

Sexual dimorphism in the proventriculus of the buff-tailed bumblebee *Bombus terrestris* (L. 1758) (Hymenoptera: Apidae)

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Funding information

Bayerisches Staatsministerium für Umwelt und Verbraucherschutz; Deutsche Forschungsgemeinschaft; Bavarian State Ministry of the Environment and Consumer Protection

Abstract

Research on eusocial bee species like *Bombus terrestris* is primarily focused on the worker caste, which is why their morphology and anatomy are already well described. This includes the alimentary tract, which is adapted for feeding on nectar and pollen. Located at the transition between crop and ventriculus is a highly specialised compartment, the proventriculus. In female workers of *B. terrestris*, the proventriculus is surrounded by muscles and consists of four anterior lips. A detailed description, however, is only provided for *B. terrestris* worker bees while studies on the proventriculus of the male reproductive caste are absent. Here, we provide a detailed analysis of the differences between the proventriculus of the *B. terrestris* males and females through morphometrics, histology and scanning electron microscopy imaging, and unravel a distinct sexual dimorphism. The male proventriculus is wider resulting in a greater volume than the female proventriculus. Histological analysis revealed 4 distinctive chambers of the male proventriculus, which are completely covered with hairs on the inside. In contrast, those chambers in the proventriculus of female *B. terrestris*, are only rudimentarily present forming only small pouches with hairs in the junctions between the proventricular folds inside the proventriculus. The morphological differences in the proventriculus may be based on different *modi vivendi*, as males do not return to the colony and fly longer distances. This and the synthesis of sperm and mating plug might require higher energy reserves, leading to the necessity of higher food storage capacities.

KEYWORDS

alimentary tract, Bombini, digestive system, invertebrate anatomy, scanning electron microscopy

1 | INTRODUCTION

Bumblebees are important pollinators of flowering plants, and as such, play a crucial role in maintaining biodiversity and ecosystem functioning (Goulson, 2010). They serve as model organisms for

social wild bees, for they are the most abundant group and easy to cultivate, which is why their anatomy, ecology and functional morphology have been studied in detail (Cameron & Sadd, 2020; Goulson, 2003; OECD, 2017). Further, bumblebees exhibit complex social structures, marked by cooperative tasks, overlapping

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generations and division of labour between sexes. This renders them a suitable model to study sexual dimorphisms (Dornhaus & Chittka, 2004; Wolf & Chittka, 2016) and how social organisation influences the evolution of morphological variations (Boomsma et al., 2005). For instance, foraging and feeding aspects distinguish these dimorphic roles. Female bumblebees, assume responsibilities primarily associated with maintaining of the nest and resource collection for the colony, which expresses itself in specialised anatomies adapted for efficient nectar and pollen gathering (Dornhaus & Chittka, 2004; Wolf et al., 2012). Conversely, male bumblebees are typically bigger and focus their efforts towards activities such as mating and dispersal (Duchateau & Marin, 1995; Dornhaus & Chittka, 2004). During the activities mentioned above bumblebees must often cover long distances, which requires a high level of energy (Brown & Brown, 2020). Therefore, they require a nutrient-rich diet, obtained through their specialised digestive system (Pyke, 1980; Wolf et al., 1999). By investigating how their digestive system processes different floral resources, such as nectar and pollen, it is possible to uncover crucial insights into the nutritional requirements of bumblebees (Dukas & Waser, 1994).

Like in other insects, the digestive system of bumblebees is composed of mouthparts, a foregut, a midgut, and a hindgut. The structural organisation of the organs belonging to the digestive system within a bumblebee species is often similar. Nonetheless, there are always specific variations that reveal small differences between, for example, worker (female) and male bumblebees. For instance, the specialised mouthparts of bumblebees are sexually dimorphic. In general, male mouthparts are smaller than the mouthparts of females and males have two prongs on their mandibles while females have five. Worker mouthparts are adapted to feed on nectar and pollen and are extensively used for constructing the hive (Inouye, 1980; Krenn et al., 2005), while males do not contribute to nectar and pollen collection or construction of the hive, which might be a reason for differences in mouthparts (Willmer, 2011). These intraspecific differences in functional traits between sexes thereby often reflect on their ecological roles and are called ecological dimorphisms (Fryxell et al., 2019). Based on the dimorphic mouthparts, it is conceivable that the digestive system shows differences in morphology or anatomy as well. Since most of the studies dealing with bumblebee morphology and anatomy are focused on the worker caste, a detailed comparison of the digestive system between the sexes has not been performed yet.

After ingestion, food enters the mouth and passes through the oesophagus to the crop (Carreck et al., 2013). The crop is a specialised pouch-like structure in bees, where nectar is temporarily stored (Dettner & Peters, 2003; Snodgrass, 1910). Located at the transition between crop and ventriculus is a highly specialised compartment of the digestive system in bumblebees and other insects, the proventriculus. However, there are differences especially in the proventricular folds. While female Bombini (bumblebees) and Euglossini (orchid bees) express proventricular folds in a columnar structure (Serrão, 2001), Meliponini [stingless bees] show a wider apical portion and a slimmer long basal part. The folds from Halictidae

[sweat bees] resemble the columnar from in Bombini, but are notably shorter (Serrão, 2001). Comparable proventricular bulbs are also found in other Hymenoptera like ants and wasps (Bution et al., 2010), but this is only true for female workers since descriptions for male individuals are elusive.

In bumblebee workers, it has a valve-like structure that plays a vital role in regulating the flow of food and controlling the rate of digestion (Dettner & Peters, 2003). In its basic structure, the proventriculus of corbiculate apidae species is similar. It consists of an anterior part called the proventricular bulb reaching partly into the crop and forming an x-shaped opening with its four triangular lips containing filiform hairs on the inside (da Cruz-Landim & Rodrigues, 1967; Snodgrass, 1910). In the middle it consists of one lumen and the posterior part ends with a cardiac valve, reaching into the midgut lumen (da Cruz-Landim & Rodrigues, 1967). It consists of four anterior lips. The proventriculus is surrounded by circular and longitudinal muscles which open and close the lips and expose the hairs, respectively (Peng & Marston, 1986). The primary function of the proventriculus in bumblebees, at least in workers, is to selectively separate pollen grains from the nectar ingested through the proboscis (Peng & Marston, 1986). This way, mainly uncontaminated nectar is retained in the crop and the pollen passes through the proventriculus, where it is packed in specialised chambers before being transported down to the ventriculus (Bailey, 1952).

While the structure and how the food is processed by the crop and proventriculus in worker bumblebees is described, such knowledge is missing for the male bumblebees. Closing this knowledge gap is important, since the relevance of males with regard to colony functioning and reproductive success is becoming more and more evident, as recent studies showed (reviewed in Belsky et al., 2020). A selective food intake might also demand a specified digestive system; therefore it is possible that there are differences between male and female bumblebees not only in the feeding apparatus, but also in morphology of the digestive system. Hence, we aimed to provide a more detailed description of the proventriculus of male bumblebees together with a comparison to the proventriculus of workers to unravel a previously unknown sexual dimorphism in the digestive system of male and female *B. terrestris*. We measured the circumference and the length of male and female proventriculi and compared them through histological analysis and scanning electron microscopy (SEM) to establish morphological differences between the sexes.

2 | MATERIALS AND METHODS

2.1 | Animal husbandry and preparation of proventriculi

Animal husbandry was performed as previously described in Hüftlein et al. (2023). In brief, we maintained five queenright colonies of the buff-tailed bumblebee *Bombus terrestris* (Linnaeus, 1758) (Biobest Group NV, Westerlo, Belgium) in a climate chamber under constant

conditions at 26°C, 70% humidity, and a 12 h/12 h light/dark cycle. Each colony was provided ad libitum sugar water (1:1 ratio of H₂O and inverted sugar solution from Apilinvert; Südzucker AG) and pollen (Imkerpur, Osnabrück, Germany). We replaced the sugar water every 3 days to prevent mould formation.

For the preparation of the proventriculi, 26 female and 26 male bumblebees were randomly selected from the five colonies and euthanized in a jar containing a paper tissue soaked with ethyl acetate (Carl Roth GmbH + Co. KG,) for 20 min. After 20 min, 15 of the 26 bumblebees were dissected for morphological measurements. We removed all the tergites using tweezers and microscissors, then severed the head to pull out the oesophagus. The crop with proventriculus will emerge by gently pulling the abdomen and thorax apart. We then removed the surrounding crop tissue from the proventriculi and stored them in 70% ethanol for the subsequent measurements. The 6 out of the 26 males and females were randomly selected for SEM preparation (see Section 2.3). The remaining five bumblebees were dissected as described above, with the difference that we took out the proventriculi, the crop and a short section of the midgut (ventriculus) in one intact piece for the histological analysis.

2.2 | Morphometrics of the proventriculi

All proventriculi were photographed using a dissecting microscope (Leica M50; Wetzlar) equipped with a digital camera (OLYMPUS-DP26, Hamburg, Germany, light: Leica KL 300 LED) using 25× magnification and measured with the Image Analysis Software CellSens Dimension (v1.11, OLYMPUS; supplementary online material, Figure S1). The width of the proventriculi was measured from the widest point of the left margin to the widest point of the right margin and the height from the bottom to the top margin to calculate the circumference. We measured the length of the proventriculi from the posterior to the anterior margin. Finally, we calculated the valves circumference's proportion to the proventriculus's total circumference (supplementary online material, Figure S1).

2.3 | Sample preparation for scanning electron microscopy

For the analysis of morphological sexual dimorphism between the proventriculi via SEM, six males and females were randomly selected from the 26 males and females isolated as described above, dissected, and their proventriculus removed. After the removal, the proventriculi were prepared for SEM analysis. In brief, samples were fixed in 2.5% glutardialdehyde prepared by diluting 25% glutardialdehyde (Carl Roth GmbH + Co. KG) in 0.1 mol L⁻¹ phosphate buffer overnight. The next day, samples were washed in 0.1 mol L⁻¹ phosphate buffer supplemented with 1.8% sucrose (Carl Roth GmbH + Co. KG) with pH=7.2 (3× for 10 min each). Then samples were dehydrated via an ethanol dilution series of increasing concentrations (60%, 70%, 80%, 90%, 96%, and 100%), each step was performed

twice for 10 min. Then samples were transferred in 100% EtOH to a critical point dryer (Leica EM CPD300; Leica Microsystems GmbH) and dried for 88 min at 26°C and 1 bar. After the drying, two proventriculi were placed with their anterior end upwards and two with their posterior end upwards on a stub (0.5 SEM Pin Stub, 6 mm length, Agar Scientific Ltd.) equipped with a carbon pad (spectro-tabs, 12 mm diameter, Plano GmbH). The remaining two proventriculi per sex were cut in half, to identify inner morphological differences. They were also placed on a stub with a carbon pad, two halves with the open side upwards and two with the lateral side upwards. Subsequently, the mounted proventriculi were sputtered with a sputter coater (EM ACE600, Leica Microsystems GmbH) using platinum (4 nm). The proventriculi were studied using a JSM-IT500 scanning electron microscope (JEOL Ltd.) equipped with a LaB₆ cathode electron gun. Operating conditions of the SEM were 3 kV accelerating voltage with a beam current of 20 μA and a working distance between 5 and 7 mm.

2.4 | Histological preparation and analysis

For the preparation of the histological sections, the remaining five dissected proventriculi with crop and midgut (ventriculus) from 2.1 were embedded in paraffin. For the embedding procedure, samples were kept in biopsy embedding cassettes (Microsette I, Simport Scientific Inc) for better handling of the samples. After fixation in paraformaldehyde (4% PFA in PBS), samples were dehydrated in an ethanol dilution series with increasing concentration (70%, 80%, 90% and 100% EtOH in H₂O dest, 2 × 15 min each step) followed by incubation in 100% isopropanol (2 × 15 min), clearing twice in cold xylene (10 min each) and once in warm xylene (60°C, 10 min). Then samples were incubated in a 1:1 mixture of molten paraffin and xylene (1 × 30 min at 60°C). Afterwards, samples were incubated in pure molten paraffin overnight at 60°C. On the next day, the paraffin was exchanged and samples were incubated for 4 h at 60°C. Finally, the samples were transferred to embedding trays filled with molten paraffin with the help of tweezers and rearranged on a heating plate. Then the paraffin was allowed to solidify in a water bath filled with iced water. Paraffin blocks were stored at 4°C for 1 day and then at room temperature until sectioning.

For sectioning, a sledge microtome (Reichert-Jung, Mod. Hn 40, Cambridge Instruments GmbH, Nussloch, Germany) with broadband disposable blades (Leica 818, Leica Biosystems) was used. Sections were produced at a thickness of 6 μm and mounted on adhesion microscope slides (Superfrost Plus, Gerhard Menzel GmbH). After sectioning, slides were stored in a glass tray inside an incubator (IN450, Memmert GmbH + Co. KG) at 50°C for 2 h.

For the histological analysis, sections were stained with haematoxylin and eosin. We removed paraffin by incubating the glass slides in xylene (2 ×, 10 min each) and rehydrated the sections using an ethanol dilution series with decreasing concentration (1 × 100% Isopropanol for 10 min, 1 × 100% EtOH for 10 min, 90%–70% EtOH for 30 s each). After rehydration of the sections,

they were briefly washed in H₂O and incubated in Mayers hemalum solution (Carl Roth GmbH + Co. KG) for 10 min. Then sections were blued in running tap water for 10 min. Afterwards, slides were incubated in 0.1% eosin G (Carl Roth GmbH + Co. KG) for 90 s and dehydrated in increasing ethanol solutions (70%–90% EtOH for 30 s each, 100% EtOH for 10 min, 2 × isopropanol for 10 min each) followed by clearing twice in xylene (10 min each). The sections were then covered with a glass coverslip and the mounting media Eukitt (Sigma-Aldrich, Merck KGaA).

The sections were photographed using a Zeiss Axio Lab. A1 microscope (Carl Zeiss AG), equipped with an Axiocam 105 colour (Carl Zeiss AG) and the software ZEN 2 core v2.5 (Carl Zeiss AG).

2.5 | Statistical analysis

Since male *B. terrestris* are generally larger than female workers, we corrected for differences in body size between male and female by using the data set of Duchateau and Marin (1995) on mean radial cell length of the wings, which is used as a proxy for body size (Medler, 1962). These calculations resulted in proventricular morphometrics relative to the body size. All statistical analyses were performed using R version 4.2.2 (2022-10-31 ucrt; R R Core

Team, 2020). To test for the homoscedasticity and normality of residuals, plots of response variables' residuals were generated using the R package *DHARMA* (Hartig & Hartig, 2017). Then general linear models (glm) for comparing the circumference of the valve and the opening and for the length of the proventriculus were produced using the built-in function *glm* with subsequent production of F-statistics with the function *ANOVA* to calculate p-values for differences between treatments. For calculating differences in the ratio of valve opening to total circumference, a binomial glm using the *glmer* function from the *lme4* package was used, as we added the replicate as a random effect (Bates et al., 2015). Plots were generated using the *ggstatsplot* package using the *ggbetweenstats* function (Patil, 2021).

3 | RESULTS

3.1 | Morphometrics of the proventriculi

The measurements of the proventriculi revealed that the relative circumference of the male proventriculus with a mean of 1439 $\mu\text{m} \pm 28.4$ SE is significantly larger than the female proventriculus with a mean relative circumference of 893 $\mu\text{m} \pm 13.3$ SE (GLM, $X^2 = 330.82$,

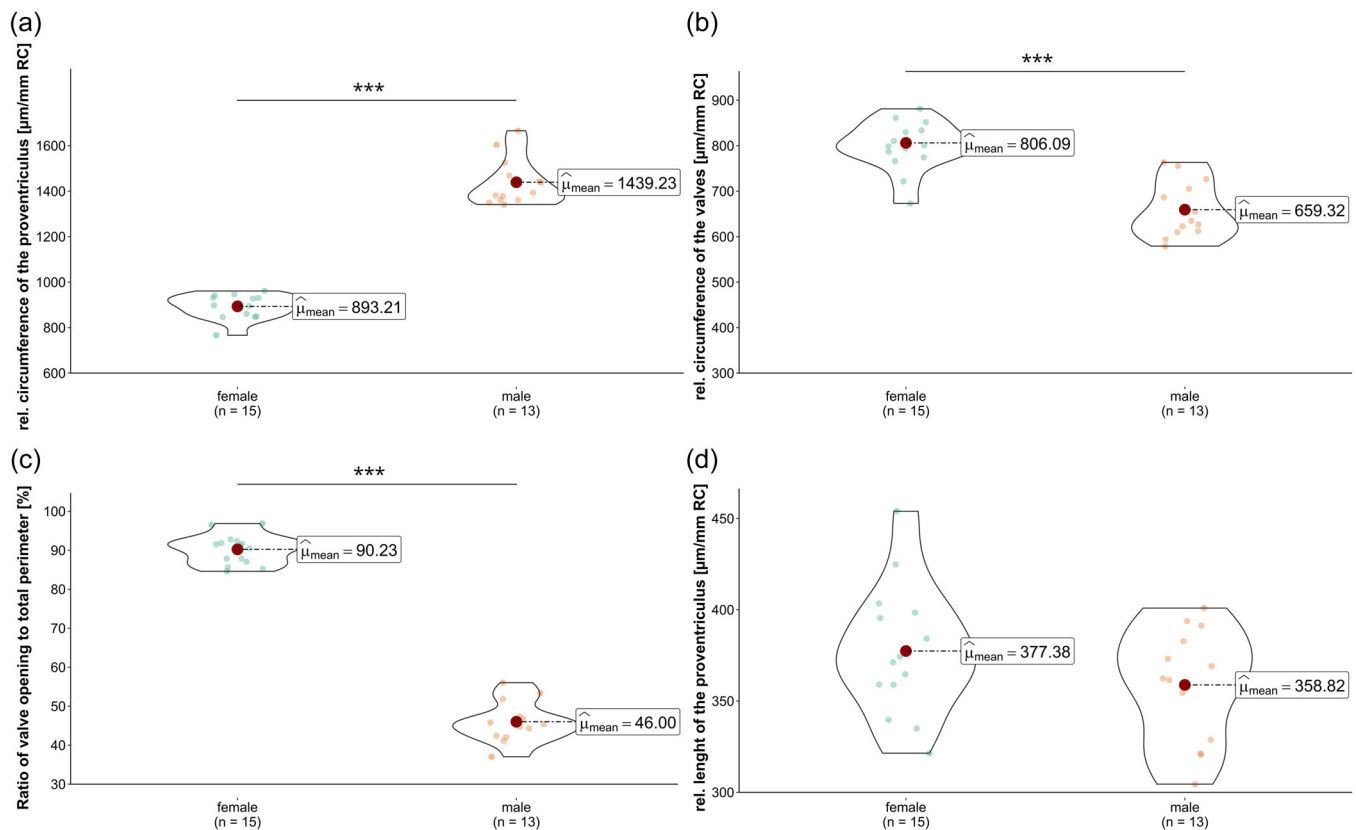


FIGURE 1 *Bombus terrestris*, morphological differences in the proventriculus corrected for body size between the sexes. (a) Difference in the mean relative circumference of the proventriculus. (b) Differences in the mean relative circumference of the valves of the proventriculi. (c) Difference of the mean ratio between valve opening and total circumference of the proventriculi. (d) Difference in mean relative length of the proventriculi. Asterisks indicate statistical significance (***) ($p < .001$).

df = 1, $p < .001$; Figure 1a). In contrast, the mean relative circumference of the valve openings of the female proventriculi with $806 \mu\text{m} \pm 15.5 \text{ SE}$ was significantly larger compared to the male valve mean relative circumference of $659 \mu\text{m} \pm 17.2 \text{ SE}$ (GLM, $X^2 = 40.407$, df = 1, $p < .001$; Figure 1b). Consequently, the mean ratio of valve opening to total circumference of the females' proventriculi is with $90.23\% \pm 0.98 \text{ SE}$ significantly larger than the males with only $46\% \pm 1.45 \text{ SE}$ (GLMM with binomial distribution, $X^2 = 501.91$, df = 1, $p < .001$; Figure 1c). We found no significant differences in the mean length of the proventriculi between the sexes (GLM, $X^2 = 2.1869$, df = 1, $p = .1392$; Figure 1d). Further, there were no differences in the proventriculus between workers and queens, as workers are basically

“dwarf queens” and these two castes are morphologically and anatomically identical apart from their size (Cnaani & Hefetz, 2001; Michener, 1974, supplementary online material, Figures S2 and S3).

3.2 | Morphology of the proventriculi

The male and female proventriculi possess four densely haired anterior valves, with their hairs directed posterior to the ventriculus (Figure 2a,d). The male proventriculus has four distinct lateral chambers and is narrowed at the anterior and posterior end with its greatest circumference in the middle part (Figure 2a–c). The female

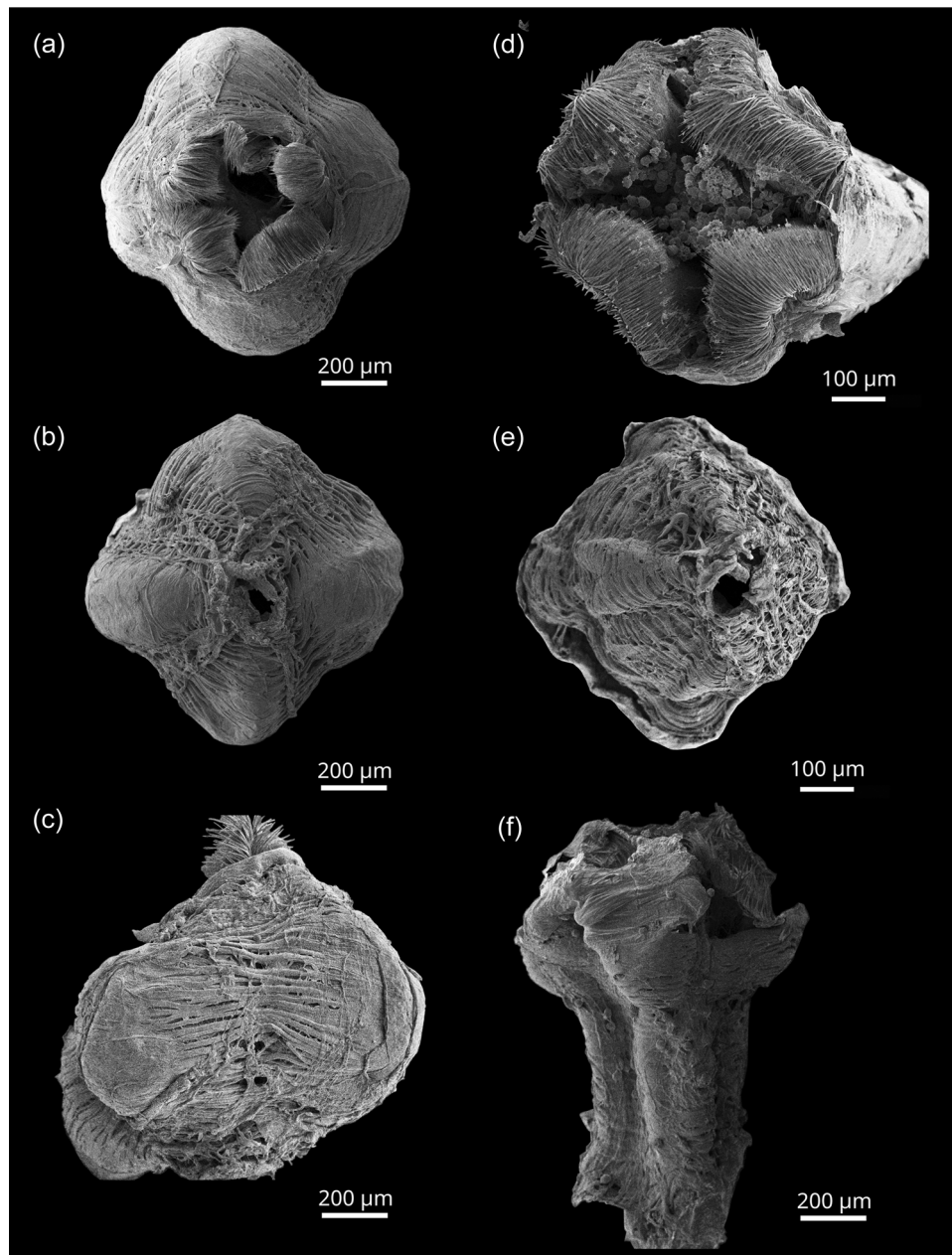


FIGURE 2 Scanning electron micrographs of the exterior anatomy of the proventriculus of *B. terrestris*; males (a–c) and females (d–f). Anterior end facing the crop (a + d); posterior end facing the ventriculus (b + f) and lateral view of the proventriculus (c + f).

proventriculus has its greatest circumference at the anterior end and becomes narrower towards the posterior end (Figure 2d–f). The posterior end of the proventriculi of both sexes are morphologically similar, as they are both densely surrounded by muscles acting as a sphincter (Figure 2b,e). The lateral view shows that the male proventriculus is more spherical in shape than the female proventriculus, which has a conical form already described by Peng and Marston (1986; Figure 2c,f). Further, the female proventriculus only consists of rudimentary chambers, which have been previously described by Bailey (1952) for worker honeybees (Figures 2e,f and 3b,d).

The female proventriculus is equipped with four triangular lips (Figure 2d). Upon further examination using a longitudinal section of the proventriculus we realised that each triangular lip corresponds to the anterior portion of the proventricular fold (Figure 3a). A pouch is formed beneath the adjacent points of the anterior lips and between the basal plates, which is equipped with hairs (Figure 3a,b,d). The SEM analysis of the longitudinal section of the male proventriculus (Figure 4a,c) revealed four basal plates with anterior triangular lips, which are densely packed with filiform hairs (Figure 4b). The male proventriculi differ from the female proventriculi in the heavily extended membranous folds between the basal plates, forming four

chambers. In addition, these chambers are densely covered with long filiform hairs (Figure 4d). The four basal plates form a duct connecting the crop with the ventriculus.

3.3 | Histology of the proventriculi

The histological analysis of the female proventriculi confirmed the elongated lumen surrounded by transverse and longitudinal muscles, reaching into the ventriculus (Figure 5). They have small rudimentary chambers located between the four lips to store pollen packages (Bailey, 1952). The basal plates of the proventricular folds are connected by circular muscles, forming a proventricular lumen (PL).

The histological analysis of the male proventriculi revealed four lateral chambers, surrounded by longitudinal and transversal muscles (Figure 6a). The chambers are connected through a channel (Figure 6b) and separated from each other by intimae and longitudinal muscles (Figure 6b,c). Each chamber is densely lined with hairs reaching into the chamber, originating from the inner chamber epithelia, confirming the SEM observations (Figure 6c magnified section).

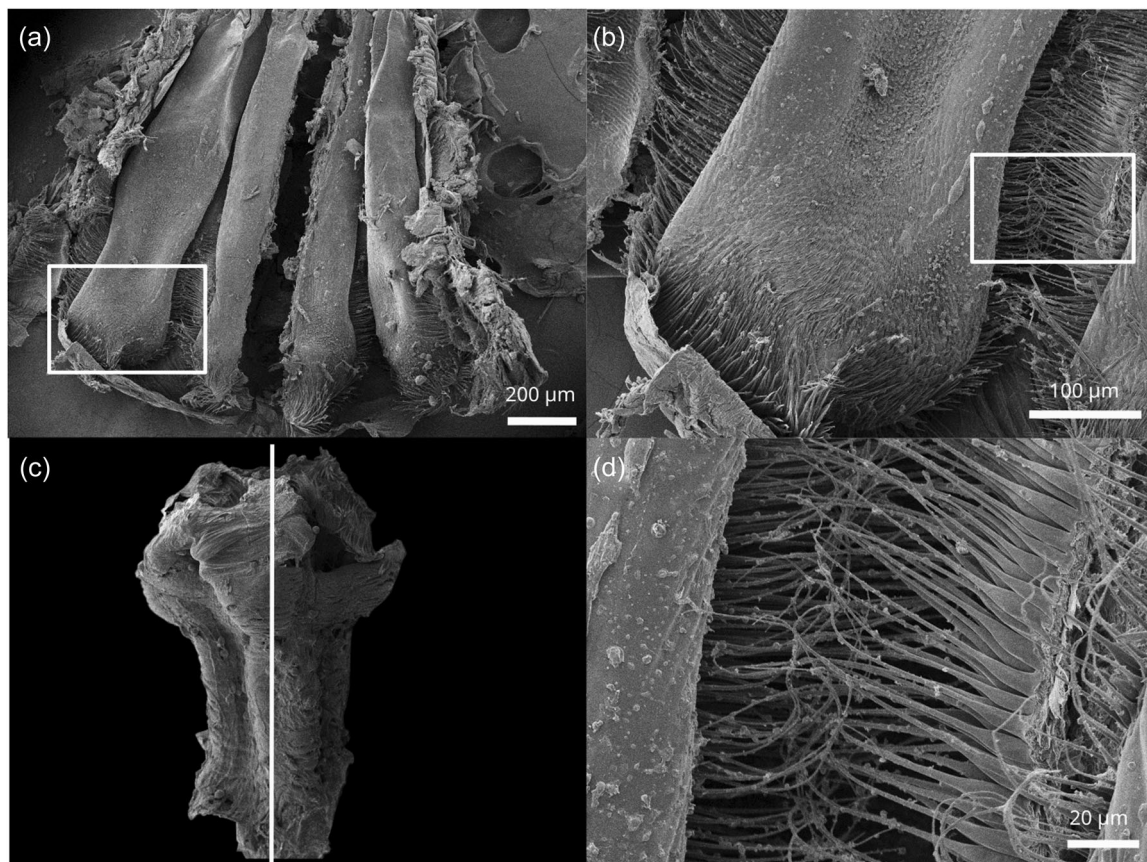


FIGURE 3 Scanning electron micrographs of the inner anatomy of the female proventriculus of *B. terrestris*. View inside a dissected proventriculus of a female *B. terrestris* individual. The cutting plane is indicated in C (a). Zoom in on one of the lips of the proventriculus, the area is indicated as white box in A (b). Exemplary SEM image of the lateral view on a proventriculus of a female *B. terrestris* individual. The cutting plane for dissection is indicated by a white line (c). Zoom in on the hairs on the edges of the lips forming the proventricular canal, the area is indicated as white box in B (d).

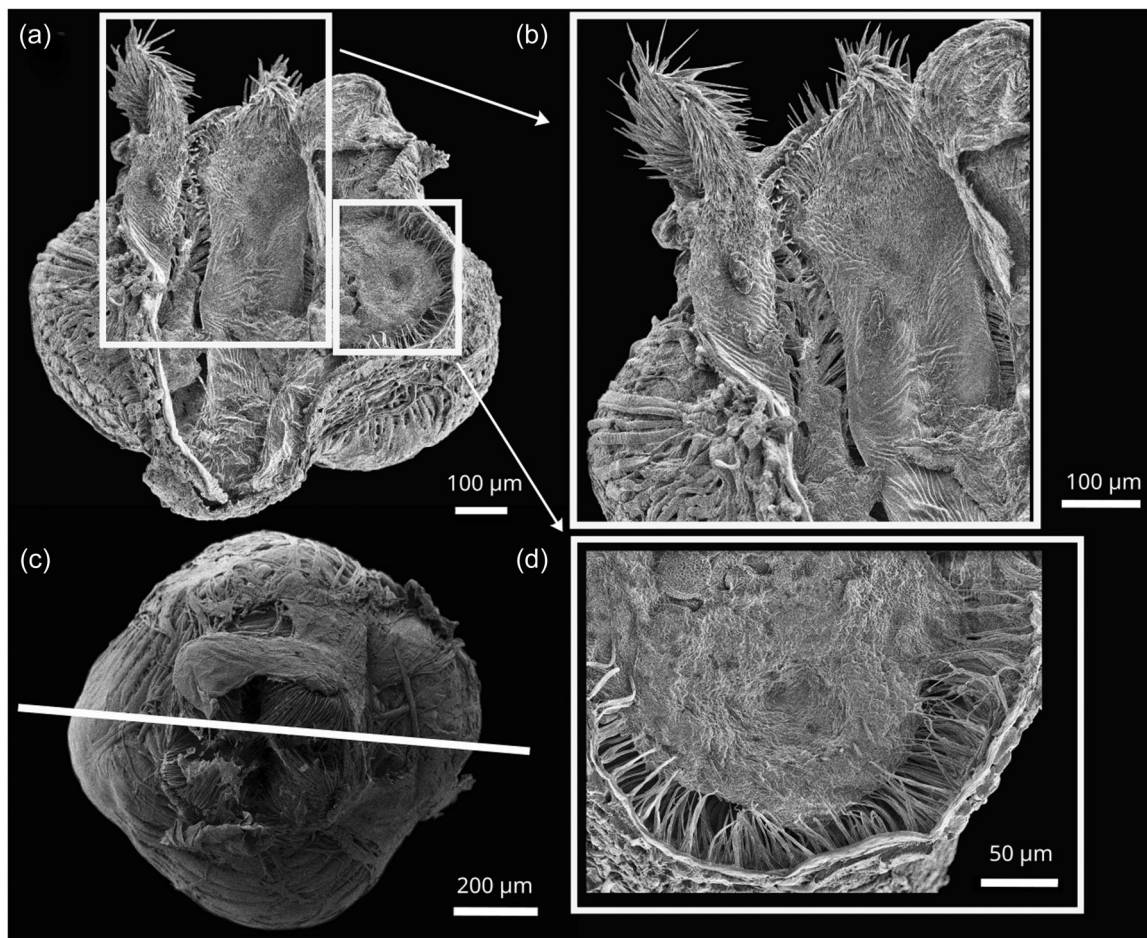


FIGURE 4 *Bomb* Scanning electron micrographs of the inner anatomy of the male proventriculus of *B. terrestris*. View inside a dissected proventriculus of a male *B. terrestris* individual. The cutting plane is indicated in C (a). Zoom in on one of the lips of the proventriculus, the area is indicated as white box with respective arrow in A (b). Exemplary SEM image of the lateral view on a proventriculus of a male *B. terrestris* individual. The cutting plane for dissection is indicated by a white line (c). Zoom in on the hairs on the edges of the chambers of the proventriculus, the area is indicated as white box with respective arrow in A (d).

4 | DISCUSSION

In our study, we document a sexual dimorphism in morphology of the proventriculus of male and female *B. terrestris*. Overall, the male proventriculus is wider in its middle part than the female proventriculus. While the female proventriculus has a conical shape and its valve openings pose the area with the highest circumference, the males' proventriculus is spherical with a significantly larger circumference, but smaller valve openings. Further, the formed chambers of the male proventriculus are lined with hairs on the inside. Chambers are only rudimentarily present in females, forming small pouches lined with hairs can be found between the basal plates constituting the proventriculus. The enlarged chambers of the males' proventriculus may serve the bumblebees as pollen filtering and storage apparatuses and help forming food boluses for concentrated pollen discharge into the ventriculus (Bailey, 1952; Peng & Marston, 1986). The controlled discharge of the pollen thereby supports the effective, concentrated enzymatic digestion within the ventriculus (Bailey, 1952). Comparable proventricular bulbs are also found in other Hymenoptera like ants and

wasps (Bution et al., 2010), but this is only true for female workers since descriptions for male individuals are elusive.

The observed differences between the proventriculi in male and female *B. terrestris* could be explained by the different tasks they perform in the colony, as the results of an ecological dimorphism (Fryxell et al., 2019). As the reproductive caste, males are crucial for maintaining and even increasing the genetic effective population size (Kraus et al., 2009; Schmid-Hempel et al., 2007; Shykoff & Schmid-Hempel, 1991). In contrast, females, especially the worker bees, focus primarily on foraging and colony maintenance while they are not involved in mating (Goulson, 2010). Consequently, males might require larger storage capacities to sustain themselves during their extensive flight activities and mate-seeking endeavours. This is also reflected in longer distances per flight (2.6–9.9 km) which male *B. terrestris* undertake when searching for mating partners (Kraus et al., 2009). By covering larger territories, male bumblebees increase the likelihood of encountering a suitable queen, thereby maximising their reproductive success (Belsky et al., 2020; Paxton, 2005). Further, some bumblebee species such as *B. terrestris* are known to be monandrous, where males insert a mating plug

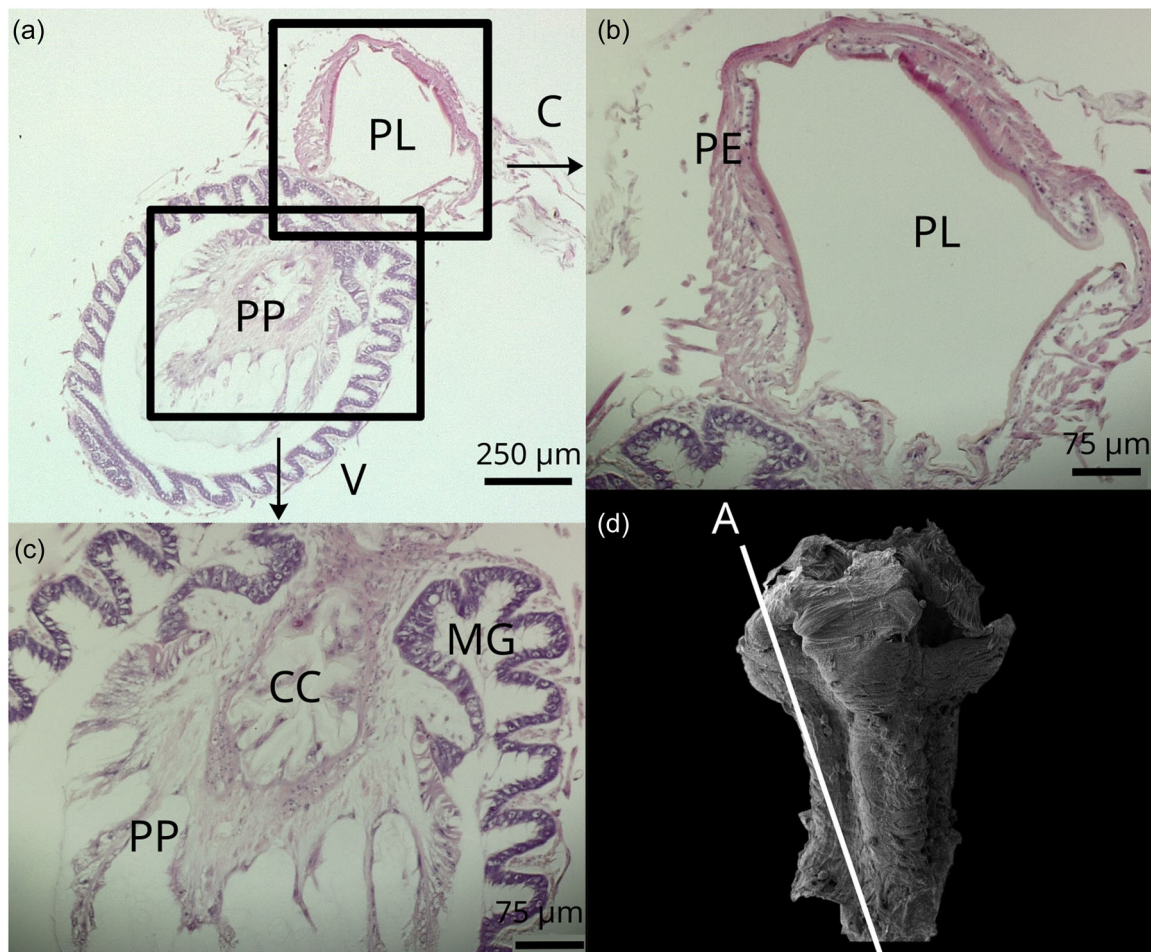


FIGURE 5 *Bomb* Histology of female proventriculus of *B. terrestris*. Paraffin section, stained with haematoxylin and eosin (a–c). Display of a lateral section, showing the proventriculus together with the adjacent ventriculus (a). Black boxes and respective arrow indicate a zoom-in on the morphological structures displayed in (b) and (c). Zoom-in on the proventriculus of the female showing that it consists of one lumen, with no separated chambers (b). Zoom in on the connection between the proventriculus and the midgut (ventriculus) (c). Exemplary SEM image of the lateral view on a proventriculus of a female *B. terrestris* (d), the white line indicates the cutting plane through the proventriculus for the respective image in (a). C, crop; CC, connecting channel; MG, midgut (ventriculus); PE, proventricular epithelium; PL, proventricular lumen; PP, posterior part of the proventriculus.

into the reproductive tract of the queen to prevent her from further mating (Ayasse et al., 2001; Baer et al., 2001). This forces other males to travel longer distances to find a queen for mating. The ability to undertake longer flights during mate search is crucial for gene transfer among subpopulations of bumblebee colonies (Kraus et al., 2009; Wolf et al., 2012). Longer flight distances require adequate energy reserves and the increased size of the proventriculus chambers in male *B. terrestris* may be attributed to their increased nutritional requirements during mate search. These enlarged chambers together with their specialised crop might serve for storing and accessing the previously acquired resources, such as nectar in the crop and pollen in the proventriculus, which sustain them during the prolonged flight and mate searching endeavours without the need for frequent food collection (Bertsch, 1984). This feeding strategy likely allows them to allocate more time and energy to mate searching and courtship behaviours. Additionally, the allocation of large quantities of pollen might be crucial for the production of the mating plug since the main compounds of the latter are linoleic, oleic, palmitic, as well

as stearic acid and pollen represents the main lipid provider for bumblebees (Baer et al., 2000; Campos et al., 2008). Next to lipids, pollen also supply the bumblebees with protein, which are crucial elements for sperm production (Collins et al., 2006; Dallai et al., 2016). These larger proventriculus chambers together with an optimal sugar intake during mate search is likely an adaptation that contribute to maximise the reproductive success of male bumblebees ensuring the viability and resilience of bumblebee colonies.

5 | CONCLUSION

We report a sexual dimorphism in morphology of the proventriculus of male and female *B. terrestris*. This highly specialised compartment of the alimentary tract differs between the sexes in size, shape and inner morphology. This difference between sexes might reflect the dimorphism in the ecological role of the male *B. terrestris* and may be crucial

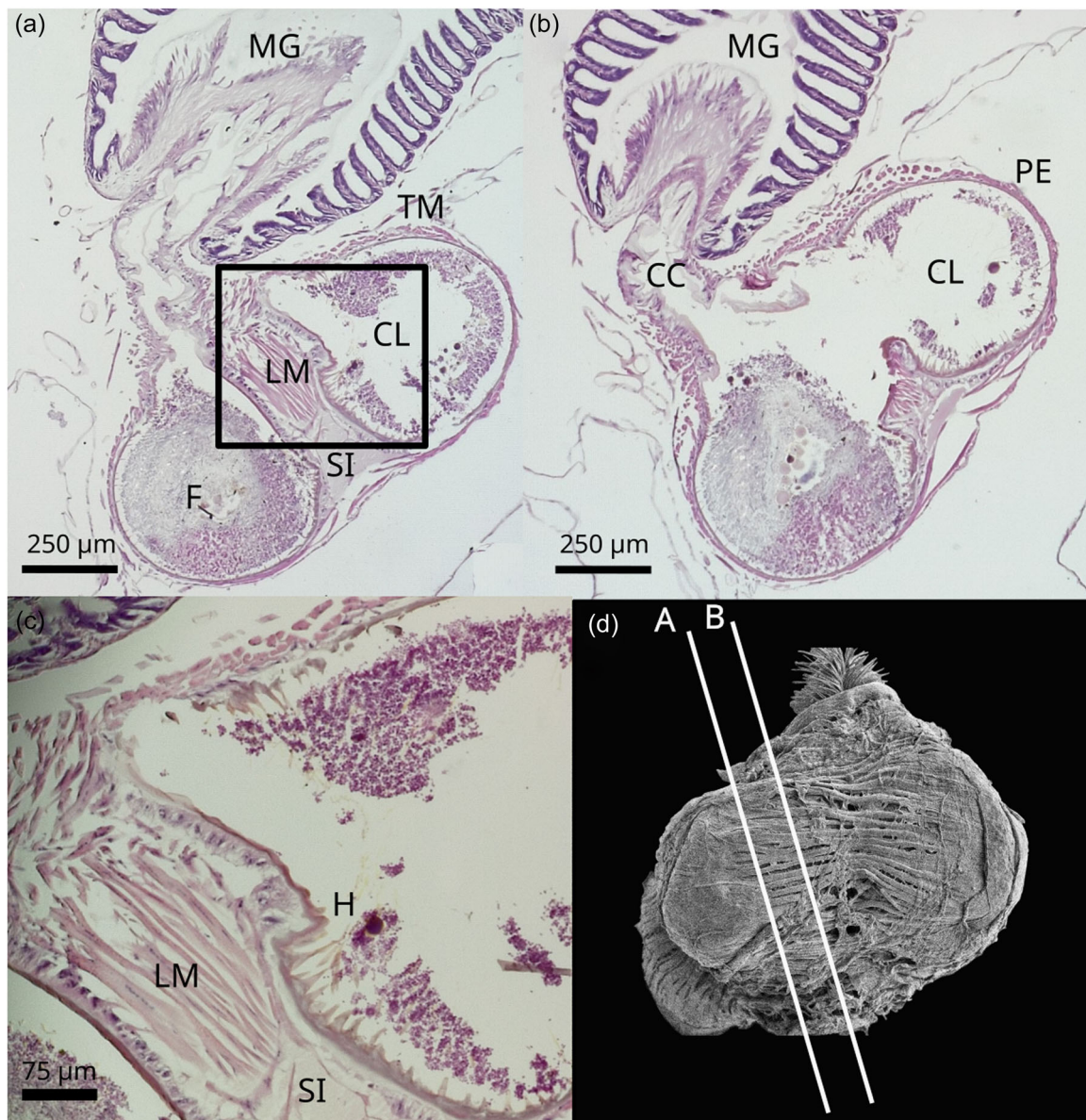


FIGURE 6 Histology of male proventriculus of *B. terrestris*. Paraffin section, stained with haematoxylin and eosin (a–c). Display of a lateral section going through the middle of the proventriculus, revealing the connecting channel of the chambers as well as the musculature and chamber-separating intima between each chamber (a). Black boxes and respective arrow indicate a zoom-in on the morphological structures displayed in (b) and (c). Display of a more lateral section, revealing interconnection of the chambers leading into the connecting channel in direction of the midgut (ventriculus) (b). The image shows the muscle fibres surrounding the epithelia layer of the chamber of the proventriculus. Inwards the intima of the chamber is covered with hairs (c). Exemplary SEM image of the lateral view on a proventriculus of a male *B. terrestris* (d), white lines indicate the cutting plane through the proventriculus for the respective images (a, b) (d). CC, connecting channel; CL, chamber lumen; F, food particles; H, hairs; LM, longitudinal muscles; MG, midgut (ventriculus); PE, proventricular epithelia; SI, separating intima.

for reproductive success. Our study highlights that research on male social wild bee pollinators has been underrepresented, given the crucial role they play in colony fitness and survival. Consequently, we suggest that the investigation of impacts from major anthropogenic stressors, like habitat fragmentation, climate change, chemical and particulate pollutants, such as microplastics, should not be restricted on female workers but also include the male caste, considering their ecological role.

AUTHOR CONTRIBUTIONS

Frederic Hüftlein: Conceptualisation; methodology; data curation; investigation; validation; formal analysis; visualisation; writing—original draft; writing—review and editing. **Sven Ritschar:** Conceptualisation; investigation; methodology; validation; data curation; formal analysis; writing—original draft; writing—review and editing. **Christian Laforsch:** Funding acquisition; writing—original draft; writing—review and editing; supervision; resources.

ACKNOWLEDGEMENTS

We would like to thank Prof. Dr. Heike Feldhaar for letting us perform the animal husbandry in her climate chambers. We further like to thank Dr. Oliver Otti for proof-reading the manuscript and for his productive feedback. Lastly, we like to thank the two anonymous reviewers for improving our manuscript. Frederic Hüftlein is financed by the Bavarian State Ministry of the Environment and Consumer Protection as part of the project network BayOekotox. Sven Ritschar is funded by the Deutsche Forschungsgemeinschaft (DFG; German Research Foundation)–Project Number 391977956-SFB 1357.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at <https://zenodo.org/record/8335000>, reference number 8335000.

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PEER REVIEW

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REFERENCES

- Ayasse, M., Paxton, R. J., & Tengö, J. (2001). Mating behavior and chemical communication in the order Hymenoptera. *Annual Review of Entomology*, 46(1), 31–78. <https://doi.org/10.1146/annurev.ento.46.1.31>
- Baer, B., Maile, R., Schmid-Hempel, P., Morgan, E. D., & Jones, G. R. (2000). Chemistry of a mating plug in bumblebees. *Journal of Chemical Ecology*, 26, 1869–1875. <https://doi.org/10.1023/A:1005596707591>
- Baer, B., Morgan, E. D., & Schmid-Hempel, P. (2001). A nonspecific fatty acid within the bumblebee mating plug prevents females from remating. *Proceedings of the National Academy of Sciences*, 98(7), 3926–3928. <https://doi.org/10.1073/pnas.061027998>
- Bailey, L. (1952). The action of the proventriculus of the worker honeybee, *Apis mellifera* L. *Journal of Experimental Biology*, 29(2), 310–327.
- Belsky, J. E., Camp, A. A., & Lehmann, D. M. (2020). The importance of males to bumble bee (*Bombus* species) nest development and colony viability. *Insects*, 11(8), 506. <https://doi.org/10.3390/insects11080506>
- Bertsch, A. (1984). Foraging in Male bumblebees (*Bombus lucorum* L.): Maximizing energy or minimizing water load. *Oecologia*, 62(3), 325–336. <https://doi.org/10.1007/BF00384264>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Boomsma, J. J., Baer, B., & Heinze, J. (2005). The evolution of male traits in social insects. *Annual Review of Entomology*, 50, 395–420. <https://doi.org/10.1146/annurev.ento.50.071803.130416>
- Brown, M., & Brown, M. J. F. (2020). Nectar preferences in male bumblebees. *Insectes Sociaux*, 67, 221–228. <https://doi.org/10.1007/s00040-020-00751-y>
- Bution, M. L., Caetano, F. H., & Fowler, H. G. (2010). Proventriculus of cephalotes ants: A structural and comparative analysis. *Micron*, 41(1), 79–83. <https://doi.org/10.1016/j.micron.2009.08.005>
- Cameron, S. A., & Sadd, B. M. (2020). Global trends in bumble bee health. *Annual Review of Entomology*, 65(1), 209–232. <https://doi.org/10.1146/annurev-ento-011118-111847>
- Campos, M. G. R., Bogdanov, S., de Almeida-Muradian, L. B., Szczesna, T., Mancebo, Y., Frigerio, C., & Ferreira, F. (2008). Pollen composition and standardisation of analytical methods. *Journal of Apicultural Research*, 47(2), 154–161. <https://doi.org/10.1080/00218839.2008.11101443>
- Carreck, N. L., Andree, M., Brent, C. S., Cox-Foster, D., Dade, H. A., Ellis, J. D., Hatjina, F., & Van Englesdorp, D. (2013). Standard methods for *Apis mellifera* anatomy and dissection. *Journal of Apicultural Research*, 52(4), 1–40. <https://doi.org/10.3896/IBRA.1.52.4.03>
- Cnaani, J., & Hefetz, A. (2001). Are queen *Bombus terrestris* giant workers or are workers dwarf queens? Solving the chicken and egg problem in a bumblebee species. *Naturwissenschaften*, 88, 85–87. <https://doi.org/10.1007/s001140000202>
- Collins, A. M., Caperna, T. J., Williams, V., Garrett, W. M., & Evans, J. D. (2006). Proteomic analyses of male contributions to honey bee sperm storage and mating. *Insect Molecular Biology*, 15(5), 541–549. <https://doi.org/10.1111/j.1365-2583.2006.00674.x>
- da Cruz-Landim, C., & Rodrigues, L. (1967). Comparative anatomy and histology of the alimentary canal of adult apinae. *Journal of Apicultural Research*, 6(1), 17–28.
- Dallai, R., Gottardo, M., & Beutel, R. G. (2016). Structure and evolution of insect sperm: new interpretations in the age of phylogenomics. *Annual Review of Entomology*, 61, 1–23. <https://doi.org/10.1146/annurev-ento-010715-023555>
- Dettner, K. & Peters, W., (Eds.). (2003). *Lehrbuch der Entomologie*. Springer-Verlag.
- Dornhaus, A., & Chittka, L. (2004). Information flow and regulation of foraging activity in bumble bees (*Bombus* spp.). *Apidologie*, 35(2), 183–192.
- Duchateau, M. J., & Marin, J. (1995). Sexual biology of haploid and diploid males in the bumble bee *Bombus terrestris*. *Insectes Sociaux*, 42, 255–266. <https://doi.org/10.1007/BF01240420>
- Dukas, R., & Waser, N. M. (1994). Categorization of food types enhances foraging performance of bumblebees. *Animal Behaviour*, 48(5), 1001–1006. <https://doi.org/10.1006/anbe.1994.1332>
- Fryxell, D. C., Weiler, D. E., Kinnison, M. T., & Palkovacs, E. P. (2019). Eco-Evolutionary dynamics of sexual dimorphism. *Trends in Ecology & Evolution*, 34, 591–594. <https://doi.org/10.1016/j.tree.2019.04.007>
- Goulson, D. (2003). *Bumblebees – their behaviour and ecology*. Oxford University Press.
- Goulson, D. (2010). *Bumblebees: Behaviour, ecology and conservation* (2). Oxford University Press Inc.
- Hartig, F., & Hartig, M. F. (2017). *DHARMA: An R package for residual diagnostics of generalized linear mixed models*. R Development Core Team. <http://florianhartig.github.io/DHARMA/>
- Hüftlein, F., Seidenath, D., Mittereder, A., Hillenbrand, T., Brüggemann, D., Otti, O., Feldhaar, H., Laforsch, C., & Schott, M. (2023). Effects of diesel exhaust particles on the health and survival of the buff-tailed bumblebee *Bombus terrestris* after acute and chronic oral exposure. *Journal of Hazardous Materials*, 458, 131905. <https://doi.org/10.1016/j.jhazmat.2023.131905>

- Inouye, D. W. (1980). The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. *Oecologia*, 45, 197–201. <https://doi.org/10.1007/BF00346460>
- Kraus, F. B., Wolf, S., & Moritz, R. F. A. (2009). Male flight distance and population substructure in the bumblebee *Bombus terrestris*. *Journal of Animal Ecology*, 78(1), 247–252. <https://doi.org/10.1111/j.1365-2656.2008.01479.x>
- Krenn, H. W., Plant, J. D., & Szucsich, N. U. (2005). Mouthparts of flower-visiting insects. *Arthropod structure & development*, 34(1), 1–40. <https://doi.org/10.1016/j.asd.2004.10.002>
- Medler, J. T. (1962). Morphometric studies on bumble bees. *Annals of the Entomological Society of America*, 55(2), 212–218. <https://doi.org/10.1093/aesa/55.2.212>
- Michener, C. D. (1974). *The social behavior of the bees: a comparative study*. Harvard University Press.
- OECD (2017). Guideline for the testing of chemicals. Bumblebee, acute oral toxicity test. OECD No. 247. <https://doi.org/10.1787/9789264284128-en>
- Patil, I. (2021). Visualizations with statistical details: the “ggstatsplot” approach. *Journal of Open Source Software*, 6(61), 3167. <https://doi.org/10.21105/joss.03167>
- Paxton, R. J. (2005). Male mating behaviour and mating systems of bees: An overview. *Apidologie*, 36(2), 145–156. <https://doi.org/10.1051/apido>
- Peng, Y. S., & Marston, J. M. (1986). Filtering mechanism of the honey bee proventriculus. *Physiological Entomology*, 11(4), 433–439. <https://doi.org/10.1111/j.1365-3032.1986.tb00434.x>
- Pyke, G. H. (1980). Optimal foraging in bumblebees: Calculation of net rate of energy intake and optimal patch choice. *Theoretical Population Biology*, 17(2), 232–246. [https://doi.org/10.1016/0040-5809\(80\)90008-8](https://doi.org/10.1016/0040-5809(80)90008-8)
- R Core Team. (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Schmid-Hempel, P., Schmid-Hempel, R., Brunner, P. C., Seeman, O. D., & Allen, G. R. (2007). Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. *Heredity*, 99, 414–422. <https://doi.org/10.1038/sj.hdy.6801017>
- Serrão, J. E. (2001). A comparative study of the proventricular structure in corbiculate apinae (Hymenoptera, Apidae). *Micron*, 32(4), 379–385. [https://doi.org/10.1016/S0968-4328\(00\)00014-7](https://doi.org/10.1016/S0968-4328(00)00014-7)
- Shykoff, J. A., & Schmid-Hempel, P. (1991). Parasites and the advantage of genetic variability within social insect colonies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 243(1306), 55–58. <https://doi.org/10.1098/rspb.1991.0009>
- Snodgrass, R. E. (1910). The anatomy of the honey bee. U. S. Department of Agriculture, Bureau of Entomology. *Technical Series*, 18, 1–162. <https://doi.org/10.1038/085169b0>
- Willmer, P. (2011). *Pollination and floral ecology*. Princeton University Press.
- Wolf, S., & Chittka, L. (2016). Male bumblebees, *Bombus terrestris*, perform equally well as workers in a serial colour-learning task. *Animal Behaviour*, 111, 147–155. <https://doi.org/10.1016/j.anbehav.2015.10.009>
- Wolf, S., Toev, T., Moritz, R. L. V., & Moritz, R. F. A. (2012). Spatial and temporal dynamics of the male effective population size in bumblebees (Hymenoptera: Apidae). *Population Ecology*, 54(1), 115–124. <https://doi.org/10.1007/s10144-011-0285-2>
- Wolf, T. J., Ellington, C. P., & Begley, I. S. (1999). Foraging costs in bumblebees: Field conditions cause large individual differences. *Insectes Sociaux*, 46(3), 291–295. <https://doi.org/10.1007/s000400050148>

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How to cite this article: Hüftlein, F., Ritschar, S., & Laforsch, C. (2023). Sexual dimorphism in the proventriculus of the buff-tailed bumblebee *Bombus terrestris* (L. 1758) (Hymenoptera: Apidae). *Journal of Morphology*, 285, e21668. <https://doi.org/10.1002/jmor.21668>