandibular base. As for the adductor, the insertion was realized via an apodeme with a distal sclerotized part and a proximal ligament. It originated from the lateral sides of the head and temples (Fig. 9).

Distinct differences in mandibular muscles were seen at the genus level (Tables 2, 3). The volume of all mandibular muscles in *Margarinotus* species was higher compared to the *Saprinus* species (Table 3). Both *Margarinotus* and *Saprinus* had larger adductor muscles compared to abductor muscles, and their M1b adductors were larger than M1a adductors (Tables 2, 3). The ratios of the muscle volume to the head volume showed that *Margarinotus* species had much better-developed adductor muscles than the *Saprinus* species. In turn, *Saprinus* had better-developed abductor muscles (Table 3). Another difference revealed by the SR-μCT was that the abductor apodemes were long and thin in *Margarinotus* and short and broad in *Saprinus*. Asymmetries in mandibular muscle development in *Margarinotus* and *Saprinus* could be further observed. The left mandibles of *Margarinotus* had better-developed adductor

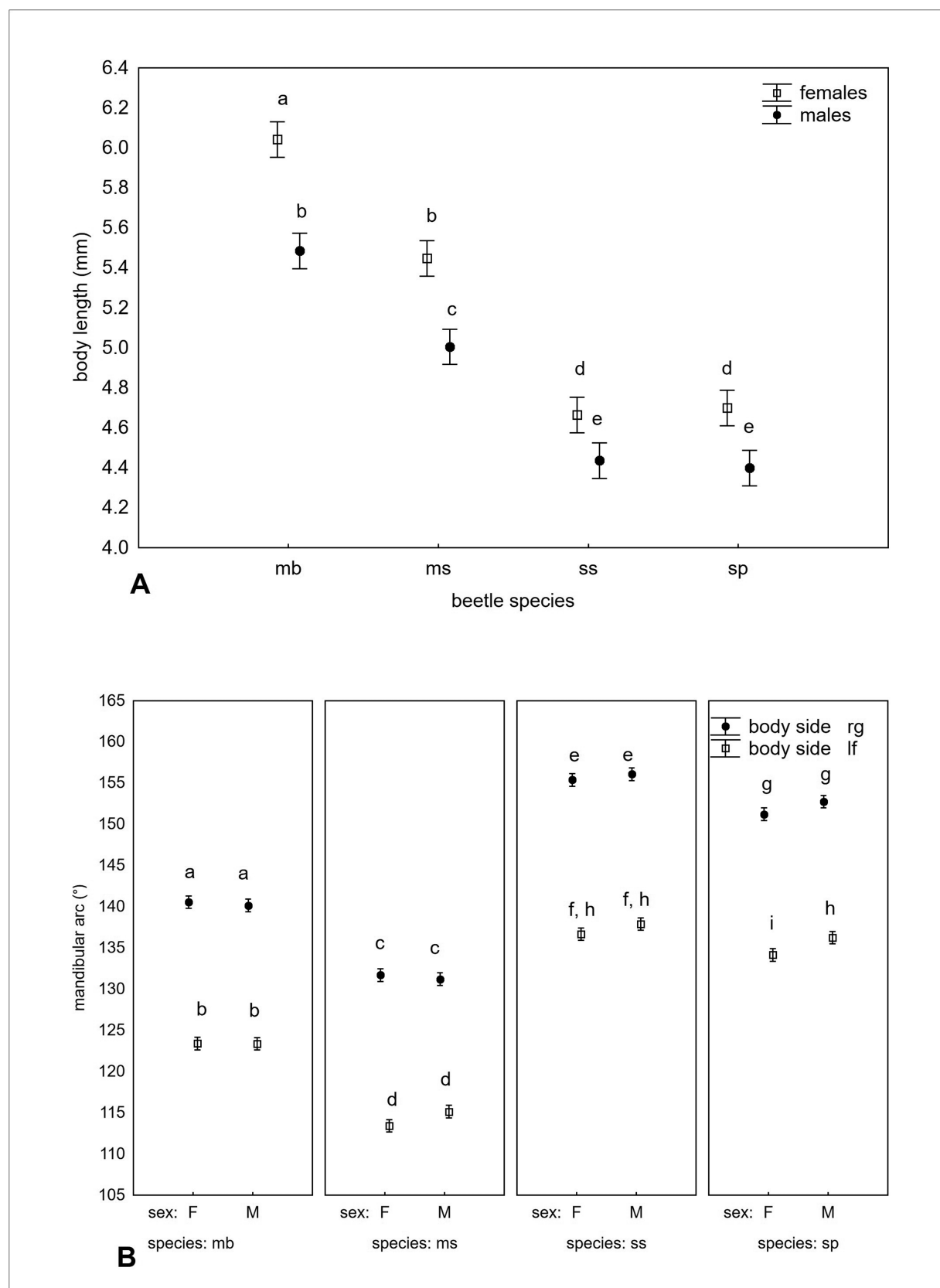
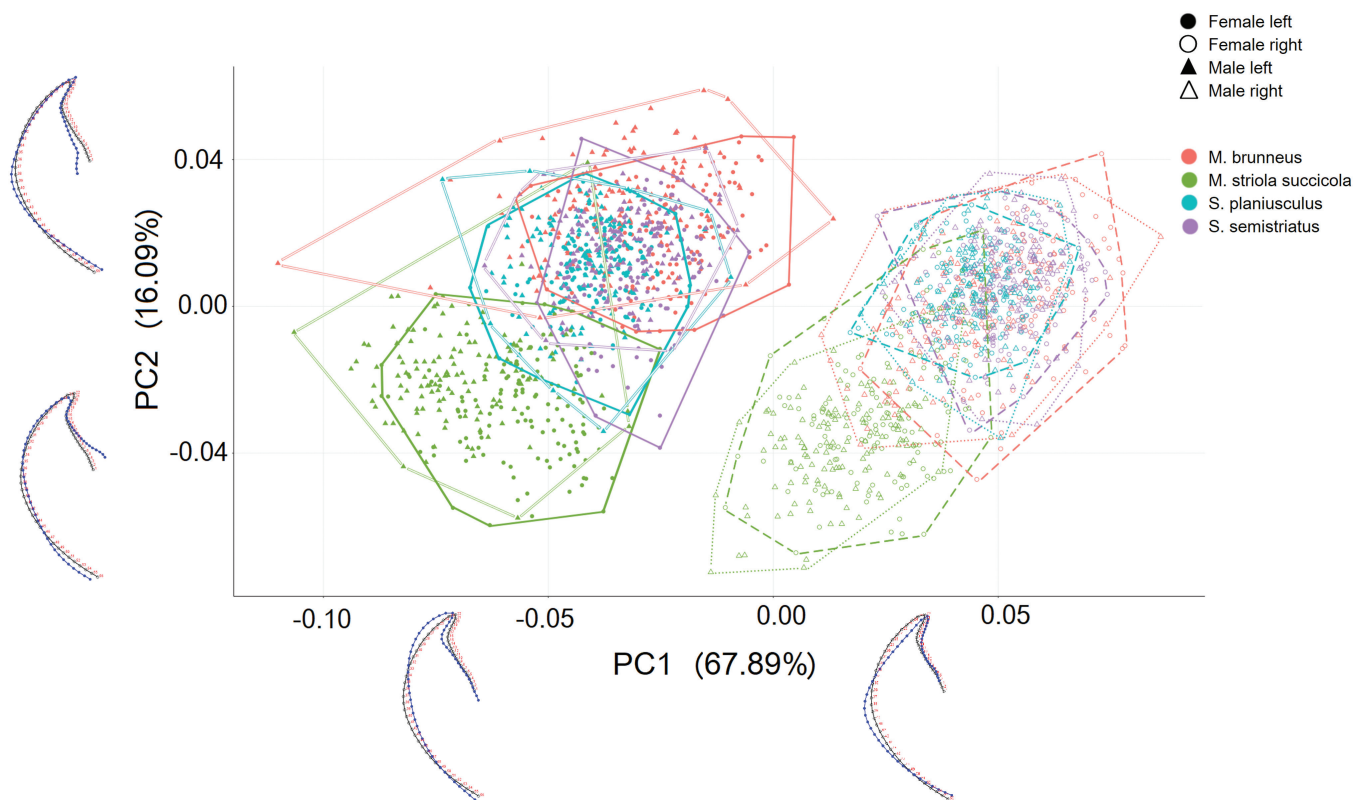


Figure 4. Body length (A) and mandible arc (B) in clown beetles (Histeridae). mb—*Margarinotus brunneus*, ms—*M. striola succicola*, ss—*Saprinus semistriatus*, sp—*S. planiusculus*, F—females, M—males, rg—right, lf—left. Different letters denote significant differences ($P < .05$) in pairwise comparisons.

Table 1. Results of the Procrustes ANOVA performed for the centroid size and shape of left and right mandibles in clown beetles (Histeridae)

Centroid size						
Effect	SS	MS	d.f.	F	P	
Individual	27721300.49	69651.50	398	0.95	.6792	
Side	4127704.09	4127704.09	1	56.56	<.0001	
Ind*Side	29046473.30	72981.08	398	0.82	.9897	
Error 1	84104263.41	89093.49	944			
Shape						
Effect	SS	MS	d.f.	F	Pillai's tr.	P
Individual	0.74	0.0000173820	42 984	1.51	52.66	<.0001
Side	3.02	0.0279636751	108	2435.43	1.00	<.0001
Ind*Side	0.49	0.0000114820	42 984	0.58	33.91	<.0001
Error 1	2.03	0.0000199669	101 952			

SS—sum of squares, MS—mean sum of squares, df—degrees of freedom, F—Goodal's F, Pillai's tr.—Pillai's trace.

**Figure 5.** PCA of shapes of mandibles of clown beetles (Histeridae), including species, sex, and body side. Black lines indicate mean shape, and blue lines indicate shape variation in the dataset along the PC axis, representing the respective PC matching the axes of the PCA.

(M1a, b) muscles (Table 3). In turn, *Saprinus* had right mandibles with a better-developed abductor (Table 3).

Differences in the development of adductor muscles were reflected in the development of beetles' temples, being more prominent in *Margarinotus* species and smaller in *Saprinus* species (Fig. 9). In *S. planiusculus* and *S. semistriatus*, the head capsule narrowed gradually behind the eyes. In contrast, in the species of *Margarinotus*, the head was just as wide along its entire length. Moreover, the head in lateral view was massive and rounded in *Margarinotus* but posteroventrally flattened in *Saprinus*. Results

of the measurements of volumes of head capsules, mandibles, and mandibular muscles are presented in Supporting information, Table S6.

A peculiar difference in the morphology of the inner mandibular base to which the adductor tendons were attached became apparent in 3D renderings of *Saprinus* against *Margarinotus*. A joint-like structure consisting of a convex and a concave was seen in the species of *Saprinus*, which were characterized by weakly sclerotized mandibles. In contrast, in the species of *Margarinotus*, which had more heavily sclerotized mandibles,

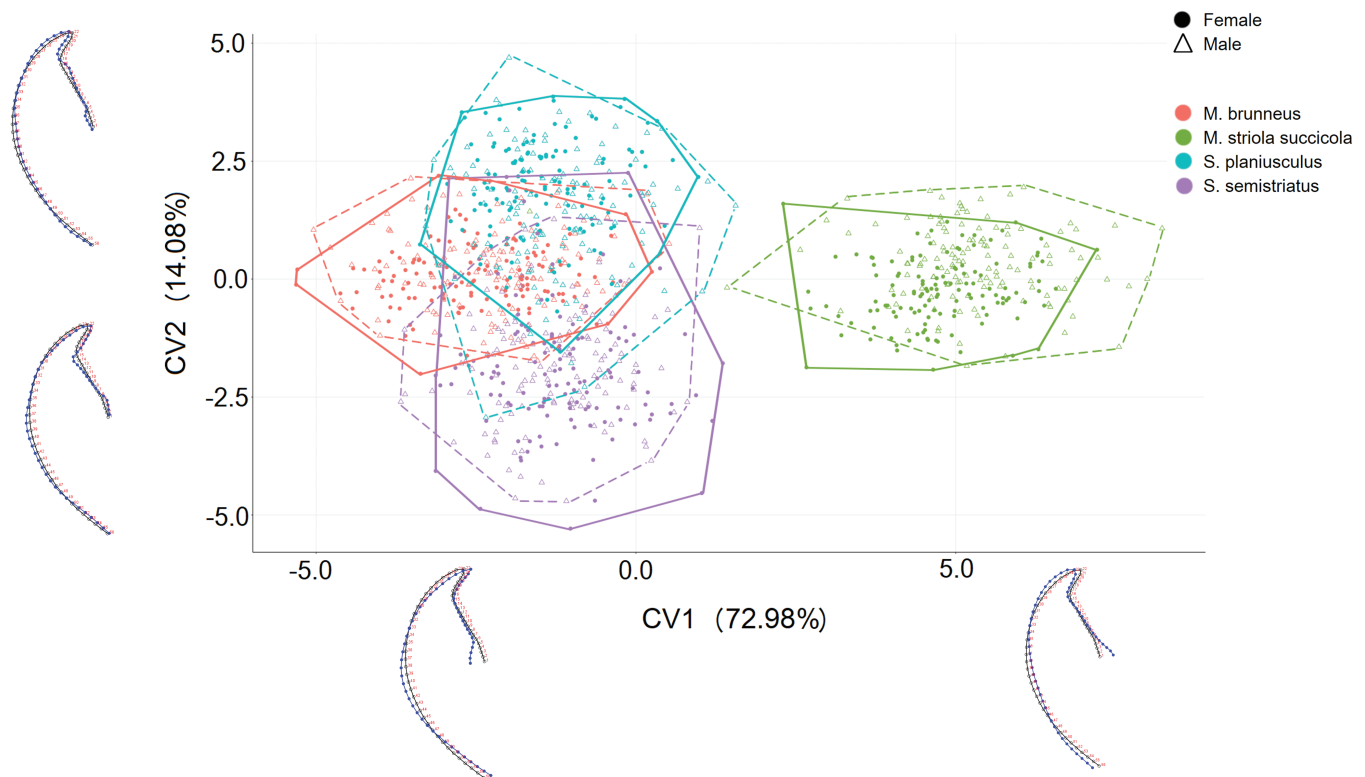


Figure 6. CVA of shapes of the left mandibles of clown beetles (Histeridae). Black lines indicate the mean shape and blue lines indicate the shape, representing a specific CV.

the mandibular base lacked such structures (Fig. 9). Overall, this unusual feature observed in *Saprinus* was suggestive of special properties in the motion of their mandibles.

Mandibular arc, angular range, and tilt of mandibles

Species, sex, and body side significantly influenced mandibular arc as main effects on mean mandible angle (each $P < .001$) (Table 4, see Fig. 3 for measurement of mandible angle). Combined effects for species*sex and species*body side interactions were also significant ($P = .010$ and $P = .015$, respectively), but the effects for sex*body side and the three-way interaction turned out to be insignificant (each $P > .05$). Comparing genera, the larger *Margarinotus* species had lower angles than the smaller *Saprinus* species (Fig. 4B). However, there was no obvious continuous relationship between body size and mandibular arc as the medium-sized *M. striola succicola* exhibited the lowest angles among the four measured species.

All species differed significantly in their mandibular angles with a relatively lower amount of variation among the two *Saprinus* species (Fig. 4B). The largest mandibular angles were found for *S. semistriatus* ($146.52 \pm 9.59^\circ$ mean \pm SD), slightly smaller for *S. planiusculus* ($143.61 \pm 8.93^\circ$), then for *M. brunneus* ($131.87 \pm 9.17^\circ$), and the smallest for *M. striola succicola* ($122.87 \pm 9.18^\circ$) (Fig. 4B; Supporting information, Table S7). Despite sex being statistically significant, overall, there was no strong effect of sexual dimorphism on mandibular angle when comparing males ($136.61 \pm 13.20^\circ$) and females ($135.83 \pm 13.21^\circ$). For the body side, in our dataset, it was always the right mandible that had a wider arc with a higher angle

than the left (Fig. 4B). Results of the body side effect showed that the right mandibles ($144.89 \pm 10.01^\circ$) consistently had this more obtuse angle when compared to the left mandibles ($127.55 \pm 9.91^\circ$).

Further, mandibular asymmetry measured as the difference in angle between left and right mandibles was consistent across all clown beetles examined despite the measured species-specific angles (Figs 4B, 10, 11; Supporting information, Table S8). The relationship between the left and right mandibles was best described as an ANCOVA model where the asymmetry in each species changed as a linear function with a slope of 0.46, which meant that the arc of the right mandible widened more slowly than the left arc. This model fit indicated that if each beetle species had higher variability in mandibular angles, the asymmetry would change within a species; there would be beetles within a species that were hugely asymmetrical, some would be symmetric, and at some point, the asymmetry would reverse. However, in fact, all beetles clustered around a roughly 17° difference between angles (Fig. 10). This overall 17° asymmetry could be described as a linear model with a slope of nearly exactly 1 and an intercept of 17.2 (dashed line, Fig. 11) that still fitted our data well and indicated a conserved asymmetry across the four species of observed histerid beetles.

From the SR- μ CT data, we could further observe that mandibular asymmetry extended into the third dimension. The right mandibles were tilted at a greater angle (min-max: $7.5\text{--}10.9^\circ$) compared to the left mandibles (min-max: $5.9\text{--}9.5^\circ$) (Fig. 12). The angular ranges of both mandibles were larger in species of *Margarinotus* compared to species of *Saprinus* (Fig. 12).

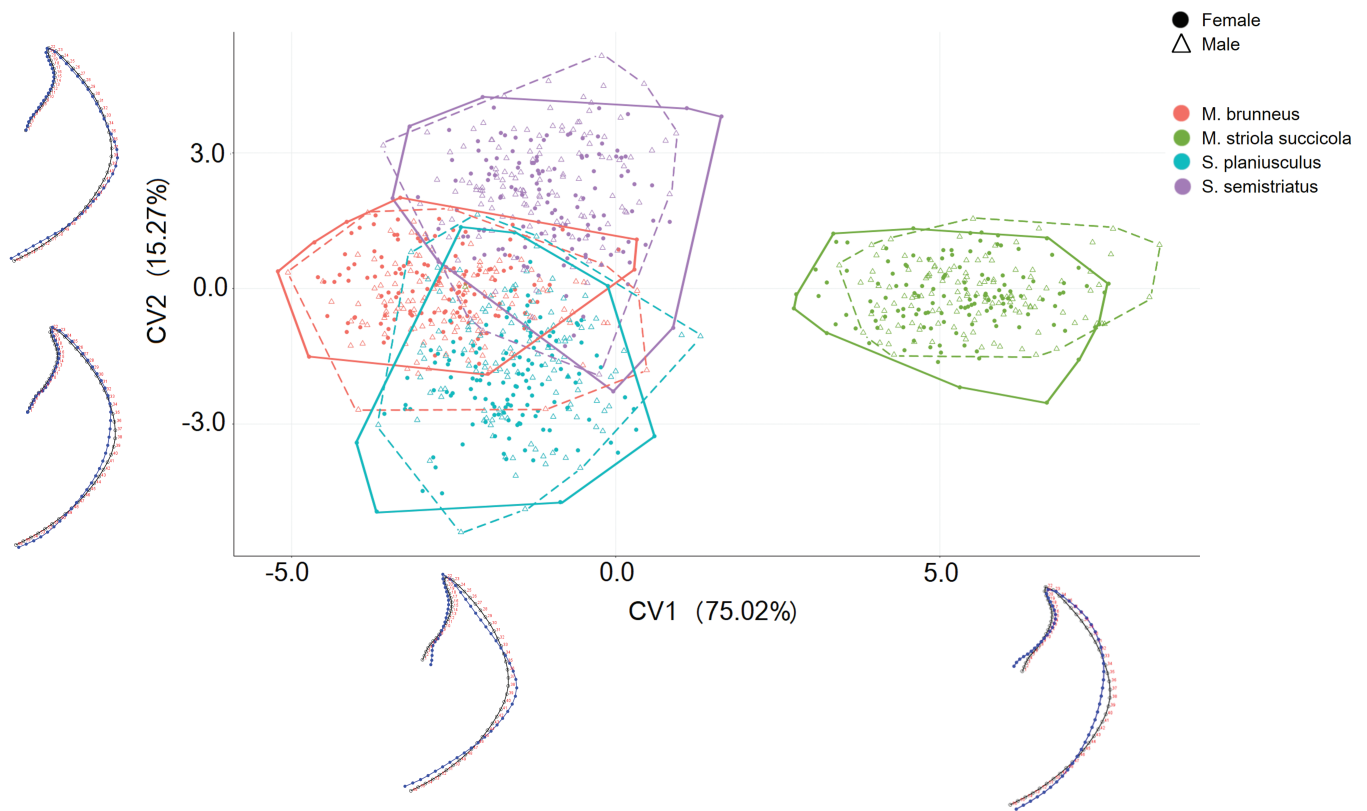


Figure 7. CVA of shapes of the right mandibles of clown beetles (Histeridae). Black lines indicate the mean shape and blue lines indicate the shape, representing a specific CV.

SEM analysis

The inner margins of the left and right mandibles of *Saprinus* were very sharp and thin (Supporting information, Figs S1–S2). In the case of the left mandibles, the area where the dorsal surface turned into a thin razor-like edge was clearly visible (Supporting information, Figs S1A–D, I–L, S2A–D, I–L). In turn, the shape of the right mandibles of *Saprinus* resembled a cleaver (Supporting information, Figs S1E–H, M–O, S2E–H, M–O). In some individuals, the margins of both mandibles showed signs of wear or their parts were more or less broken off (Supporting information, Figs S1–S2).

Some *Margarinotus* mandibles also had sharp inner margins (Supporting information, Figs S3A–D, S4A, D, I), but they were thicker than the inner margins of the mandibles of *Saprinus*. However, most of the examined mandibles of *Margarinotus*, particularly the left ones, had worn inner margins (Supporting information, Figs S3, S4). They were often more or less arched (Supporting information, Figs S3L, S4J–L), particularly in males. Similarly to *Saprinus*, a relatively large variation in the length of the apices of both mandibles of *Margarinotus* could be observed. In some individuals, they were relatively long and sharp (Supporting information, Fig. S3A, G), but more often, they were rounded and worn (Supporting information, Figs S3F, O, S4B, C, L). Some apices were extremely worn in *Margarinotus* species (Supporting information, Figs S3F, O, S4L).

Table 5 summarises the functional morphology results of clown beetle mandibles at the genus level.

DISCUSSION

Inter-specific variation and functional interpretation

Although insect mandibles can be used for various tasks such as nesting, social care, and defending against predators or rivals, their primary function is grasping, cutting, or crushing food. The clown beetles examined are not known to exhibit any specific behaviour using their mandibles, such as male fights over females or building nests. Therefore, most probably, differences in mandible shapes and the development of mandibular muscles between *Margarinotus* and *Saprinus* beetles suggest differences in their diets. While previous studies mainly focused on the functional morphology of mandibles and their relation to feeding habits in predatory beetles from the families Carabidae (e.g. Wizen and Gasith 2011, Sasakawa 2016, Hayashi and Sugiura 2021), Staphylinidae (e.g. Weide *et al.* 2010, Li *et al.* 2011, Stocker *et al.* 2022), and Dytiscidae (e.g. Wall *et al.* 2006, Barman *et al.* 2008, Hayashi and Ohba 2018), this paper presents in-depth results on the functional morphology of mandibles in histerid beetles.

Differences in mandible shape and muscles are most pronounced at the genus level. Mandibles in *Saprinus* beetles are gracile and have long and distinctly pointed apices. The inner margins of the left mandibles are sharp and thin, and the right mandibles look like cleavers. The morphology of mandibles in *Saprinus* beetles indicates their adaptations for cutting and puncturing soft-bodied prey. *Saprinus semistriatus* and *S. planiusculus* seem to be specialized predators of blow fly larvae.

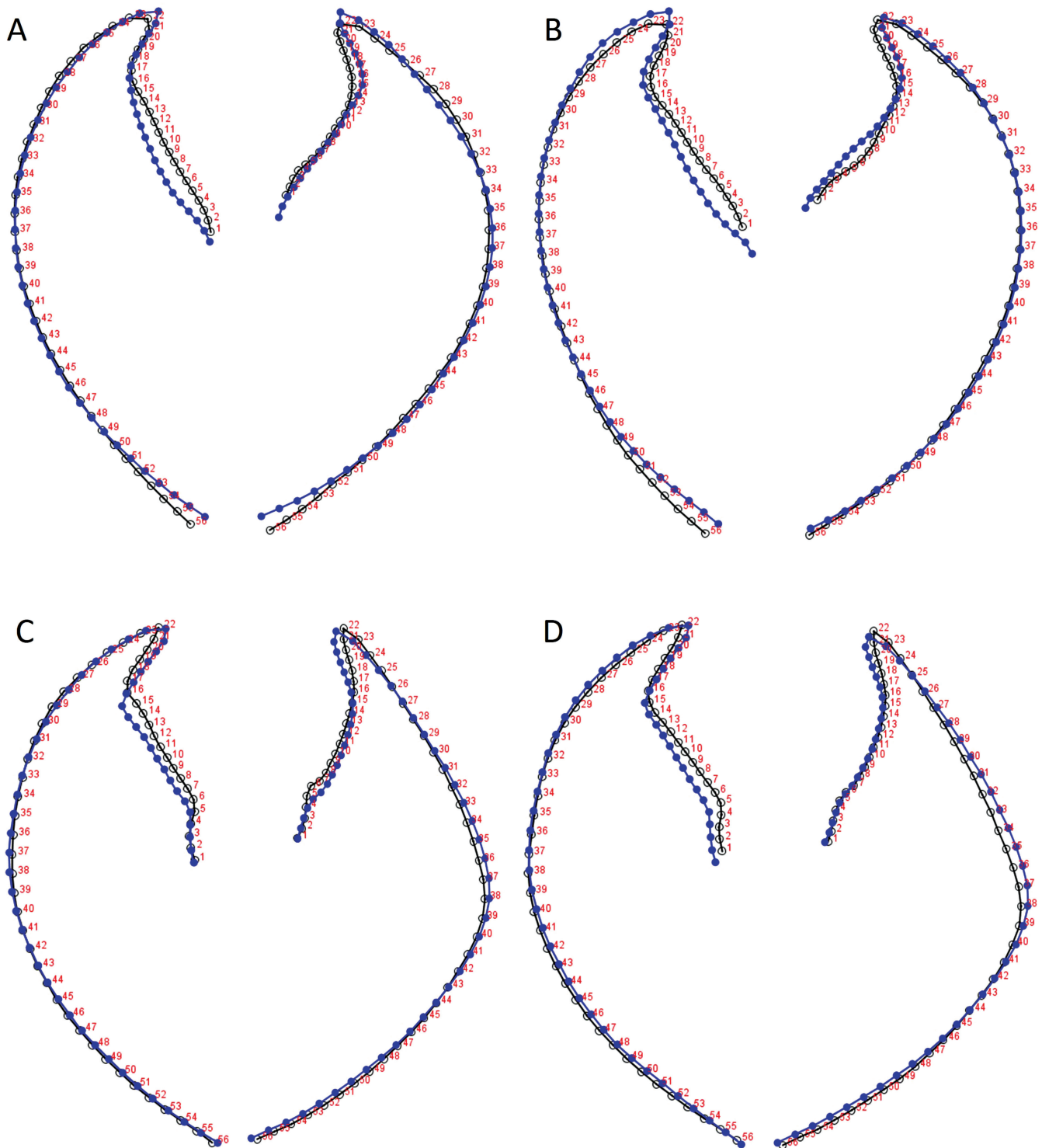


Figure 8. Sexual differences in the shapes of the left and right mandibles of clown beetles (Histeridae) based on semilandmark localization. A, *Margarinotus brunneus*. B, *Margarinotus striola succicola*. C, *Saprinus planiusculus*. D, *Saprinus semistriatus*. Black contours indicate females, and blue contours indicate males.

Previous studies showed that *S. semistriatus* is present on carrion at the same time as blow fly larvae (Calliphoridae) (Bajerlein et al. 2011).

In turn, the mandibles in *Margarinotus* beetles are more robust and convex. SEM analysis has shown that both mandibles

of some individuals have pointed apices, and thin, razor-blade-like inner margins characterize the left mandibles. Nevertheless, most of the studied individuals of *Margarinotus* had distinctly rounded inner margins of the left mandibles, and more or less shortened and rounded apices in both mandibles, which suggests

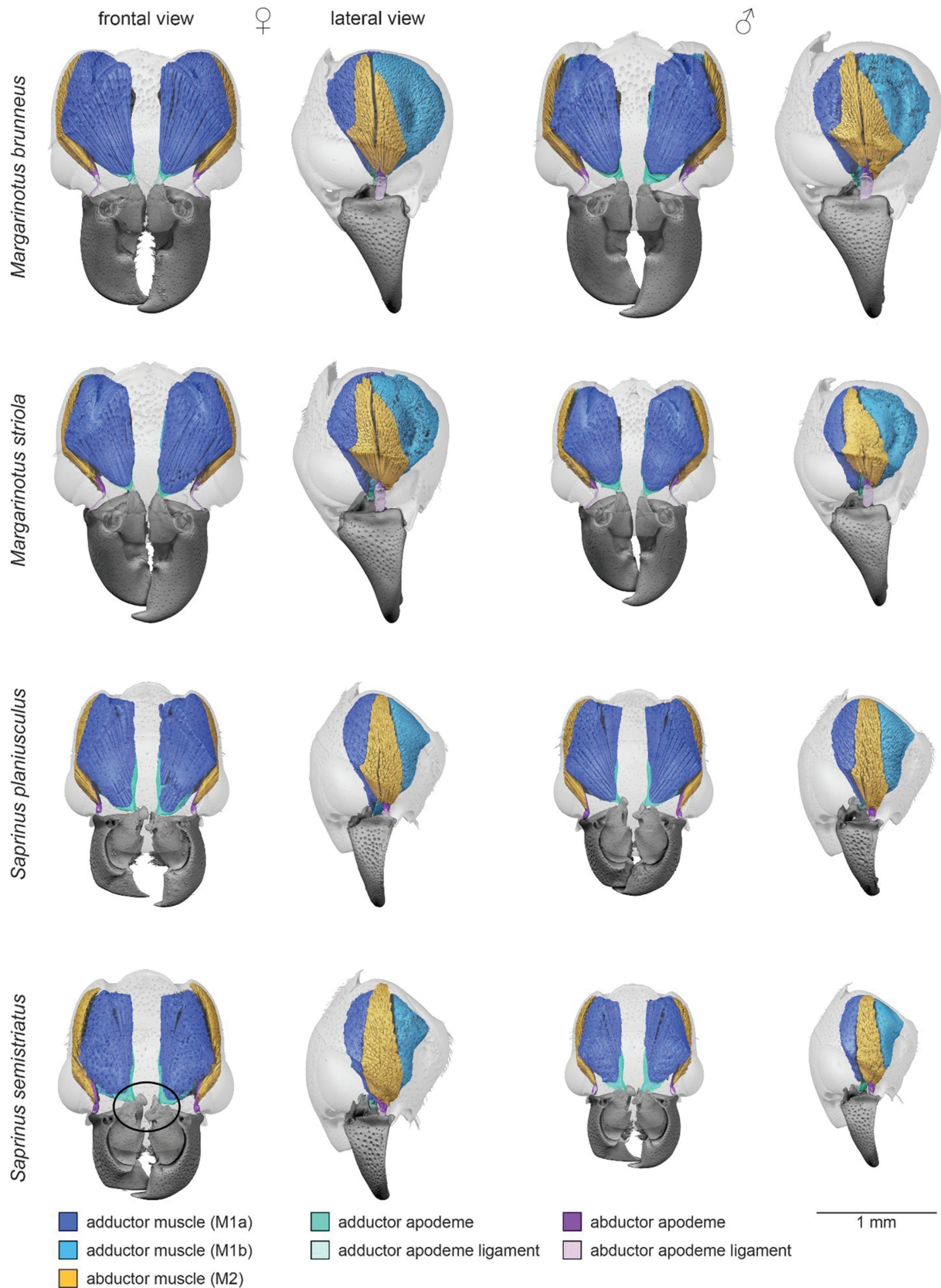


Figure 9. Surface renderings based on segmented SR- μ CT data highlighting mandibles and attached musculature in clown beetles (Histeridae). A black oval indicates the joint-like structure in *Saprinus* beetles.

Table 2. Differences in the development of mandibular muscles in *Margarinotus* and *Saprinus* beetles^a

Genus	Adductor M1a/M1a left ratio	Adductor M1a/M1a right ratio	Adductor M1b/M1b left ratio	Adductor M1b/M1b right ratio	Abductor M2/M2 left ratio	Abductor M2/M2 right ratio	Adductor/abductor M1(a + b)/M2 left ratio	Adductor/abductor M1(a + b)/M2 right ratio
<i>Margarinotus</i> / <i>Saprinus</i>	2.0	1.8	2.3	1.9	1.5	1.4		
<i>Margarinotus</i>							9.1	8.2
<i>Saprinus</i>							6.5	6.2

^aFor each beetle genus, we calculated the mean volume of a given muscle (for raw data, see [Supporting information, Table S6](#)). Next, volume ratios were calculated by dividing the mean volume of a given muscle in *Margarinotus* by the mean volume of the same muscle in *Saprinus*. The last two columns include ratios which show differences in muscle volumes within- and between genera.

Table 3. Differences in the development of mandibular muscles in *Margarinotus* and *Saprinus* beetles expressed as volume ratios of a given muscle to the head capsule^a

Genus	Head/adductor M1a left ratio	Head/adductor M1a right ratio	Head/adductor M1b left ratio	Head/adductor M1b right ratio	Head/ abductor M2 left ratio	Head/abductor M2 right ratio
<i>Margarinotus</i>	20.4	22.3	16.0	18.0	81.0	81.2
<i>Saprinus</i>	24.2	24.5	21.2	21.1	73.6	70.7

^aWe calculated a ratio of a given muscle volume to the head volume for each beetle species. Next, ratios were averaged within a genus (for raw data, see [Supporting information, Table S6](#)).

Table 4. Results for three-factor ART ANOVA in a study of mandibular arc in clown beetles (Histeridae)

Effect	F	d.f.1	d.f.2	P
Species	1919.65	3	944	<.001
Sex	17.04	1	944	<.001
Body side	2834.41	1	944	<.001
Species*Sex	3.82	3	944	.010
Species*Body side	3.49	3	944	.015
Sex*Body side	3.63	1	944	.057
Species*Sex*Body side	1.20	3	944	.308

F—F-value, d.f.—degrees of freedom, P—P-value.

their wear during use. Presumably, mandibles with relatively thin inner margins and pointed apices are present in younger individuals. In contrast, mandibles with distinctly worn margins and apices can be observed in older beetles. The following questions arise: are there any differences in diets between younger and older individuals of *Margarinotus*? Are the mandibles initially used to puncture prey with a harder body surface, and does contact with such a surface cause them to wear out gradually? Could the clear traces of wear also indicate that they are used to crush prey with a hard body surface, e.g. blow fly pupae or adult beetles? *Margarinotus brunneus* and *M. striola* remain present on carrion long after the masses of blow fly larvae have disappeared. Therefore, they seem to have a wider range of prey than *Saprinus*.

Other results from our studies further indicate differences in diet between *Saprinus* and *Margarinotus* beetles. First, mandibles in *Saprinus* and *Margarinotus* are equipped with differentially developed musculatures. *Margarinotus brunneus* and *M. striola* have better-developed adductor muscles, i.e. the closer muscles, compared to adductor muscles in both species of *Saprinus*, which

suggests that the work of their mandibles requires a high level of muscular power, for instance, during crushing. In turn, species of *Saprinus* have better-developed abductor muscles, i.e. the opener muscles, which suggests that they may need force to retract the mandibles from their prey. Moreover, abductor apodemes, which convey forces to the mandibular base, are also differentially developed in *Margarinotus* and *Saprinus*. Second, *Saprinus* beetles have larger mandibular arcs, but mandibles in *Margarinotus* have larger angular ranges. These findings may suggest differences in the size of their prey. Third, the presence of an additional joint, which apparently articulates the mandibles in *Saprinus* with each other, points to another level of functional specialization.

The best-known species of clown beetle in terms of diet is *Carcinops pumilio* (Erichson, 1834). It can feed on larvae of all stages of *Drosophila melanogaster* (Meigen, 1830) but only on eggs and larvae of the first stage of a much bigger fly species—*Musca domestica* Linnaeus, 1758 ([Achiano and Gilomee 2007](#)). This result may suggest that *S. semistriatus* and *S. planiusculus*, the smallest species we studied, feed on smaller prey than *M. brunneus* or *M. striola*. Both species of *Margarinotus* may have a wider prey range—larger predators eat prey with a larger range of body sizes than smaller predators ([Cohen et al. 1993](#)). Differences in the body size of predators may reduce their competition for food and enable their co-occurrence.

We hypothesized no differences in shape within the genera, i.e. between the mandibles of *S. semistriatus* and *S. planiusculus* and between *M. brunneus* and *M. striola*. Therefore, a similar diet between species within a genus should be expected, and competitive exclusion should be expected between species in a genus. The results of previous studies ([Bajerlein et al. 2011](#)) indicate this by showing the co-existence of *S. semistriatus* and *M. striola* on domestic pig carcasses decomposing in a forest habitat. *Saprinus planiusculus* and *M. brunneus* reached much lower abundances here. However, geometric morphometrics revealed

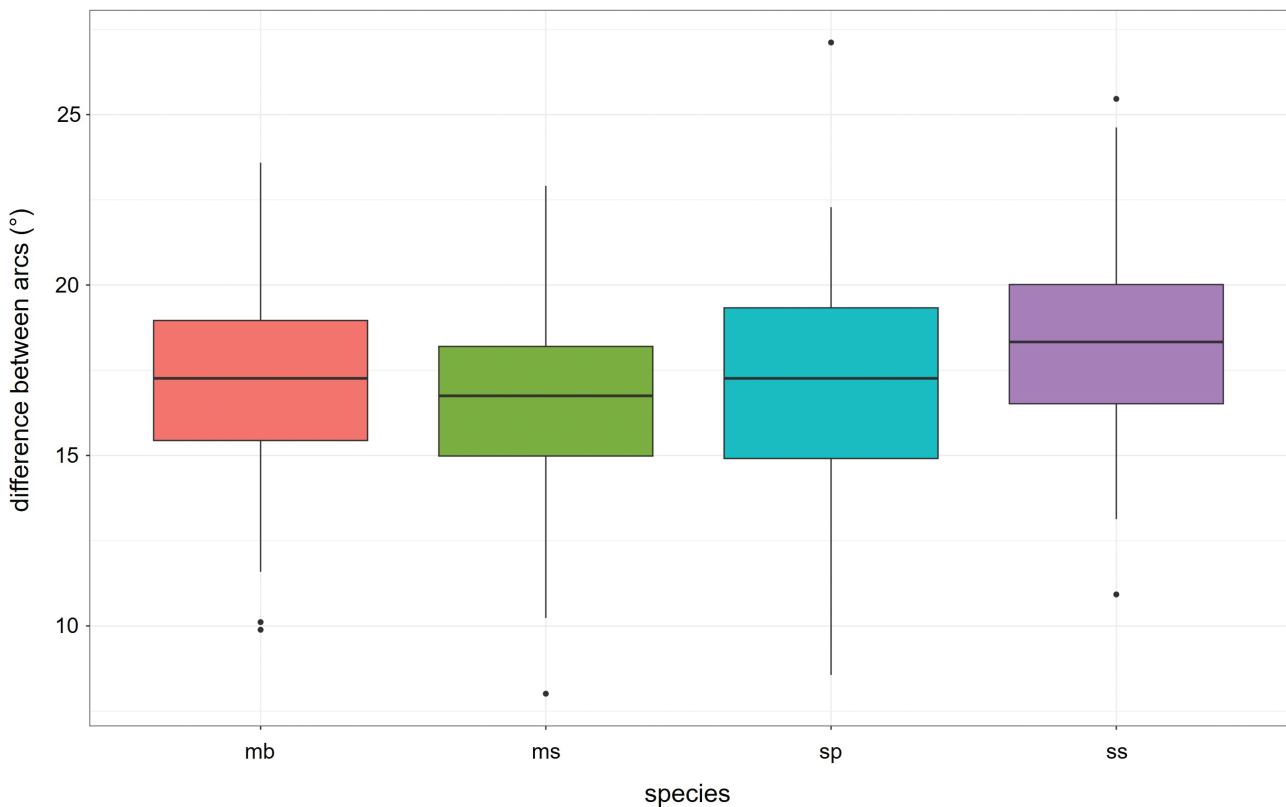


Figure 10. Differences in arc (asymmetry) between right and left mandibles of clown beetles (Histeridae). The horizontal line in the box plot indicates the median; the box indicates the first and the third quartiles; the vertical lines represent the minimum and maximum values; and the black dots indicate the outliers. Abbreviations are the same as in Figure 4.

differences in mandibular shape between all these species, and *M. striola* is especially distinct in the geometric morphometric analyses. Nevertheless, these differences are mostly subtle, and overall shape patterns can be well summarized within the genera. The fact that different species within a genus do not co-exist may suggest that the variability in the shape of their mandibles does not indicate different diets.

SR- μ CT has revealed the presence of a joint-like structure at the basal margin of the mandibles in *Saprinus* species. Their mandibles are less heavily sclerotized compared to mandibles in *Margarinotus*. Presumably, this joint can prevent breakage of the mandible by transmitting load from one mandible to the other (Supporting information, Movie S1). Beetles of the genus *Margarinotus* lack such joints. However, the inner edges of the mandibles may fulfil their function at the base, which is flat, but they will presumably press against each other when the mandibles open. A putative additional joint within at least some Histeridae represents an evolutionary novelty, and this morphological feature should be examined further in comparative, behavioural, and biomechanical frameworks.

Asymmetry

Our study has shown conspicuous directional asymmetry in the mandibles of all beetle species we examined. Above all, left and right mandibles differ in shapes and arcs. The asymmetry of the mandibles in beetles may be involved in feeding (e.g. Hayashi and Sugiura 2021), intersexual combats (e.g. Okada et al. 2008)

or egg laying (Toki et al. 2016). The function of mandibular asymmetry in the studied clown beetles is unknown, but it is probably related to diet or intersexual interactions. Tests that enable the observation of attack, feeding, and potential behavioural conflicts between competing individuals will be necessary to explain the function of asymmetry in this beetle group.

Although the examined histerid species differed in the body size, mandible shape, and mandibular arc, all retained a roughly 17° asymmetry, with the right mandible always having the higher arc. This finding may suggest that the function of both mandibles interacting is based on this conservative asymmetry, which remains stable even in species with various diets. It is difficult to explain why this difference is approximately 17° in all the species we studied. It can be supposed that it is biomechanically determined, i.e. it is influenced by the mechanical forces the mandibles experience and ensures their optimal feeding functions. Further studies are needed to recognise whether mandibles of other species of Histeridae retain the asymmetry around 17° and whether this asymmetry occurs in other predatory beetles with asymmetric mandibles.

Our study showed that the difference between the left and right mandibles of Histeridae is the most striking shape feature, and yet, this difference appears to be predictable. In this respect, our results align with recent findings on the head morphology of the grasshopper *Schistocerca gregaria* (Forskål, 1775) presented by Ginot et al. (2024). The authors showed strong integration between this orthopteran's left and right mandibles, although

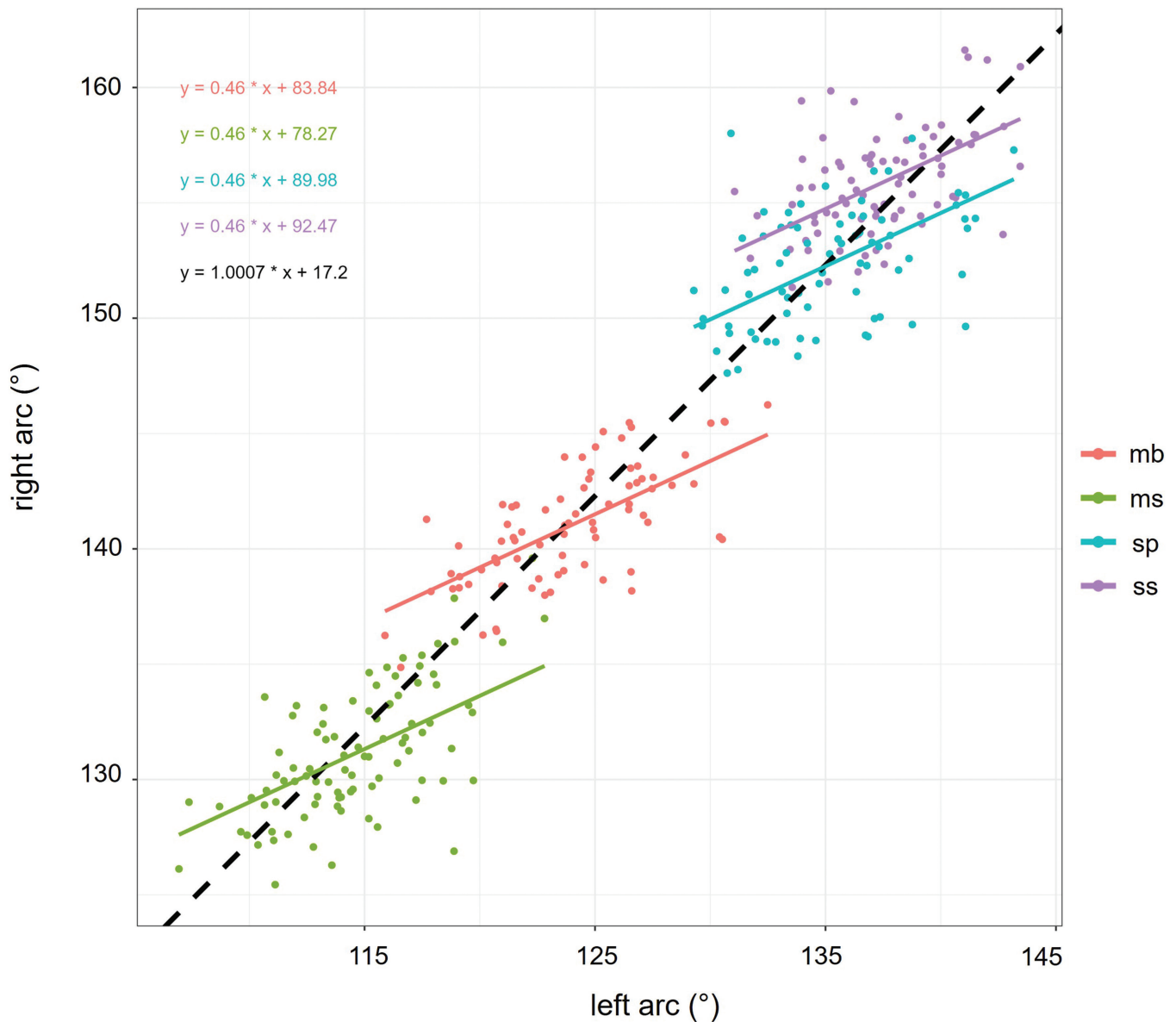


Figure 11. GLS_ANCOVA model and linear regression illustrating relationships between left and right mandibular arc within particular species of clown beetles and across all species (the black line). Abbreviations are the same as in Figure 4.

they display asymmetric phenotypes. [Ginot et al. \(2024\)](#) hypothesized that ‘each mandible’s optimal shape depends on the shape of the opposite mandible. Accordingly, achieving optimal feeding function relies on an optimal degree of asymmetry between both mandibles.’ The fit of the mandibles is also visible in the development of the joint-like structure at the basal part of the mandibles of the *Saprinus* species. If our hypothesis about the function of this structure is correct, the asymmetry of the mandibles will determine not only the achievement of optimal feeding function but also their movement. Further research may prove that the function of histerid mandibles entails precise integration with several potential morphological adaptations.

Moreover, findings by [Ginot et al. \(2024\)](#) support the idea that the insect head system is also a modular system. Their studies revealed significant but subtle directional asymmetry in the grasshopper head ([Ginot et al. 2024](#)). The heads of the clown

beetles we examined do not show apparent signs of asymmetry. However, the head asymmetry in clown beetles may occur similarly to the head asymmetry in the grasshopper, which is very subtle and detectable only using quantitative methods. Our research suggests head asymmetry at the anatomical level in terms of the differences in the development of musculature of the left and right mandibles. However, clear conclusions in this regard require further analysis using more individuals.

Sexual dimorphism in mandible shape

Our results revealed the existence of previously unknown morphological variation in clown beetles reflected in sexual dimorphism in the shape of their mandibles. These differences in all studied species were primarily related to apices and inner margins, hence parts involved in cutting or crushing. Moreover, differences in the curvatures of outer margins were observed, but

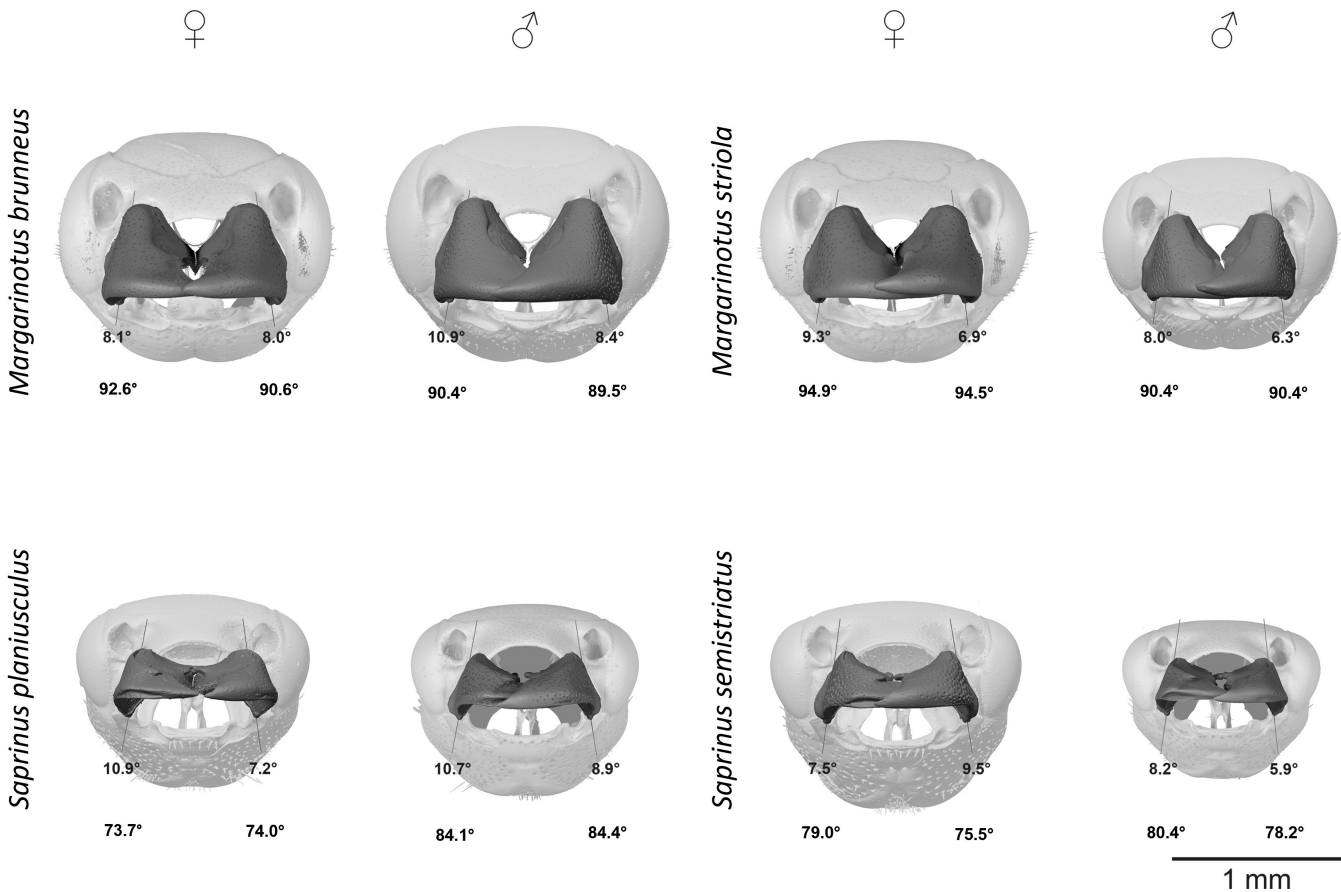


Figure 12. Mandibles’ tilt (values in upper rows) and angular range (values in lower rows) in clown beetles (Histeridae).

Table 5. Summary of results of functional morphology of mandibles in histerid beetles at the genus level

Genus	Body size	Head		Mandibles			Mandibular muscles				
	Size	Size	Temples	Size	Asymmetry	Apex	Arc left, right	Angular range	Adductor M1a left, right	Adductor M1b left, right	Abductor M2 left, right
<i>Margarinotus</i>	Larger	Larger	Large, prominent	Larger	Present	Shorter, rounded	Smaller	Larger	Well-developed	Well-developed	Weaker developed
<i>Saprinus</i>	Smaller	Smaller	Small, less prominent	Smaller	Present	Longer, pointed	Larger	Smaller	Weaker developed	Weaker developed	Well-developed

these were more distinct in species of *Margarinotus* compared to species of *Saprinus*. The question arises about the reason for the observed differences. Are they observed in freshly emerged adults and related to differences in diet between females and males, or have they developed due to their different diets or behaviours?

Unfortunately, sex-specific colonization of carrion has not been evaluated yet in these species. Therefore, it is difficult to conclude whether females and males differ in their presence period, which could suggest differences in their diet. Previous studies have shown that the abundance and appearance of rove

beetles (Staphylinidae) on carrion were not sex-biased (Peschke 1987, Mądra-Bielewicz et al. 2017). The co-occurrence of both sexes on carrion increases the probability of finding mates. On the other hand, previous studies on feeding habits in insects have revealed differences in diets between sexes or even between individuals of the same sex. For instance, Tsukamoto et al. (2014) have shown that mated female crickets preferred food with higher protein content than virgin females. In most insects, females are larger than males because the energetic investment in the progeny is more significant in females than in males. Carrion is a temporary resource which is unpredictable and inconsistent

in its availability and locality (Braack 1987). Due to high competition for resources, it must be located quickly; therefore, clown beetle females may require a more protein-rich diet.

The fact that males' left mandibles are indented may suggest that they come into contact with prey with a harder body than the females, and the contact causes the mandibles to wear down.

Can geometric morphometrics tell more?

Geometric morphometrics is a useful tool for quantifying differences in the shape of morphological characters between sexes in insects. However, most previous studies have focused on beetles with a distinct sexual dimorphism reflected in exaggerated morphological traits such as enlarged mandibles in stag beetles (Lucanidae) (e.g. Romiti et al. 2017, Cáceres et al. 2023) or elongated horns in dung beetles (Scarabaeidae) (e.g. Crabtree et al. 2020, Rohner et al. 2024). The fact that our analysis revealed sexual dimorphism in mandible shape in species of clown beetles was somewhat surprising since these differences were not visible at first sight. The comparison of the effectiveness of traditional and geometric morphometrics is still being investigated (e.g. Romiti et al. 2017, Parés-Casanova et al. 2020, Powell et al. 2020). However, we did not use both approaches, which would allow us to conclude their effectiveness in the study of the morphology of mandibles in histerid beetles. Whether the observed differences in mandibular shape between sexes could be revealed using traditional morphometrics should be studied. Nevertheless, many previous studies showed that geometric morphometrics possesses advantages over the more traditional approach based on linear measurements (e.g. Fruciano et al. 2011, Fabre et al. 2014, De Souza et al. 2015, Schmieder et al. 2015, Romiti et al. 2017, Parés-Casanova et al. 2020). Most importantly, this method was shown to be more sensitive to the variation among samples and more effective in describing a structure as a whole, particularly when the changes are subtle. The mandibles of the species we examined were almost toothless; therefore, the number of available homologous points and possible landmarks was low. Since the curvature of the mandibles is difficult to determine using linear measurements, we placed a high number of semilandmarks along their inner and outer margins for a better characterization of their shapes. Our analysis has shown sex-biased differences in mandible shape within areas not usually included in linear measurements. Therefore, geometric morphometrics seems to be more effective in studies of the shape of mandibles with a low number of homologous points.

Final remarks

Using a multimodal approach to study the functional morphology of mandibles provided numerous new and exciting findings. So far, necrophilous clown beetles have been regarded as predators of immature stages of Diptera. The results of our studies suggest differences in diet, at least at the genus level. We also showed variation in mandibular shape at the species and sex levels. The fact that we have found sexual dimorphism in the mandible shape of clown beetles was surprising because the observed differences are not apparent at first glance, and no differences in diet between males and females have been reported yet. They were revealed using geometric morphometrics, which indicates the sensitivity and effectiveness of this method

in studies of shape variability, even if this variability is hidden from view. Understanding the functional morphology of insect mouthparts may allow for better planning of experimental research. The results of our study indicate that future experiments on the diet of necrophilous *Saprinus* and *Margarinotus* beetles should include observations of both females and males of different ages. Their diet should consist of all immature life stages of various European blow fly species, particularly from the genera *Calliphora* Robineau-Desvoidy, 1830 and *Lucilia* Robineau-Desvoidy, 1830, which can vary significantly in size. Overall, morphological variation in histerid beetle mouthparts and ecologies will be even higher because both adults and their larvae are predatory and co-exist in carrion ecosystems. Future studies in histerid biology should verify our diet assumptions and further focus on larval feeding habits to understand intra-specific dietary variations and niche partitioning.

SUPPORTING INFORMATION

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

Figure S1. Inner margins and apices of left and right mandibles in *Saprinus semistriatus*.

Figure S2. Inner margins and apices of left and right mandibles in *Saprinus planiusculus*.

Figure S3. Inner margins and apices of left and right mandibles in *Margarinotus striola succicola*.

Figure S4. Inner margins and apices of left and right mandibles in *Margarinotus brunneus*.

Movie S1.

Table S1. Results for two-way ANOVA in a study of body size in clown beetles (Histeridae).

Table S2. Body length (mm) of females and males of clown beetles (Histeridae).

Table S3. Results of the Procrustes ANOVA for two error levels in a study of the mandibles in clown beetles (Histeridae).

Table S4. Intra-specific allometry: multivariate regression of shape on centroid size.

Table S5. Results of the permutation test for pairwise distances in CVA for all species, sexes, and body sides.

Table S6. Volumes (μm^3) of head capsules, mandibles, and mandibular muscles of clown beetles (Histeridae).

Table S7. Mandible arc ($^\circ$) according to clown beetle species, sex, and body side.

Table S8. Mean arc ($^\circ$) difference between right and left mandibles according to clown beetle species and sex.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in relation to this work.

DATA AVAILABILITY

The SR- μ CT volumes analysed in this study are accessible through the RADAR4KIT repository at <https://dx.doi.org/10.35097/1hpbj8rtp0cyfs6x>. The other data supporting this study's findings are available in the Supporting information. The corresponding author will provide geometric morphometric semilandmark data upon reasonable request.

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