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UNDERSTANDING THE ROLE OF SPECIALIZED MOUTHPARTS IN THE SELECTIVE FEEDING OF DUNG BEETLES (COLEOPTERA: SCARABAEINAE)

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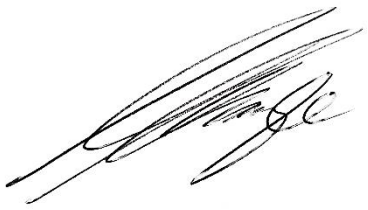
22 September 2021

Dissertation

Submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science, Johannesburg, South Africa 2020

Declaration

I hereby declare that this entire dissertation is my own, unaided work except where in the research I have indicated otherwise. It has not been submitted before by me for any diploma or degree or examination at any other university or institution of higher learning



_____

Nthabiseng Gladys Mathikge

22 September 2021

Master of Science Supervisors:

Dr Claudia Tocco

Dr James du G. Harrison

Prof. Marcus J. Byrne

Dedication

This dissertation is dedicated to my late grandmother, Dimakatso Mittah Mathikge Moqhekoana, o e leng "*Mohlakoana wa mmaphola disema maila ngoathela, motho ya sa jeng sengoathana sa maobane, motho ya e tlareng a ja meriti e theye*".

Thanks for always having my back Oumie and for believing that I can do anything I set my mind to. Thank you for instilling in me principles of hard work, independence and trust in the Almighty. You taught me that "*The roots of education are bitter, but the fruit is sweet*", which has truly encapsulated my M.Sc. journey. I hope that I have made you proud.

"Not by might, nor by power, but by My Spirit, says the Lord of hosts"

- Zechariah 4:6

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Abstract

The use of a complex food substance such as herbivore dung, by dung beetles has promoted the evolution of an adaptive feeding strategy known as particle feeding. Selection of fine particulate matter elevates the food nutrient quality by up to five-fold. However, it is not known how dung beetles achieve this. An ephemeral food source such as dung is not always available, thus several dung beetle genera have resorted to exploring alternative diets such as dead plant material and mushrooms, dry dung pellets and carrion to name a few. How they use similar mouthparts to process this food is not known. Dung beetles possess highly specialized mouthparts, but how these operate is still not fully understood. Several hypotheses attempting to explain the mode of action of these mouthparts have been put forward but only one has been consistent with several experimental findings. This hypothesis suggests that dung beetles increase the nutrient quality of dung using their mouthparts as a filtering apparatus that concentrates minute and nutritious dung particles while eliminating excess water and intractable plant fragments with no nutritional value. To gain more insight on this topic, this study investigated the morphology of various internal components of dung beetle mouthparts to understand their respective roles in particle feeding. Microscopy and geometric morphometric analysis were used to examine and compare the mouthparts of 12 dung beetle species belonging to four feeding groups (generalist, wet dung, carrion and dry diet feeders). The qualitative microscopy analysis revealed that dry diet feeders possess grinding and cutting structures, which are reduced or absent in the generalist, carrion and wet dung feeders. Dry diet feeders are hypothesized to have evolved from a wet-dung feeding lineage, thus they may have gained cutting and grinding structures as a result of adaptations to environmental changes that confined them to arid habitats. The carrion feeder and generalist feeder have similar structural features as the wet dung feeders, presumably because of a pre-adaption to liquid-based food. The microscopy findings also reveal that the generalist, carrion and wet feeders possess structures such as filtration channels and the prosthecal comb that are heavily reduced in dry feeders. Based on comparisons made between the dry feeder *Pachysoma striatum* which shares morphological similarities with all of the feeding groups, it was concluded that the likely function of the filtration channels is to facilitate the movement of ready to digest food from the molar lobes to the pharynx to be ingested. The quantitative microscopy results indicate that the filtration channels are not designed to effectively expel excess water, thus Holter's hypothesis is not entirely valid and needs to be revised. The mouthpart dimensions

measured in my thesis in relation to the particle size range determined by Holter and Scholtz (2007) and Madzivhe et al. (2020) show that the mouthparts do not function as a filter in the strictest sense as proposed by Holter (2000) and I have thus proposed a rake – like mechanism. The geometric morphometrics results indicate that distinct structural features of dung beetle mouthparts across feeding groups are determined by taxonomic relationships shared between species rather than food habits. Thus, the use of the shape of the epipharynx for geometric morphometrics is more useful for understanding phylogenetic relationships between species than it is for mapping feeding behavioural differences across feeding groups.

Keywords: Dung beetles, particle feeding, mouthparts, morphology, microscopy, geometric morphometrics

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Chapter 1: Introduction

1.1 Feeding biology of dung beetles

1.1.1 Coprophagy and the specialization of dung beetle mouthparts

According to Davis et al. (2009), coprophagy is a newly derived feeding behaviour that involves feeding on dung (primarily that of herbivorous and omnivorous mammals (Miller, 1961; Hata and Edmonds, 1983; Holter et al, 2002; Holter, 2016)) by adult dung beetles and their larvae (Holter, 2016). While there may be approximately 6000 to 8000 recognized extant dung beetle species worldwide (Holter, 2016), coprophagy is mostly dominant within the Scarabaeinae and Aphodiinae subfamilies because "not all dung beetles are dung specialists" (Davis et al, 2009). However, there are dung beetle species belonging to the families Geotrupidae and Hydrophilidae and subfamily Sphaeridiinae that also feed on dung (Holter, 2004; Holter and Scholtz, 2007). Coprophagy is suggested to have evolved from a detritus-feeding lineage (by members of the Scarabaeoidea superfamily) (Cambefort, 1991) and is likely to have occurred concurrently with the radiation of the terrestrial herbivores (Halffter and Edmonds, 1982) that increased in numbers due to the expansion of grassland and savanna biomes during the Miocene period (Scholtz et al., 2009).

An increase in herbivores yielded considerable amounts of wet dung that were conducive for the use of dung and the evolution of insects that feed on dung (Scholtz et al., 2009). Thus, the exploitation and manipulation of dung required the evolution of a special feeding technique (Verdú and Galante, 2004), which must have forced wet dung feeding beetles (wet feeders) to undergo morphological changes to their mouthparts (which are hypothesised to have been formerly heavily sclerotized (Scholtz et al., 2009; Holter and Scholtz, 2011)) to now being membranous and hairy (Miller, 1961; Hata and Edmonds, 1983; Holter and Scholtz, 2011; Bai et al., 2015). Specialization of the mouthparts subsequently caused wet feeders to lose the ability to incise and grind food during the initial steps of digestion (Holter and Scholtz, 2011).

Although dung beetles were able to successfully transition to wet dung from a saprophagous diet, they also had to develop ways of protecting that dung from resource competitors and changes in

environmental conditions that could negatively impact its moisture and nutrient content (Scholtz et al., 2009). Dung beetles confronted this obstacle by evolving various nesting strategies to protect the dung (Halffter and Edmonds, 1982). These nesting behaviours have been partitioned into four main ecological groups, namely: telecoprids, paracoprids, endocoprids and kleptocoprids (Halffter and Edmonds, 1982; Scholtz et al., 2009).

The telecoprids gather portions of the dung pile and create dung balls which they roll away from the original point of deposition where it will either be buried or placed in grass tussocks, the paracoprids form tunnels and nests beneath the dung pat, the endocoprids choose not to displace their food from the dung source but rather feed, form brood balls and construct nests within the dung source itself and the kleptocoprids exploit the dung that has been collected and buried by other dung beetle species (Halffter and Edmonds, 1982).

By undergoing ethological changes and modifying their mouthparts, dung beetles were able to exploit this ephemeral food source effectively and were able to avoid intra- and interspecific levels of competition with other dung beetle species and insects by the spatial and temporal separation of habitat use described (Verdú and Galante, 2004).

The modification of mouthparts resulted in ingestion being limited to the fluid constituent of dung, containing minute particles (Holter, 2000) of nutritious and digestible food items such as epithelia from the herbivore's gut, fungi and bacteria (Holter and Scholtz, 2005). To determine the nutritional value and impact that small particles of dung have on the physiology and overall development of dung beetles, Aschenborn et al. (1989) conducted experimental trials that indicated that the reproductive performance of the dung beetle *Euoniticellus intermedius* (Reiche) improved when it was only fed the fluid component of dung. This proved that the "fluid" component was the main supply of nutrients that dung beetles used to meet their dietary requirements. The larger particles of dung, on the other hand, are made up of unusable food items (generally portions of fibrous, indigestible plant material) that are filtered out by the mouthparts (Holter et al., 2002), because dung beetles generally possess a simple and undifferentiated gut that lacks structures for housing bacteria that break down lignocellulose contained within the plant's cell wall (Holter 2000; Holter and Scholtz 2013; Ebert et al., 2021). This plant material is considered an intractable food source with no nutritional value because the

"assimilable carbon and nitrogen" has already been used up by the herbivore (Holter and Scholtz, 2007).

With respect to dry feeder species of *Pachysoma* (MacLeay) species (dry feeders), however, the intractable food sources rejected by wet-dung feeding beetles such as plant fragments, detritus and dry dung form an integral part of their diet (Holter and Scholtz 2011). Even though the 13 known *Pachysoma* species (which are all flightless) are assumed to have evolved from a wet-dung feeding ancestor (figure 1.1) (Sole et al., 2005; Forgie et al., 2006; Sole et al., 2007), they are a coprophagous taxon that does not exploit a wet dung diet and has subsequently evolved cutting and grinding structures that are adapted for a drier diet (Holter and Scholtz, 2011). Lacking the ability to fly and being geographically confined to arid environments such as deserts (Harrison et al., 2003; Davis et al., 2008), *Pachysoma* species have had to undergo the fore-mentioned morphological adaptations to their mouthparts (Holter and Scholtz, 2011) and changes to their gut structure that enable them to process dry food efficiently (Holter and Scholtz, 2013) which involved elongating the hindgut for the effective mechanical digestion of dry food (Holter and Scholtz, 2013).

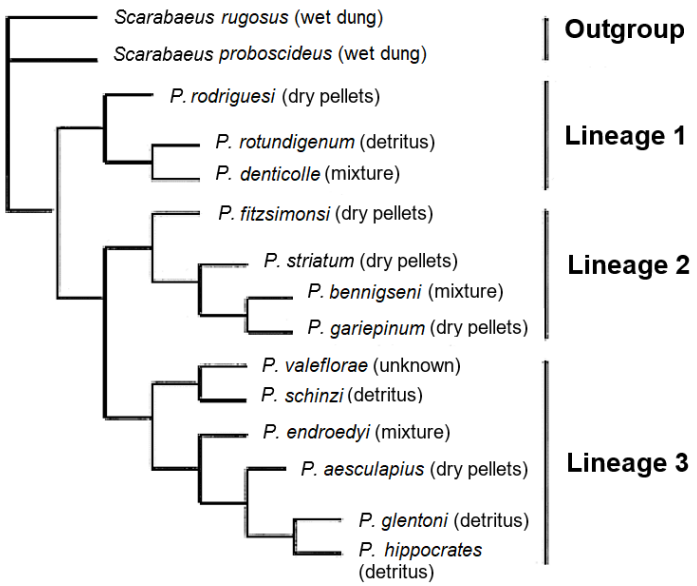


Figure 1.1. Adapted phylogenetic tree of *Pachysoma* species based on Sole et al. (2005) and Davis et al. (2008). The food preferences of each species are in brackets. Mixture represents a combination of detritus and dung pellets of "small ruminants" (Harrison et al., 2003; Holter et al., 2009). Source of phylogram: Holter and Scholtz (2011).

To establish how *Pachysoma* beetles derive nourishment from this dry diet, Holter et al. (2009) studied the nutritional ecology of *Pachysoma glentoni* (which primarily feeds on detritus). This tested three hypotheses: (1) *Pachysoma* beetles rehydrate plant litter by storing it in below the ground moisture line, (2) moist plant litter stimulates the production of fungi and (3) fungi is the major food source of these beetles because their *Scarabaeus* relatives target minute particles which include fungal matter (Holter et al., 2009; Scholtz et al., 2009).

These hypotheses were, however, refuted as Holter et al. (2009) revealed that (1) there is no evidence of water absorption by the plant litter (2) there was no sign of any fungal growth and (3) the plant litter that *P. glentoni* feeds on is of good quality because the average carbon:nitrogen (C/N) ratio (a food quality index for heterotrophic animals (Dorgelo and Leonards, 2001)) is 35, which is almost the same quality as found in terrestrial plant species consumed by herbivores (Holter et al., 2009). The beetles were able to assimilate 60% of nutrients in the plant litter (Holter et al., 2009).

Holter and Scholtz (2011) later studied the morphology of the cutting and grinding structures in *Pachysoma* mouthparts suggesting that these structures are newly evolved and aid in digestion by mechanically breaking-down the food (Holter and Scholtz, 2011).

1.1.2 Exploration of alternative diets

While herbivores may have afforded dung beetles an abundant food source, some geographical areas may have experienced a shortage in dung which may have pressured dung beetles to explore alternative diets (Scholtz et al., 2009). This is true for wet feeding Neotropical species belonging to the genera: *Coprophanaeus, Deltochilum* and *Canthon*, which switched to carrion (Carrion feeders) (Scholtz et al., 2009) following the extinction of mega-herbivores during the Pleistocene era (Halffter and Matthews 1966).

The mechanisms that wet-dung feeders employ when feeding on the liquid component of dung provided a pre-adaption to alternative liquid-based food sources such as carrion and decaying fruits (Scholtz et al., 2009). Hence, the transition to carrion might have proved to be easier than the morphological transformation that *Pachysoma* had to undergo when transitioning to dry food.

In Africa, dung beetles that feed on vertebrate carrion are rare because the animal remains are often consumed by a large number of scavengers before the beetles can locate the food (Halffter

and Matthews 1966). Thus, dung beetles from the *Scarabaeus* subgenera *Sceliages* (Westwood) (Scholtz et al., 2009) have resorted to being obligate millipede feeders (Davis et al., 2008). *Scarabaeolus* (Balthasar) species have been found to exploit both dung and invertebrate carrion (Davis et al., 2008).

1.1.3 Selective feeding

The basis of foraging by organisms (including insects) is to obtain key nutrients required to promote growth and development and reproduction for the subsistence and propagation of the organism (Behmer, 2009). Depending on the food source, insects are able to acquire a suitable mixture and equilibrium of essential nutrients in the form of carbohydrates, amino acids, vitamins, minerals, phospholipids and fatty acids (Chapman, 1998; Schoonhoven et al., 2005).

Many insects have devised strategies of attaining nutrient levels required to meet their dietary requirements; some of which include but not limited to (1) the consumption of large amounts of food (Behmer, 2009) and (2) selective feeding. When afforded the opportunity, insects lean towards feeding selectively (Bernays et al., 1994; Chambers et al., 1996; Singer and Stireman, 2001) and one way of doing this is through a feeding behaviour known as “dietary self-selection” (Richter et al., 1938), sometimes referred to as "optimization of the nutrient mix" (Westoby, 1974). Dietary self-selection explains how a variety of organisms consume food in a way that promotes a “favourable balance of nutrients” (Waldbauer et al., 1984). To show the occurrence of dietary self-selection in insects, various insects such as beetles (*Tribolium confusum*) Jacquelin du Val (Tenebrionidae) (Waldbauer and Bhattacharya, 1973) and (*Harmonia axyridis*) Pallas (Coccinellidae) (Soares et al., 2004)), grasshoppers (*Locusta migratoria*) Linnaeus (Acrididae) (Chambers et al., 1993), and ants (*Ectatoma ruidum*) Roger (Formicidae) (Cook and Behmer, 2010) have been studied. These indicate that the food they choose encompasses an equilibrium of carbohydrates and proteins that the insect can digest (Morales-Ramos et al., 2011).

Selective feeding strategies have also been observed in filter feeders, which are defined as organisms that possess a sieve-like feeding apparatus that functions to procure food by filtering “suspended matter” from freshwater and marine bodies (Jørgensen, 1966). These types of feeders have undergone a series of morphological adaptations that essentially enable them to “sieve” food before its consumption (Wallace and Merritt, 1980). The morphological adaptations include

filtering structures such as bristles, fans, silk secretions (Wallace and Merritt, 1980) and keratinous plates of baleen (Goldbogen et al., 2017). A few examples of organisms that exhibit filter feeding include insects such as *Isonychia* nymphs, caddisflies and black fly larvae (Wallace and Merritt, 1980), small bodied animals such as sponges (Leys and Eerkes-Medran, 2006), bivalve molluscs (Soto and Mena, 1999) and fish, and large bodied animals such as flamingos (Zweers et al., 1995) and baleen whales to name a few (Goldbogen et al., 2017).

Dung beetles (Scarabaeidae: Scarabaeinae) are also known to selectively feed and self-regulate their nutrient levels to meet their dietary requirements by means of particle feeding (Holter, 2000; Holter et al., 2002; Holter and Scholtz, 2007). According to Holter and Scholtz (2005), selective feeding behaviour exhibited by dung beetles is perhaps an adaptation to a food source that is fundamentally a combination of high volumes of water and intractable, valueless food items (Holter and Scholtz 2005).

The selection of dung particles allows dung beetles to concentrate the quantity of the nutritious food items in dung (Holter and Scholtz, 2005) in order to elevate the level of nutrients obtained by many folds (Holter and Scholtz, 2007; Madzivhe et al., 2020). The concentration of nutrients is usually attained by eliminating large portions of worthless plant material before ingestion (Holter, 2000; Holter et al., 2002; Holter, 2004).

1.1.4 Particle size selection and nutrient manipulation of dung

Selective feeding behaviour exercised by dung beetles brings into effect a limitation on the particle sizes of dung that can be ingested (Madle, 1934; Miller, 1961; Hata and Edmonds, 1983; Holter, 2000). Hata and Edmonds (1983) proposed a size range of ingested particles between 2 and 200 µm for adult dung beetles. However, particle size studies by Holter (2000), Holter et al. (2002), Holter (2004), Holter and Scholtz (2005), Holter and Scholtz (2007) and Madzivhe et al. (2020) found particle size ranges that differed substantially from those.

Holter (2000) approximated the "maximum diameter of ingested particles" (MDIP) to be between 5 and 25 µm for six small-bodied endocoprids Aphodiinae (Scarabaeidae) dung beetle species. This was determined by blending latex balls of known diameters with dung, feeding the mixture to *Aphodius* species (Scarabaeidae, Aphodiinae): *A. fossor* (Linnaeus), *A. contaminatus* (Herbst), *A. rufipes* (Linnaeus), *A. erraticus* (Linnaeus), *A. ater* (Degeer) and *A. fimetarius* (Linnaeus) and then measuring the ingested latex balls located in the mid-gut against those that

were not ingested in order to make the MDIP approximation. This method of particle size approximation would later be used in all the particle size studies by Holter et al. (2002), Holter (2004), Holter and Scholtz (2005), Holter and Scholtz (2007).

The MDIP estimates made by Holter (2000) proved to be useful in highlighting the presence of a filtering system, however, as far as understanding the dynamics of particle size, the estimates are not that informative as they do not consider the effects that a wider range of taxa and body size, ecological groups and dung preferences might have on the MDIP. Therefore, Holter et al. (2002) comprehensively evaluated the relationship between the MDIP and the aforementioned variables by determining the particle size ranges of 12 Scarabaeinae dung beetle species belonging to the tribes: Onitini, Onthophagini, Coprini, Oniticellini and to the paracoprid and endocoprid ecological groups that had a broader body size range. The outcome of the study revealed a particle size range of 8 to 50 µm where particle size increased slightly with increasing body size (Holter et al., 2002). The study also showed that taxon, ecological groups and dung preference did not have a considerable impact on the MDIP (Holter et al., 2002).

However, Holter and Scholtz (2005) later found that nesting behaviour does in fact have an impact on the MDIP because their study on 11 telecoprid Scarabaeinae dung beetle species from the tribes Scarabaeini, Gymnopleurini, Sisyphini, with wide-ranging body sizes, presented a particle size range of 4 to 85 µm. The study showed that telecoprids and endocoprids of similar body sizes select particles of different sizes where endocoprids were found to select smaller dung particles than telecoprids. Holter and Scholtz (2005) concluded that (1) the extent of selectivity by endocoprids could be attributed to the greater availability of food than to the telecoprids, that must quickly form a dung ball and roll it away and (2) the inclusion and subsequent ingestion of large particles by large telecoprids resulting from difficulties in rejecting large particles during the formation of food dung balls using their large heads and legs. Thus, smaller telecoprids were found to select smaller particles than large telecoprids. Holter and Scholtz (2007) later suggested a particle size range that is typical for many adult dung beetles that is between 2-5 and 130 µm after having observed the MDIP of the large telecoprid *Circellium bacchus* Fabricius to be between 125 – 130 µm (P. Holter and L. Stenseng, unpublished) and concluded that the nutritional ecology and the body size of dung beetles has a significant effect on the MDIP (Holter and Scholtz, 2007; Holter, 2016).

Madzivhe et al. (2020) directly measured the size of all the ingested dung particles in the foregut of the telecoprid dung beetle *Scarabaeus goryi* (Castelnau) and found that these beetles ingest an overall particle size range that is between 1.4 and 2000 µm compared to that of raw dung that is between 2 and 3000 µm. The maximum volume (9%) of the foregut sample of *Scarabaeus goryi* consisted of particles that ranged between 30 and 180 µm compared to that of raw dung that is between 180 and 250 µm. The mean maximum particle size diameter found in the foregut was around 1500 µm in contrast to that of raw dung that was found to be around 3000 µm. This confirms that dung beetles do indeed select small dung particles, that the approximations of Holter underestimate the actual size of ingested dung particles.

Nonetheless, these studies were instrumental in pointing out that both small and large dung beetles regardless of their phylogenetic origins all possessed a similar feeding system that is biased towards retaining minute particles of dung and filtering out hard – to – digest large portions of plant material (Holter et al., 2002). Furthermore, the findings from these studies have raised a very pertinent question. How does the selection of dung particles relate to nutrient content?

Holter and Scholtz (2007) and Madzivhe et al. (2020) both showed that particle size has an inverse relationship with nutrient levels i.e minute particles have a higher nitrogen content than larger particles. Holter and Scholtz (2007) measured the effects of varying MDIPs on dung nitrogen content and C/N ratios by sieving dung into three fractions of differing particle size ranges (0 – 20 µm, 0 – 106 µm and bulk dung) representing different types of selective feeding on four dung types (elephant, rhino, buffalo and sheep). Nitrogen concentrations of the 0 – 20 µm fraction were almost double the concentration of bulk dung. The C/N ratio decreased with selective feeding in contrast to the larger C/N ratio of bulk dung probably because the carbon and nitrogen available to the dung beetles originates from microbial matter (Holter and Scholtz, 2007).

Madzivhe et al. (2020) directly measured the nitrogen content from foregut samples of the dung beetle *Scarabaeus goryi*. He found that the nitrogen content increased from 1.40% (dung sample with mean particle size of 240 µm) to 5.14% (foregut sample with a mean particle size of 80 µm) with a C/N ratio (10) that was three times lower than the C/N ratio (35) of raw dung.

1.2 Basic morphology of dung beetle mouthparts

Members of the superfamily Scarabaeoidea possess mouthparts that are characteristically distinctive of insects since they contain sclerotized mandibles for the basic purpose of incising and pulverizing food (Davis et al., 2009). However, dung beetles have substantially diverged from this rudimentary morphological configuration (Davis et al., 2009).

As a result of their adaptation to dung, dung beetle mandibles typically comprise a soft and membranous mandibular incisor lobe and a somewhat sclerotized molar lobe (Hata and Edmonds, 1983) (figure 1.2). The incisor lobe is dorsoventrally flattened and fitted with dual lines of bristle-like structures known as the prosthecal comb (pc) on its innermost boundary (figure 1.2) occurring on both the dorsal and ventral sides of the mandible (Miller, 1961), and a dorsal row of setae (dr) occurring medially on the incisor lobe (figure 1.2) (Miller, 1961; Hata and Edmonds, 1983). The tip of the incisor lobe holds a closely packed brush of setae known as the apical fringe (fr) (Hata and Edmonds, 1983) (figure 1.2). These hairs are regarded as filtering setae and involved in filtering out and excluding large particles of dung (Holter et al., 2002).

The molar lobes are encased in a cavity known as the cibarium (figure 1.3) and are asymmetrically shaped where the right mandible possesses a convex surface and the left mandible a concave surface (Holter, 2000) (figure 1.4.1). The molar lobes are positioned in such a way that the blind/closed side of the molar faces the distal and external part of the mouth whereas the open side of the mouth faces the inner part of the mouth and the pharynx (figure 1.3) (Hata and Edmonds, 1983).

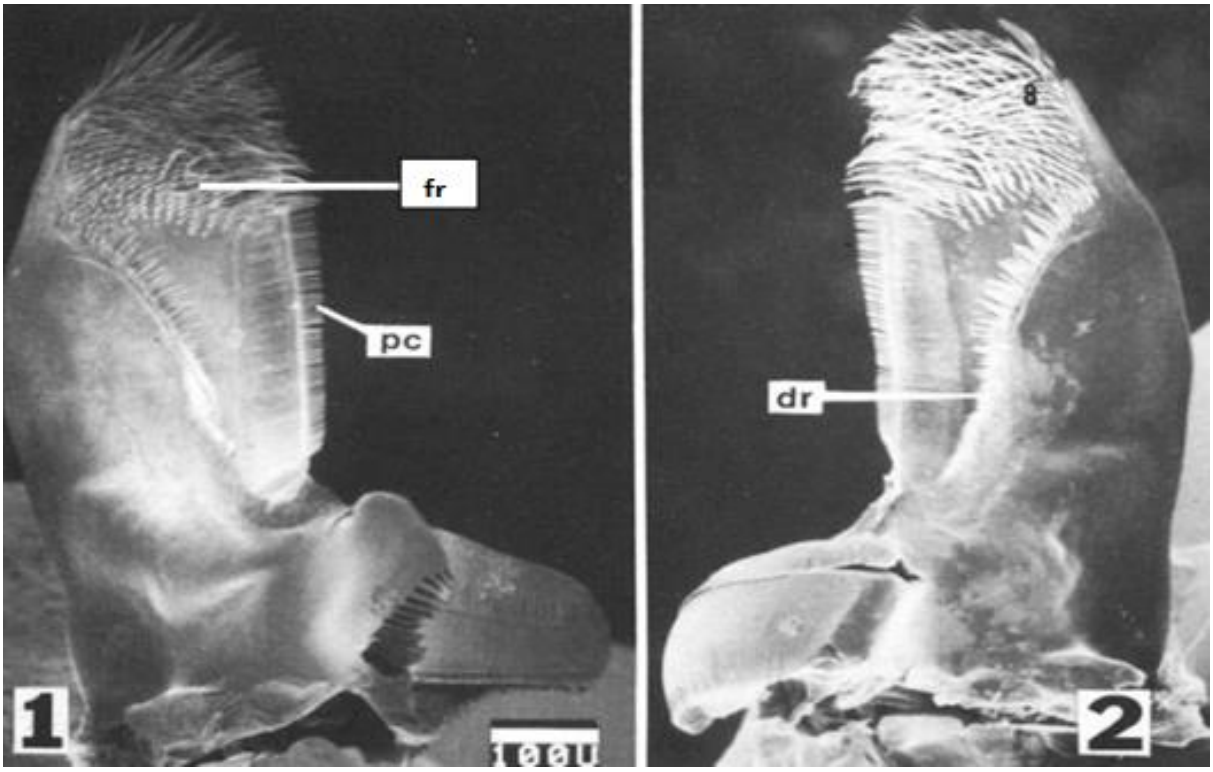


Figure 1.2. *Canthon pilularius.* 1. Left mandible, dorsal view (pc = prosthecal comb); fr = apical fringe. 2. Right mandible, dorsal view (dr = dorsal row of setae). Source: Hata and Edmonds (1983).

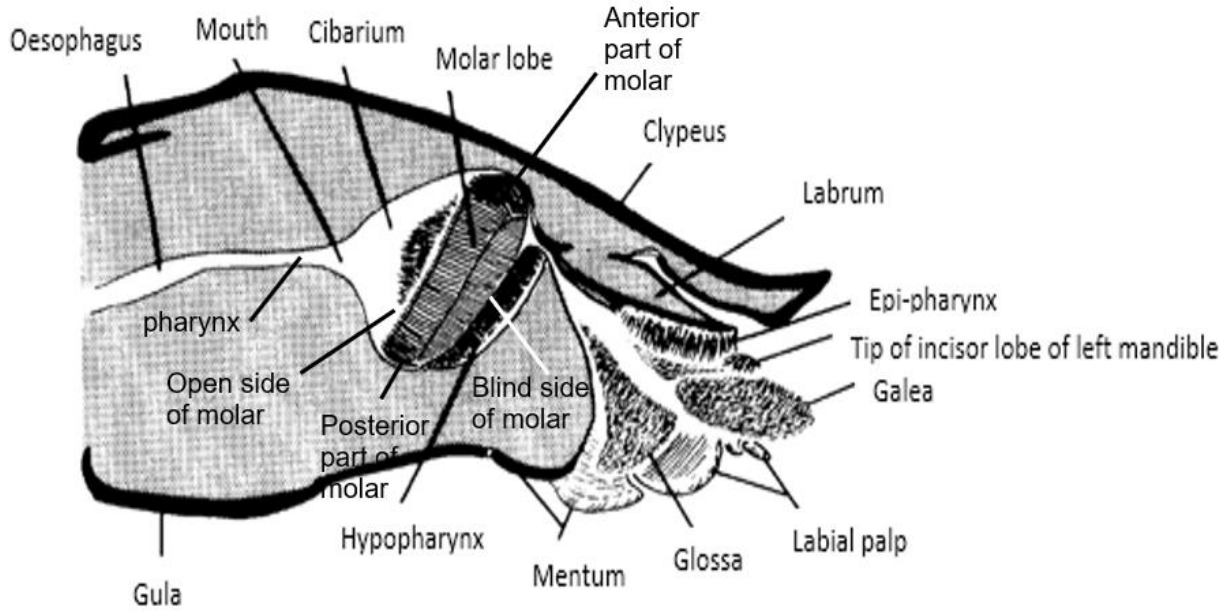


Figure 1.3. *Canthon pilularius.* Adapted semi-diagrammatic sagittal section through head. Source: Hata and Edmonds (1983).

A single molar lobe consists of many crosswise molar ridges (figure 1.4.1 – 3) that are divided by furrows known as filtration channels (figure 1.4.4) (Madle, 1934; Holter 2000). According to Hata and Edmonds (1983) and Miller (1961), each molar ridge is partitioned into “tightly packed scalloped blocks” known as triators (figure 1.4.3).

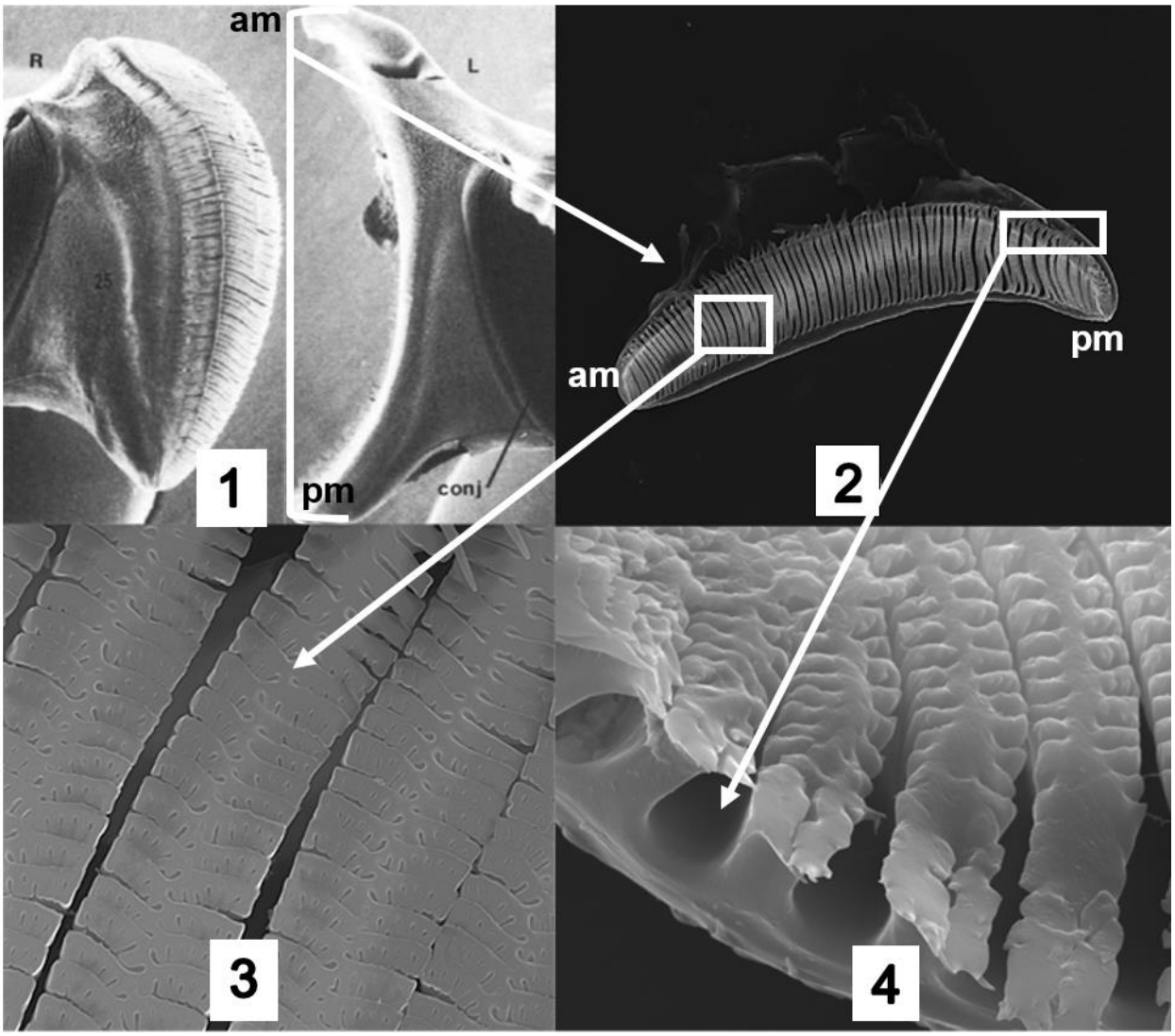


Figure 1.4. *Canthon pilularius*, 1. Frontal view of the right (R) and left (L) molar lobes. Source: Hata and Edmonds (1983). 2. *Euoniticellus intermedius*, mesal view of the left molar lobe. 3. *Euoniticellus triangulatus*, tritors of the mandibular ridges. 4. *Euoniticellus triangulatus*, filtration channels. Abbreviations: am, anterior region of the molar lobe; pm, posterior region of the molar lobe. Source: Tumi Mathikge

Apart from the mandibles apparently being the main mechanism underlying particle feeding, the mouth also possesses more membranous structures such as the epipharynx (figure 1.5) that probably contribute to particle feeding. The epipharynx is transparent, roughly square-shaped, and has setae across its surface and is located horizontally beneath the clypeus (figure 1.5) (Lopez-Guerrero, 2011). The epipharynx's position above the mandibles indicates some sort of interaction between both structures when dung particles are being filtered (figure 1.6) (Hata and Edmonds, 1983). The setae across the surface of the epipharynx are also suggested to have a "gustatory and tactile" function (Miller 1961).



Figure 1.5. 1. Ventral view of the epipharynx of *Chalcocopris hesperus* Oliver which mainly feeds on wet dung but has been observed feeding on dead vertebrates and rotten fruits (Rossini and Vaz-De-Mello, 2015). Image source: Tarasov and Génier (2015). 2. Ventral view of the epipharynx of *Eucranium simplicifrons* Fairmaire that feeds on dry dung pellets of small mammals. Image source: Palestrini et al., (2020).

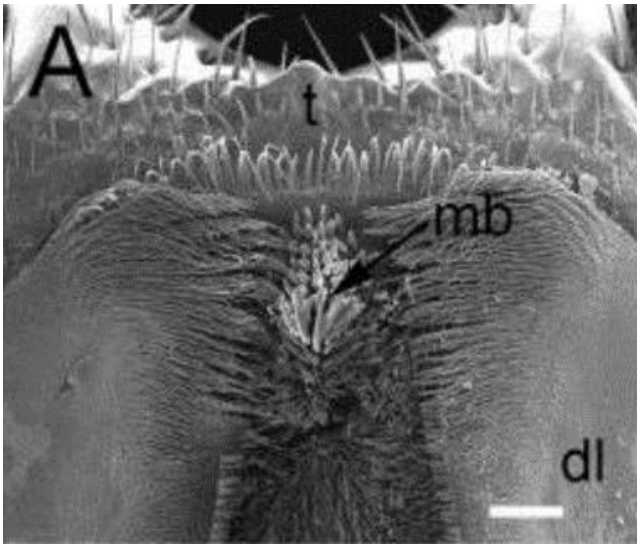


Figure 1.6. Ventral view of the mouthparts of *Scarabaeus rugosus*. Abbreviations: dl, distal lobe of the mandible; mb, median brush of the epipharynx; t, median tooth on the median ridge. Source: Holter and Scholtz (2011)

1.3 Hypotheses on the mode of action of dung beetle mouthparts during feeding

While several studies on the feeding ecology of dung beetles have documented what they eat (Holter and Scholtz, 2007) and the specialization of their mouthparts (Miller, 1961; Hata and Edmonds, 1983; Nel and Scholtz, 1990), how these mouthparts operate is still not completely understood (Holter, 2000). Three hypotheses have been proposed (two of which have been reviewed by Holter (2000)) to explain how the mouthparts function during particle feeding (Holter et al., 2002).

The first hypothesis presented by Madle (1934) suggests that the external components of the mouth such as the maxilla, galeae and hirsute laciniae help gather small portions of dung that are then transferred to the mandibles located inside the mouth. The dung is subsequently transported to the mandibular molars where a heterogeneous solution of water and minute particles of dung are extracted through squeezing (Holter et al., 2002) and later flows into the pharynx via the filtration channels (Holter et al., 2002). However, given that the filtration channels are of a particular width, only dung particles of a diameter that does not exceed the channel width can presumably pass through (Holter et al., 2002). The remaining larger constituents of dung are expelled out of the mouth (Madle, 1934). The hypothesis has since then been disproved by Holter (2000). Holter's study on six *Aphodius* dung beetle species revealed that the maximum diameter of ingested particles (MDIP) (Holter, 2000) found in the beetle's guts far exceeds the

width of the channels. For instance, he found the channel widths of *A. fimetarius* and *A. fossor*, to be between 3 – 6 µm and 4 – 8 µm (P. Holter, unpublished observation) respectively, whereas dung particles as large as 14 µm and 18 µm respectively, were found in the gut (Holter, 2000).

The second hypothesis proposed suggests that dung is carried into the oral cavity by the incisor lobes and then pulverized by the grinding surfaces of the mandibular molars composed of the triators that supposedly break down large particles into smaller particles (Miller, 1961; Holter et al., 2002) which are ingested (Miller 1961, Hata and Edmonds, 1983). According to Holter (2000), this idea has been accepted by Halffter and Matthews (1966), Macqueen (1975), Halffter and Edmonds (1982), Scholtz (1989), Cambefort (1991) and Mathison and Ditrich (1999).

Support of this hypothesis might have stemmed from evidence derived from a study conducted by Miller et al. (1961) who explored the fate of helminth eggs that were fed to dung beetles. The outcome of the study emphasized the damaged condition of the eggs before ingestion as a result of the supposed grinding action of the mouthparts. Holter (2000) tested this hypothesis by feeding *Aphodius* dung beetles a mixture of stained plant fragments with a 50 – 100 µm particle size range, 5 µm latex balls and fresh dung and comparing it to dung mixed with 5 µm latex balls to determine whether the plant fragments would be ground up or not. The grinding up of food ought to result in a larger ratio of minute stained particles (2 -5 µm) to 5 µm latex balls than in the dung but instead the findings of this experiment show the small particles of dung in the gut do not result from the pulverization of large portions of plant material but rather from the filtering action of mouthparts that retain small particles of dung while eliminating the larger particles because the ratio (of stained particles to latex balls) was not different to that of the dung. Furthermore, Holter (2000) reasoned that the nutritional value that would be gained from grinding and consuming the plant material would be minimal because of the insufficiency of the simple gut to break down lignocellulose. The refutal of the second hypothesis was also confirmed by the large particle size range found by Madzivhe et al. (2020) which affirms that dung particles as large as 3000 µm were present in the gut because they were not ground up. Therefore, the second hypothesis, like the first, is improbable (Holter, 2000; Holter et al., 2002).

Since then, a third hypothesis has been put forward by Holter (2000). Based on the experimental findings of Holter (2000), the hypothesis explains how the larger particles of dung are filtered out by the setae of the incisor lobe while the remnants (being the smaller particles) are

concentrated by the molar lobes by means of squeezing whilst releasing superfluous liquid that flows away from the pharynx and out of the mouth (Holter, 2000; Holter et al., 2002; Holter, 2016). This hypothesis has then been accepted by various authors (Davis et al., 2009; López-Guerrero, 2011; Bai et al., 2015).

1.4 Rationale for the study

Despite the research conducted for decades on the feeding dynamics of dung beetles, the exact mechanism of how they feed remains an enigma (Holter 2016). To date, we understand the structural configuration of the mouthparts and some of their functions (Miller, 1961; Hata and Edmonds, 1983; López-Guerrero, 2011; Holter and Scholtz, 2011; Bai et al., 2015). We have recognised that dung beetles are particle feeders (Holter, 2000; Holter et al., 2002; Holter, 2004; Holter and Scholtz, 2005; Holter and Scholtz, 2007; Holter, 2016; Madzivhe et al., 2020) and have become more knowledgeable on what dung beetles eat (Holter and Scholtz, 2007; Holter, 2016; Madzivhe et al., 2020). However, regardless of these breakthroughs, the major knowledge gaps that need to be addressed are those concerning the mode of operation of dung beetle mouthparts, and the processes involved in manipulating a metabolic waste-product into a substrate that dung beetles can subsist on (Holter 2016).

Holter and Scholtz (2007) and Madzhive et al. (2020) made an interesting discovery in uncovering that dung beetles can increase their food's nutrient levels, especially increased nitrogen levels. This idea brought into question how dung beetles elevate nutrient levels during ingestion? It further questions how components of the mouthparts are involved in this selective process?

Considering this, this study has assumed a morphological and quantitative approach to investigate these aforementioned concerns. This study seeks to make inferences on how particle feeding is carried out by the mouthparts through interpretation of the morphology of these mouthparts. This will be achieved by examining and comparing mouthparts of dung beetles adapted to different diets so that the role of the various structural components of the mouth in particle feeding can be inferred by comparison, so that the mode of operation of the mouthparts can be better understood.

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Chapter II: Materials and Methods

2.1 Taxon selection

The 12 species used in this study belong to four different genera (*Scarabaeus*, *Neosisyphus*, *Anachalcos* and *Pachysoma*) and they were chosen to represent three different feeding groups (wet, dry and carrion feeders). *Anachalcos convexus* Boheman, 1857 was selected to represent a generalist feeder because it is an intermediate between the feeding groups (Davis et al., 2008). It was included to understand the anatomical modifications that may result from adapting to a wide-ranging diet. The diet preference, nesting behaviour and geographical information of the species examined is outlined in Table 2.1

Table 2.1: Background information on the biology of the study species. Sources: Harrison et al. (2003), Davis et al. (2008), Scholtz et al. (2009), Daniel et al. (2018). Abbreviations: GMA, geometric morphometric analysis; SEM, scanning electron microscope.

#	Species name	SEM sample size (n)	GMA sample size (n)	Nesting behaviour	Diet Preference	Inhabited climatic zone
1	*Scarabaeus* (*Kheper*) *lamarcki* (MacLeay, 1821)	5	10	Telecoprid	Wet dung	Humid to arid savanna
2	*Scarabaeus* (*Kheper*) *nigroaeneus* (Boheman, 1857)	5	7	Telecoprid	Wet dung	Humid Savanna
3	*Scarabaeus zambesianus* (Péringuey, 1901)	5	10	Telecoprid	Wet dung	Humid to arid Afrotropical
4	*Scarabaeus rusticus* (Boheman, 1857)	Not included in the analysis	10	Telecoprid	Wet dung	Afrotropical (Northern South Africa)
5	*Scarabaeus* (*Scarabaeolus*) *damarensis* (Balthasar, 1965)	5	8	Telecoprid	Wet dung	Kalahari sand
6	*Scarabaeus* (*Scarabaeolus*) *carniphilus* (Balthasar, 1965)	5	5	Telecoprid	Wet dung, carrion	Kalahari sand
7	*Anachalcos convexus* Boheman, 1857	5	10	Telecoprid	Generalist	Dry savanna
8	*Neosisyphus mirabilis* (Arrow, 1927)	Not included in the analysis	3	Telecoprid	Wet dung	Summer rainfall coastal forests
9	*Neosisyphus rubus* (Paschalidis, 1974)	Not included in the analysis	4	Telecoprid	Wet dung	Coastal, moist grasslands and savanna
10	*Pachysoma endroedyi* (Harrison, Scholtz and Chown, 2003)	2	4	Telecoprid, (dragging)	Plant detritus	Arid, Fynbos, Namaqualand
11	*Pachysoma hippocrates* (MacLeay, 1821)	4	6	Telecoprid, (dragging)	Plant detritus	Arid, Fynbos, Namaqualand
12	*Pachysoma striatum* (Castelnau, 1840)	4	4	Telecoprid, (dragging)	Dry dung pellets (rodents)	Arid, Namaqualand

2.2 Field sampling process

The species examined in this study (except the *Scarabaeolus* sub-genus) were all taken from dry specimens housed at the Wits Life Sciences Museum and previously collected from the localities listed in the table below:

Table 2.2: Location data of study species.

Species	**Locality**	**Collector**
Pachysoma hippocrates	Port Nolloth, Namaqualand (29°15'S, 16°53'E)	M. Byrne (2014 – 2018; these are donated samples hence the month of their collection is unknown)
Pachysoma endroedyi	Kommandokraal Farm, Namaqualand (31°30'S, 18°12'E)	
Pachysoma striatum	Strand Fontein, Namaqualand (30°33'S, 17°26'E)	
Kheper lamarcki, *Scarabaeus zambesianus*	Stonehenge Farm, Vryburg, North West Province, South Africa (26°28'S, 24°20'E), November 2017	C. Tocco (November 2017; 2018)
Kheper nigroaeneus *Scarabaeus rusticus,* *Anachalcos convexus*	Wits Pullen Farm, Nelspruit, Mpumalanga Province, South Africa (25°34'S, 31°10'E)	
Scarabaeus (*Scarabaeolus*) *damarensis*	Stonehenge Farm, Vryburg, North West Province, South Africa (26°28'S, 24°20'E)	C. Tocco November 2017
Scarabaeus (*Scarabaeolus*) *carniphilus*		N. Mathikge (November 2018)

The *Scarabaeolus* species were collected from the field by means of baited pitfall traps (figure 2.1). These dung beetles were collected from traps baited with approximately 250g of cattle dung and subsequently preserved in an aqueous solution of 75 % ethylene glycol. The traps were regularly checked in the morning and in the evening. Some individuals were also found and collected from dung pats.



Figure. 2.1. Pitfall trap baited with cow dung; photographer: Macphee Madzhive.

2.3 Morphological Analyses

2.3.1 Scanning Electron Microscope (SEM) analysis

Aim 1: Understand the morphology in order to infer the mode of operation of dung beetle mouthparts.

Objective 1: Use microscopy (light and scanning electron microscope (SEM)) to obtain qualitative data in the form of micrographs depicting morphological features of dung beetle mouthparts involved in the particle feeding process.

Objective 2: Compare and describe the morphology of dung beetle mouthparts across feeding groups.

Aim 2: Understand the relationship that particle size selection and nutrient levels of dung have with dimensions of the various structural components of the mouthparts

Objective 3: Use Image J (measuring software) (Rasband, 2012) to obtain quantitative data of the basic dimensions (length, height and width) of the structural components of the mouthparts from SEM micrographs and relate these to particle size selection of dung by the different dung beetles.

Pre-dissection measurements

The species used for the SEM analysis were: *K. lamarcki, K. nigroaeneus, S. zambesianus, S.* (*Sc.*) *damarensis, S.* (*Sc.*) *carniphilus, A. convexus, P. endroedyi, P. hippocrates and P. striatum.*

Two measurements were used as proxies for body size: the pronotum width (mm) (the widest part of the pronotum was measured each time and no objective method was applied) (figure 2.2) and the dry weight (g). The pronotum width was measured using Cocraft digital callipers (0-150mm) and was used in determining relative measures of the mouthpart structures being studied so that a comparison between absolute and relative values could be made. The relative measures were calculated as ratios between the structure of interest and body size. The dry weights were taken after the specimens were oven dried at 60°C for 72 hours.

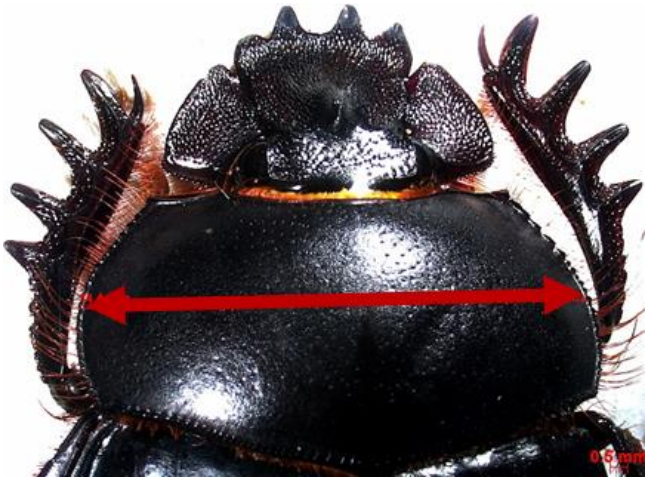


Figure 2.2. Dorsal view of the pronotum width of *Scarabaeus zambesianus.* This method of measuring was applied across all the species used in this study.

SEM specimen preparation

Dried samples (see table 2.1 for species sample sizes) were rehydrated by boiling them in distilled water for approximately 8-10 minutes (depending on the body size of the individual). The mandibles and epipharynx were dissected out of the head capsule and immersed in 5% potassium hydroxide for 4-10 minutes (at room temperature) to disintegrate any unwanted membranous and fatty tissue, then rinsed in distilled water for 5 minutes and dehydrated in a series of 75%, 95% and 99% ethanol. The mandibles were then air dried in an oven (60°C) for

approximately 24 hours, mounted on aluminium stubs using carbon adhesive and then coated with two layers of carbon and one layer of gold palladium. The epipharynx was not air-dried (to avoid structural damage from desiccation) but immediately mounted on the stubs following the dehydration procedure with ethanol.

Scanning electron micrographs

The images were taken using the Tescan Vega 3 SEM and the FEI Nova Nanolab 600 SEM. From these micrographs, ImageJ (version. 1.50i) (Rasband 2012) was used to measure several mouthpart dimensions to obtain quantitative data (figure 2.3-2.6).

Measures were taken from both the concave and convex mandibles and included the mandible length (ml), the mandible width (mw), the prosthecal comb length (pcl) and the prosthecal comb width (pcw) (figure 2.3). The final length measure of the mandible's dimensions was the mean between the concave and convex mandible measures since the paired Student's T-test showed there was no significant difference between the left and right dimensions. However, because this study used small and unequal sample sizes, this test might have not been statistically powerful enough to capture variance or any meaningful differences.

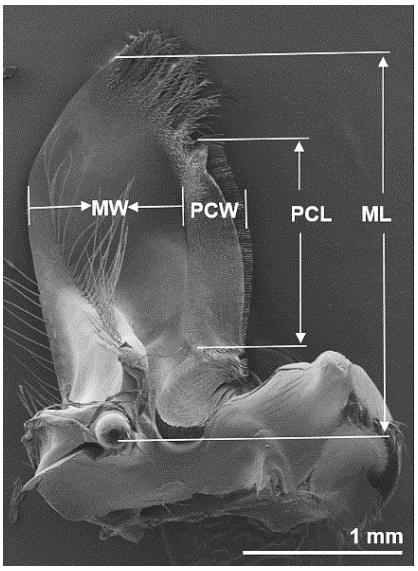


Figure 2.3. *Anachalcos convexus.* Ventral view of the convex mandible depicting the dimensions measured: mw, mandible width; pcw, prosthecal comb width; pcl, prosthecal comb length; ml, mandible length.

The dimensions that were measured from the molar lobe were as follows: the filtration channel width (cw) and the molar ridge height (figure 2.4). The dimensions of the filtration channel widths and the molar ridge height between the concave and convex molar lobes were significantly different, thus were analysed separately. The anterior and posterior filtration length measures were also significantly different to each other and also analysed separately. For each individual within a species, the size of each dimension was calculated as the mean of five length measures.

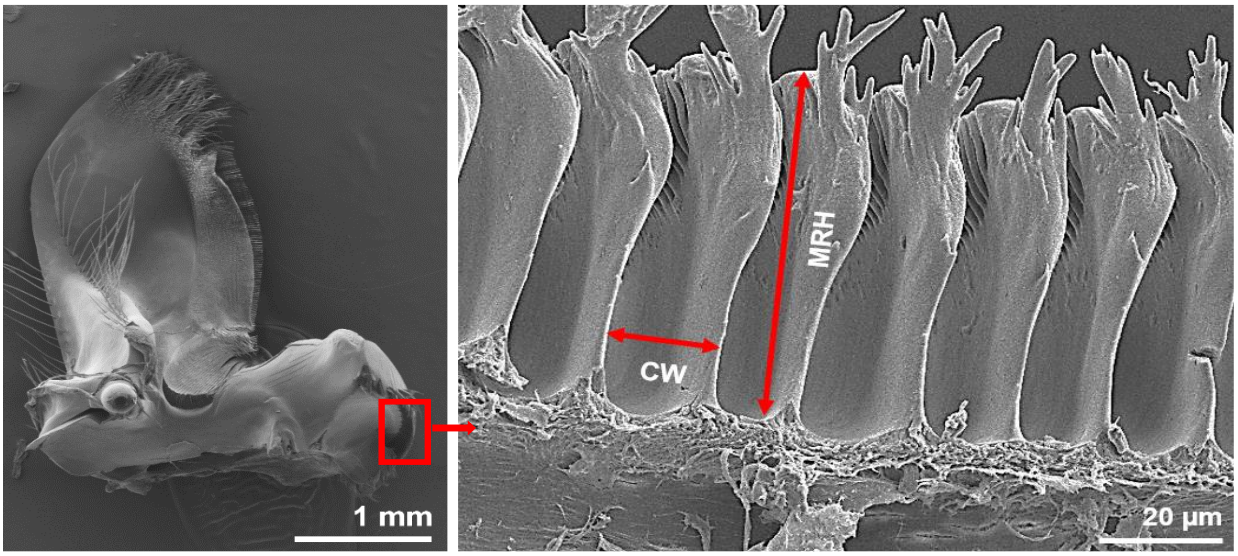


Figure 2.4 *Anachalcos convexus.* Length measures of the filtration channels of the molar lobe and their location on the mandible; cw, filtration channel width; mrh, molar ridge height.

The gaps between the bristles of the distal comb were measured (figure 2.5) as were the gaps between the bristles of the prosthecal comb (figure 2.6). The length of the bifurcations of prosthecal comb (represented by the red dots (figure 2.6)) were also measured. For each individual within a species, the mean of five length measures was calculated. The dimensions of the distal comb, prosthecal comb and bifurcations of the left and right mandible of each individual within a species were not significantly different and were thus calculated as a mean.

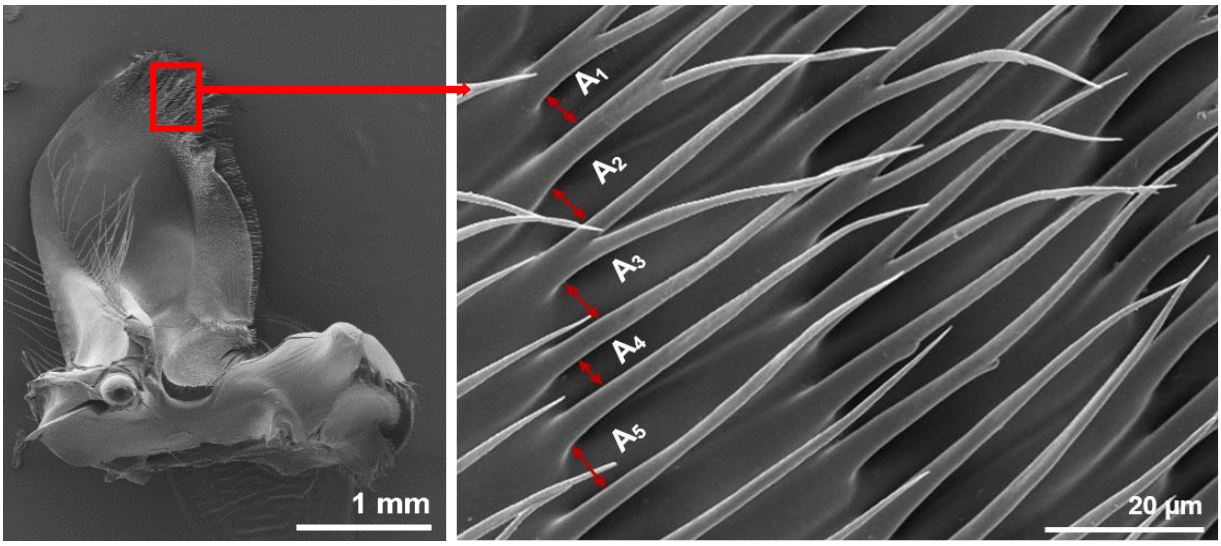


Figure 2.5 *Anachalcos convexus.* Dorsal view of the distal comb showing the length measures A_1 – A_5 of its dimensions.

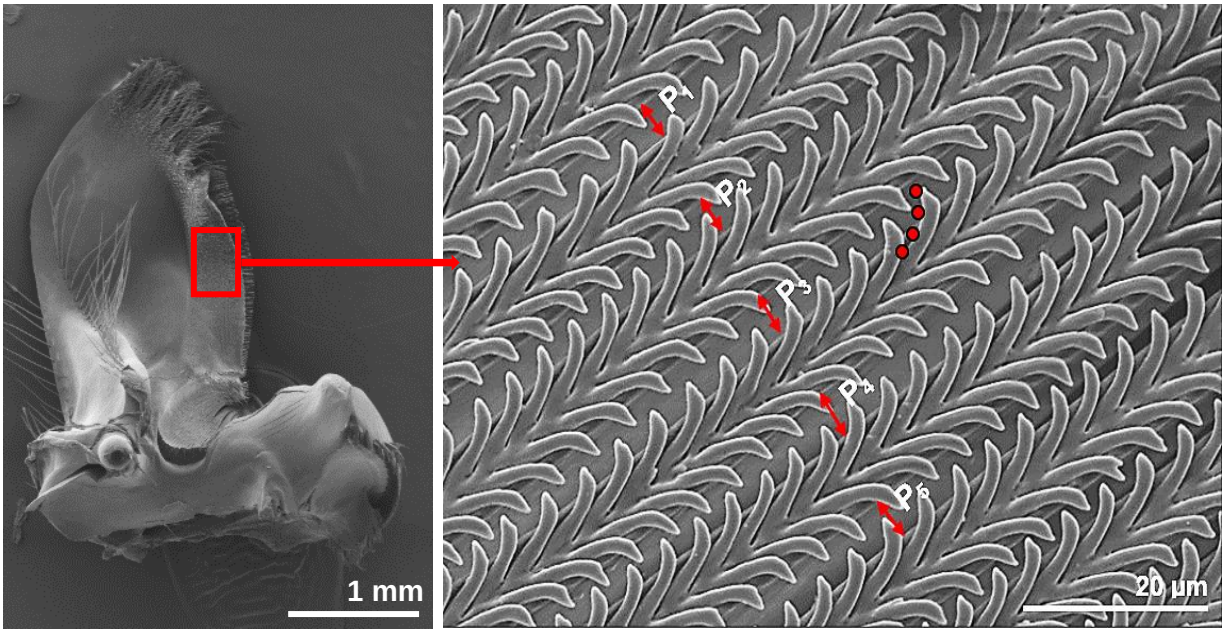


Figure 2.6. *Anachalcos convexus.* Dorsal view of the prosthecal comb showing the length measures (P_1-P_5 of its dimensions.

Statistical analysis

SEM images were measured in ImageJ. The mean values of the dimensions were analysed using statistical software: Statistica version 8.0.360.0 (Weiß, 2007), testing for normality using a Shapiro-Wilk test, then using an ANOVA and Tukey HSD test for parametric data, or a Kruskal

Wallis H test for non-parametric data, to determine the significant differences of the absolute and relative values (although using ratio data is complex and statistically tricky (Curran-Everett, 2013)) of the following variables: mandible width and length, filtration channel width and ridge height of the molars, prosthecal comb width and length of the incisor lobe, apical fringe width of the incisor lobe, and bifurcation of prosthecal comb across ten species. These parameters were measured to determine and understand the maximum size of dung particles that can potentially pass through these structures.

2.3.2 Geometric morphometric analysis (GMA)

Aim: To determine whether the structure of dung beetle mouthparts is influenced by the feeding habits of each species or by their phylogenetic origins

Objective 1: Conduct a GMA on the epipharynx of selected dung beetle species using landmark based morphometrics (Bookstein, 1991; Tatsuta et al., 2018). And simplify and analyse the quantified shape data using multivariate analyses to understand the taxonomic and behavioral relationship that these species have within their feeding groups.

GMA specimen preparation

The species used for the GMA analysis were: *K. lamarcki, K. nigroaeneus, S. zambesianus, S.* (*Sc.*) *damarensis, S.* (*Sc.*) *carniphilus, A. convexus, P. endroedyi, P. hippocrates, P. striatum*. *Neosisyphus* species (*Neosisyphus mirabilis* and *Neosisyphus rubus*) were included in this analysis to confirm whether taxonomy or feeding habits influences mouthpart morphology. Data on *Neosisyphus* species was acquired from images provided by C. Tocco.

The steps taken in removing, cleaning, degreasing and dehydrating the epipharynx for the GMA were identical to the SEM preparation methods, except after dehydration, the epipharynx was immersed in xylene for 5 minutes, mounted onto a glass slide using DPX mountant and then covered with a glass coverslip. A Zeiss, Discovery V12 stereo microscope was used to capture images for the GMA. The magnification range for large and medium sized dung beetles was between 23x and 34.5x using the 0.63x objective. The magnification for small beetles was 70x and 71x using the 1.5x objective.

Landmarks and data analysis

For landmark-based morphometrics, the shape of the structure of interest was captured, represented by sets of co-ordinates from each landmark site (Bookstein 1991). Landmarks were selected based on points that could be easily identified and showed homology across species. Only one side (right side) of the epipharynx landmarks were used because the proximal part of the epipharynx is bilaterally asymmetrical.

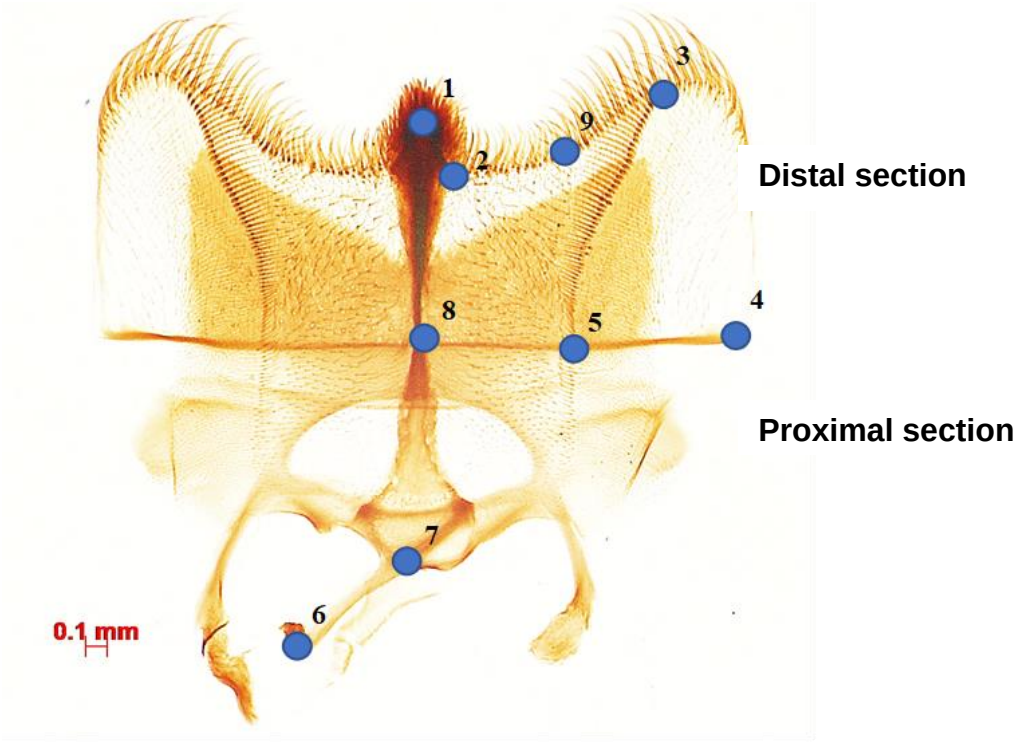


Fig 2.7. *Anachalcos convexus.* Light micrograph depicting locations of the geometric morphometric landmarks on the ventral view of the epipharynx. Landmarks were identified as follows: 1. Tip of the medio-anterior process/epitorma covered by the median brush, 2. Starting point of the apical fringe from the median brush 3. Margin of the chaetopariae, 4. Lateral margin of the clypeo-labral suture, 5. Site where the chaetopariae and clypeo-labral suture converge, 6. Tip of the posterior median tormal process, 7. Base of the posterior median tormal process, 8. Site where the clypeo-labral suture and anterior median process converge, 9. Midpoint between the inception of the apical margin from the median brush and the margin of the chaetopariae.

Two-dimensional light micrographs/images (JPEG format) were opened in the program tpsUtil version 1.78 (Rohlf, 2019) and transformed into a tps (thin-plate spline) format. TpsDig version 2.31 program (Rohlf, 2017a) was then used to place and digitize the landmarks onto the images. GMA is only concerned with analysing shapes of objects/structures irrespective of their size

differences, thus a Generalised Procrustes Analysis (GPA) (Rohlf and Slice, 1990) was carried out using Morpho J 1.06d (Klingenberg 2011) to align the landmarks of each specimen and remove the effects of size, orientation and translation so that only the shape of the epipharynx was analysed regardless of its size across species. The shape space variation (tangent vs Procrustes distance) of the epipharynx was tested using tpsSmall version 1.34 (Rohlf, 2017b). This variation must be small enough to give a good approximation of the tangent space to the Kendal shape space in order to proceed with subsequent geometric morphometric analyses. A principal component analysis was carried out to provide a visual and multivariate description of the shape variation of the epipharynx. A Discriminant function analysis was subsequently performed using Morpho J 1.06d (Klingenberg, 2011) to determine the significant difference of the mean shape of the epipharynx across the feeding groups and species.

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Chapter III: Results

3.1 Body size differences across species

The body weights and the body size of the beetles (maximum pronotum widths) varied across species (Table 3.1). The body weights and body size ranges per feeding groups are as follows respectively: wet feeders (0.04±0.00 - 1.08±0.30 g; 7.72±0.38 - 20.11±1.97 mm), dry feeders (0.17±0.02 - 0.77±0.07 g; 13.12±0.84 - 18.29±0.79 mm), the carrion feeder (0.06±0.01 g; 7.49±0.40 mm) and the generalist feeder (0.51±0.13 g; 12.57±1.07 mm). Data on the body weights and size for different species is summarised in table 3.1.

Table 3.1: Dung beetle species and their respective feeding groups, tribes and body size proxies. The body weights (dry weight) and body size (pronotum widths) are means±SD based on 2 – 5 individuals per species (cf. table 2.1 for sample sizes).

Species name	Feeding group	Tribe	Dry weight (g)	Pronotum width (mm)
K. lamarcki	Wet feeder	Scarabaeini	1.08±0.30	20.11±1.97
K. nigroaeneus	Wet feeder	Scarabaeini	0.40±0.10	17.32±1.01
S. zambesianus	Wet feeder	Scarabaeini	0.63±0.12	16.87±1.80
S. (*Sc.*) *damarensis*	Wet feeder	Scarabaeini	0.04±0.00	7.72±0.38
S. (*Sc.*) *carniphilus*	Carrion feeder	Scarabaeini	0.06±0.01	7.49±0.40
A. convexus	Generalist feeder	Canthonini	0.51±0.13	12.57±1.07
P. endroedyi	Dry feeder	Scarabaeini	0.31±0.04	14.22±0.21
P. hippocrates	Dry feeder	Scarabaeini	0.77±0.07	18.29±0.79
P. striatum	Dry feeder	Scarabaeini	0.17±0.02	13.12±0.84

3.2 Microscopy

Detailed descriptions of dung beetle mouthparts have been previously given by various authors (Miller, 1961; Hata and Edmonds, 1983; López-Guerrero, 2011; Holter and Scholtz, 2011; Bai *et al*., 2015), therefore, this study is primarily concerned with describing structures that are involved in particle feeding and comparable across different species and feeding groups. The mouth part terminology used in this study follows that of Nel and Scholtz (1990), Dellacasa and Dellacasa (2006) and Holter and Scholtz (2011).

3.2.1. Mandibles

The mandibles of all the dung beetle feeding groups typically contain a blade-like and membranous dorso-ventrally flattened incisor lobe (il) also known as the "apicalis", "distalis", or "distal lobe" and a strongly sclerotized molar lobe (ml) or "basalis" (figures 3.1 – 3.5). Covering the molar lobes is a membranous structure known as the molar tapetum (mt), which appears to have crosswise "ridges" when observed under a scanning electron microscope (figures 3.1 – 3.5). The mandibles are positioned below the epipharynx where the epipharynx's setae in ventral view, interact directly with the setae of the mandibles in dorsal view (figure 1.5).

The setal composition of the mandibles of all the feeding groups consists of the prosthecal comb (pc) lining its inner margin, the ventral comb lining the middle region (only visible ventrally) and closely packed rows of setae, the distal comb (dc), covering the apex of the incisor lobe (figures 3.1 – 3.5). Lodged between the incisor lobe and the molar lobe is a pouch-like structure known as the conjunctivuse (co) consisting of rows of small and compact setae (figures 3.1 – 3.5). For some dry feeders, the conjunctivuse is present but highly reduced in comparison to all other feeding groups (figures 3.4; 3.5).

The measurements of the mandibles' dimensions across various species are indicated in (table 3.2). Both the mandible length and width of wet feeders is significantly greater than that of carrion feeders ($p = 0.004$; $p = 0.011$ respectively), however, these dimensions between the remaining feeding groups are not significantly different (table 3.2; figure 3.6). The relative mandible length of wet feeders, carrion feeder and the generalist feeder is significantly greater than that of dry feeders ($p = 0.0002$; $p = 0.024$; $p = 0.0002$ respectively) (table 3.2; figure 3.6a). For the relative mandible width, there are no significant differences across all the feeding groups (table 3.2; figure 3.6b).

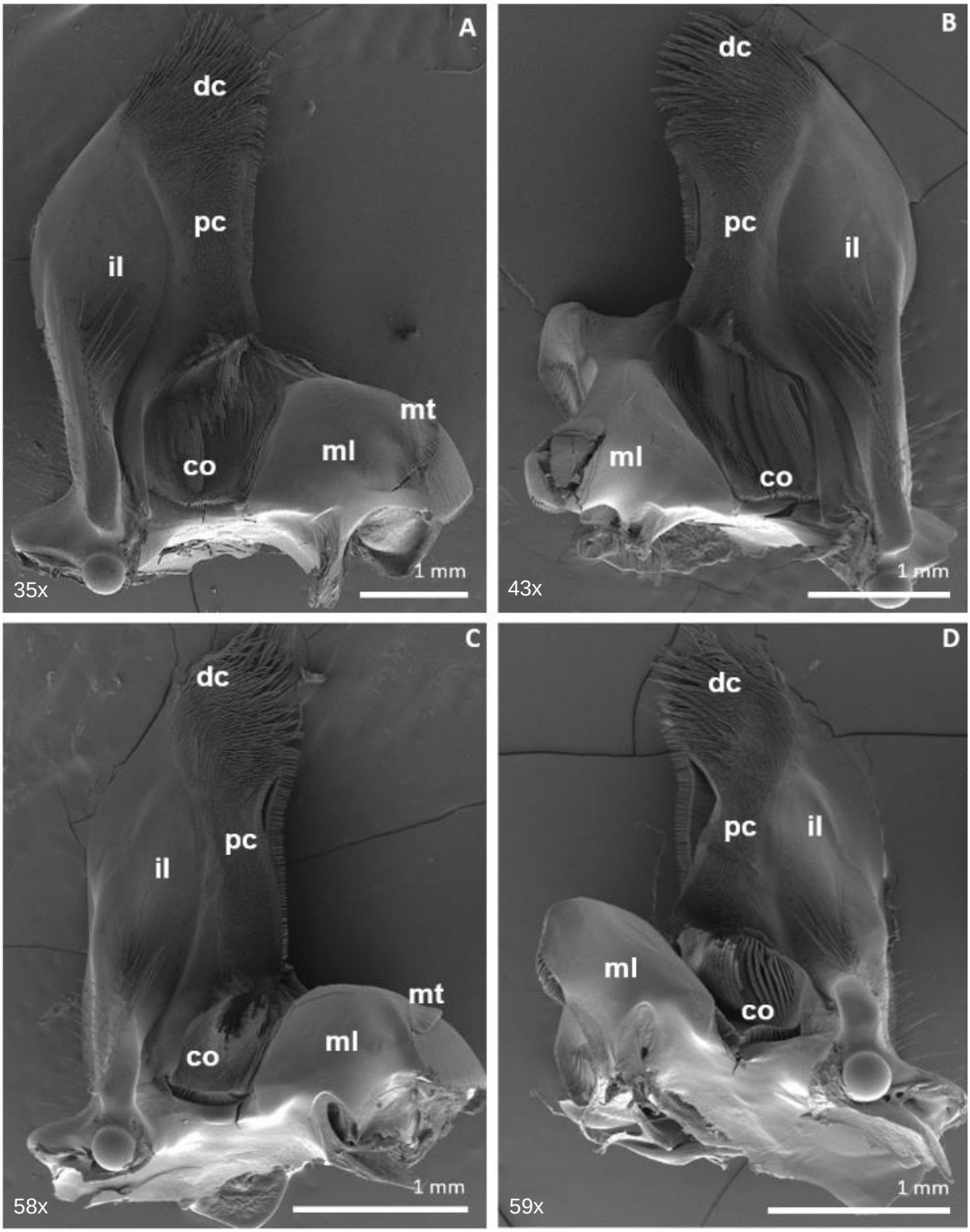


Figure 3.1. Ventral view of the convex (left) and concave (right) mandibles of dung beetle species feeding on wet dung. (A, B) *Kheper lamarcki*. (C, D) *Kheper nigroaeneus*. Abbreviations: co, conjunctivuse; dc, distal comb; il, incisor lobe; ml, molar lobe; mt, molar tapetum; pc, prosthecal comb. Scale bar = 1 mm.

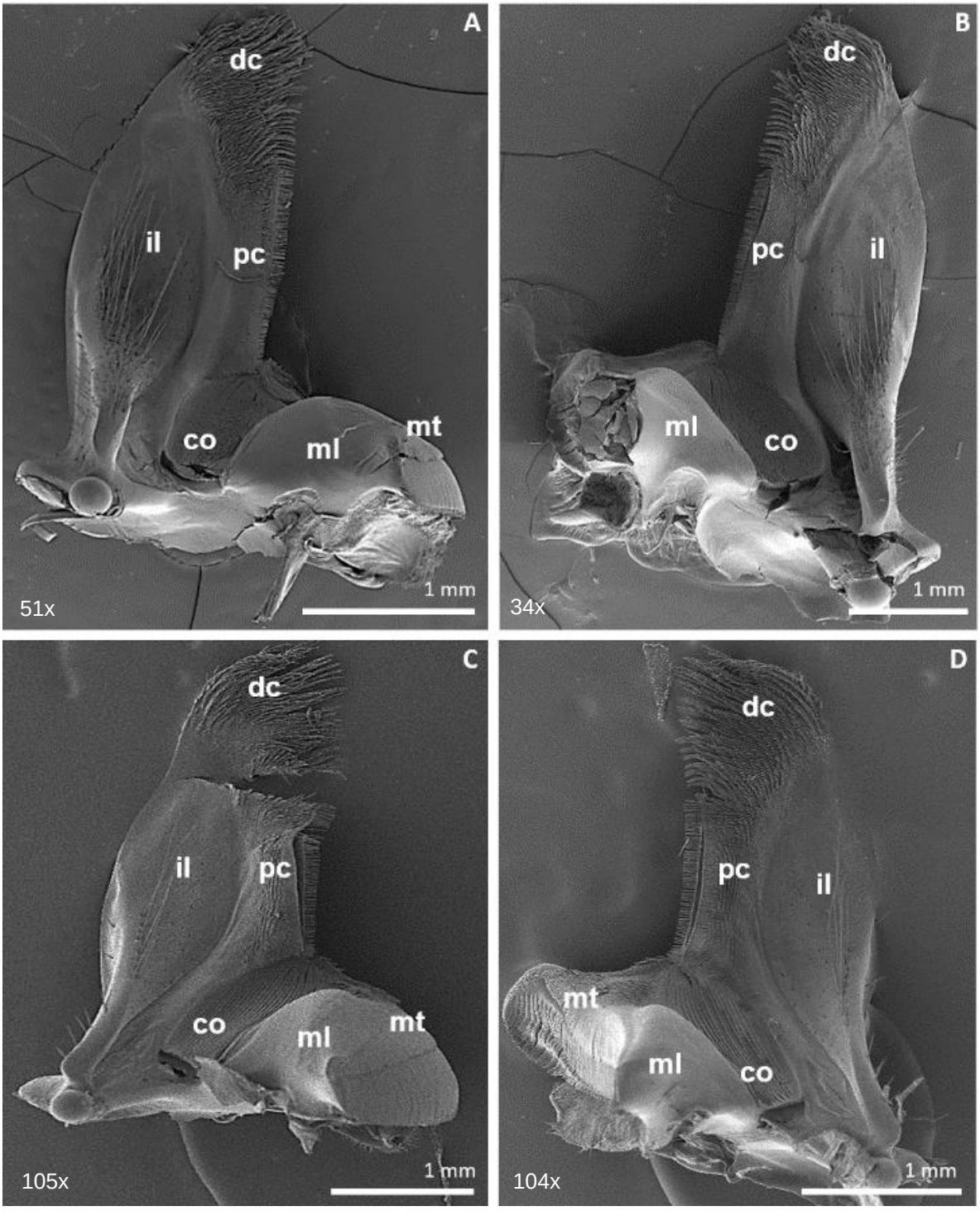


Figure 3.2. Ventral view of the convex (left) and concave (right) mandibles of dung beetle species feeding on wet dung. (A, B) *Scarabaeus zambesianus* (C, D) *Scarabaeolus damarensis*. Abbreviations: co, conjunctivuse; dc, distal comb; il, incisor lobe; ml, molar lobe; mt, molar tapetum; pc, prosthecal comb. Scale bar = 1 mm.



Figure 3.3. Ventral view of the convex (left) and concave (right) mandibles of dung beetle species feeding on carrion (A, B) *Scarabaeolus carniphilus* and a generalist feeder (C, D) *Anachalcos convexus*. Abbreviations: co, conjunctivuse; dc, distal comb; il, incisor lobe; ml, molar lobe; mt, molar tapetum (not visible on plate C); pc, prosthecal comb. Scale bar as indicated per micrograph.

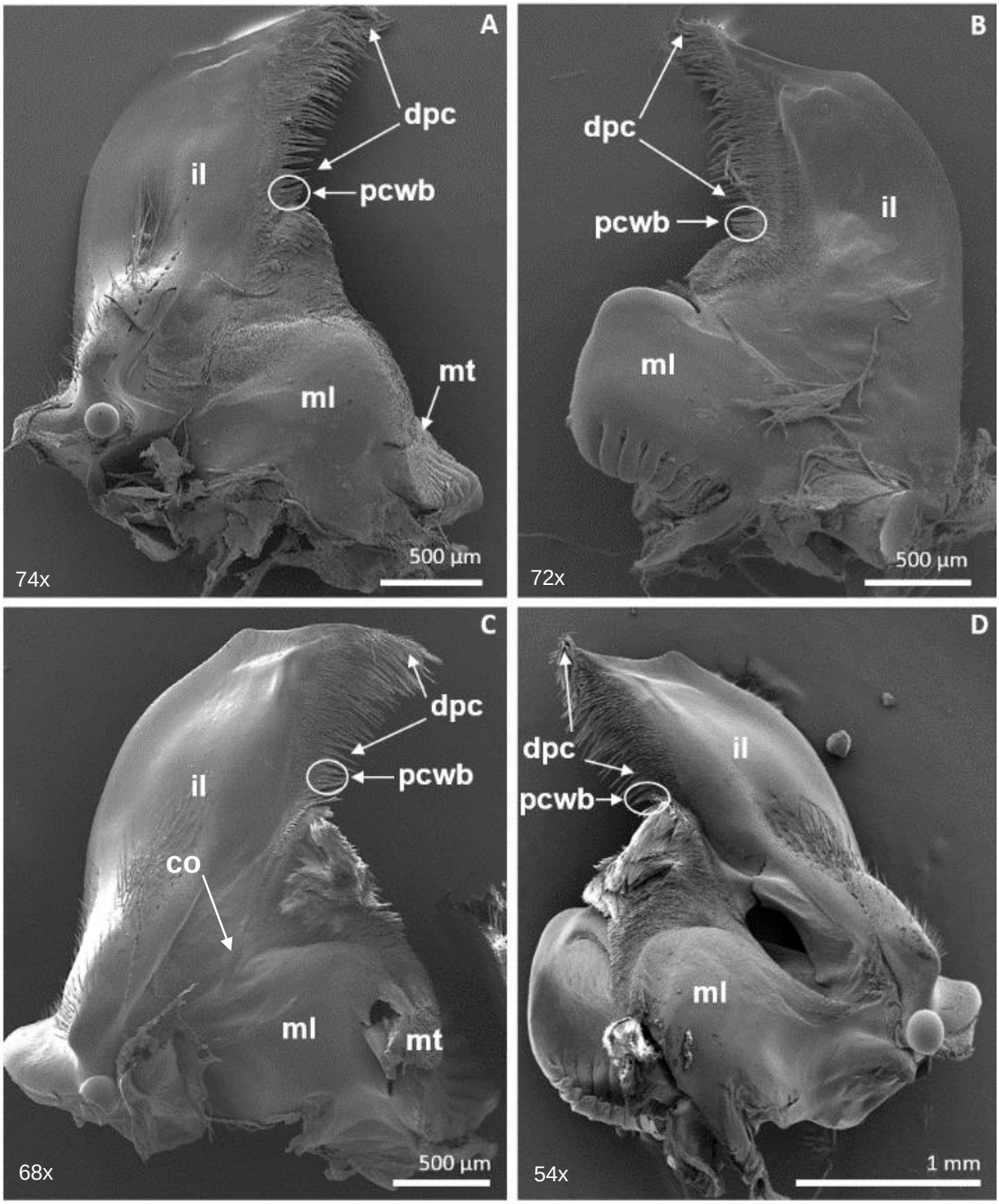


Figure 3.4. Ventral view of the convex (left) and concave (right) mandibles of dung beetle species feeding on a dry food diet. (A, B (dorsal view)) *Pachysoma endroedyi*, (C, D) *Pachysoma hippocrates*. Abbreviations: co, conjunctivuse (not visible on plates A, B, D); dpc, distal and prosthecal comb (cannot be distinguished from each other, i.e lacks bifurcations); il, incisor lobe; ml, molar lobe; mt, molar tapetum (not visible on plates B, D); pcwb, only region of the prosthecal comb with bifurcations. Scale bar as indicated per micrograph.

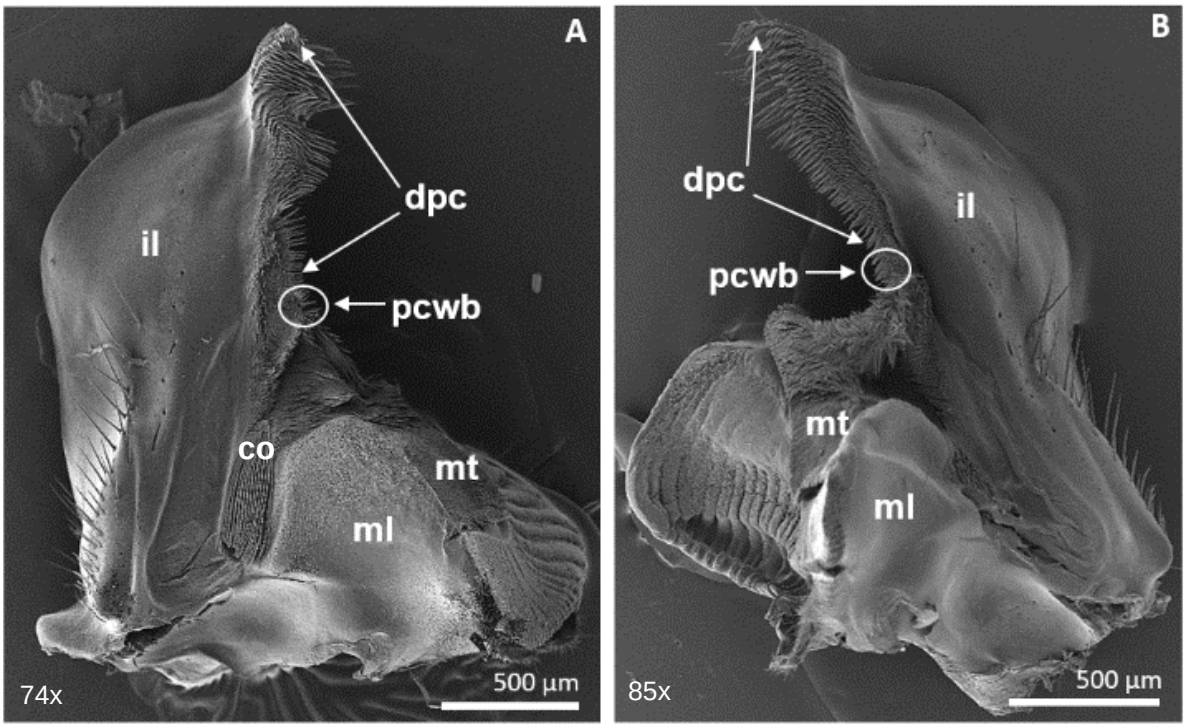


Figure 3.5. Ventral view of the convex (left) and concave (right) mandibles of dung beetle species feeding on a dry food diet. (A, B) *Pachysoma striatum.* Abbreviations: co, conjunctivuse (not visible on plate B); dc, distal comb; il, incisor lobe; ml, molar lobe; mt, molar tapetum; pc, prosthecal comb. Scale bar as indicated per micrograph.

Table 3.2. Mean±SD measurements of the mandibles' dimensions across dung beetle species of tribes: Scarabaeini and Canthonini. The relative values calculated as ratios between the dimensions and the pw. Abbreviations: cf, carrion feeder; df, dry feeder; gf, generalist feeder; ml, mandible length; mw, mandible width; pw, pronotum width; wf, wet feeder

Species	**Feeding group**	***n***	**ml**	**ml/pw**	**mw**	**mw/pw**
Units			**mm**	**mm**	**mm**	**mm**
1 Kheper lamarcki	wf	5	4.76±0.39	0.24±0.01	1.30±0.14	0.07±0.01
2 Kheper nigroaeneus	wf	5	5.60±0.24	0.33±0.02	1.36±0.11	0.08 ±0.01
3 Scarabaeus zambesianus	wf	5	3.69±0.31	0.22±0.01	0.98±0.10	0.06±0.00
4 Scarabaeolus damarensis	wf	5	1.77±0.11	0.23±0.02	0.40±0.02	0.05±0.01
5 Scarabaeolus carniphilus	cf	5	1.77±0.07	0.24±0.01	0.41±0.02	0.05±0.00
6 Anachalcos convexus	gf	5	3.34±0.14	0.27±0.02	0.84±0.04	0.07±0.01
7 Pachysoma endroedyi	df	2	2.53±0.08	0.18±0.00	0.75±0.04	0.05±0.00
8 Pachysoma hippocrates	df	4	3.34±0.23	0.18±0.01	1.28±0.05	0.07±0.01
9 Pachysoma striatum	df	4	2.30±0.15	0.18±0.02	0.71±0.08	0.06±0.01

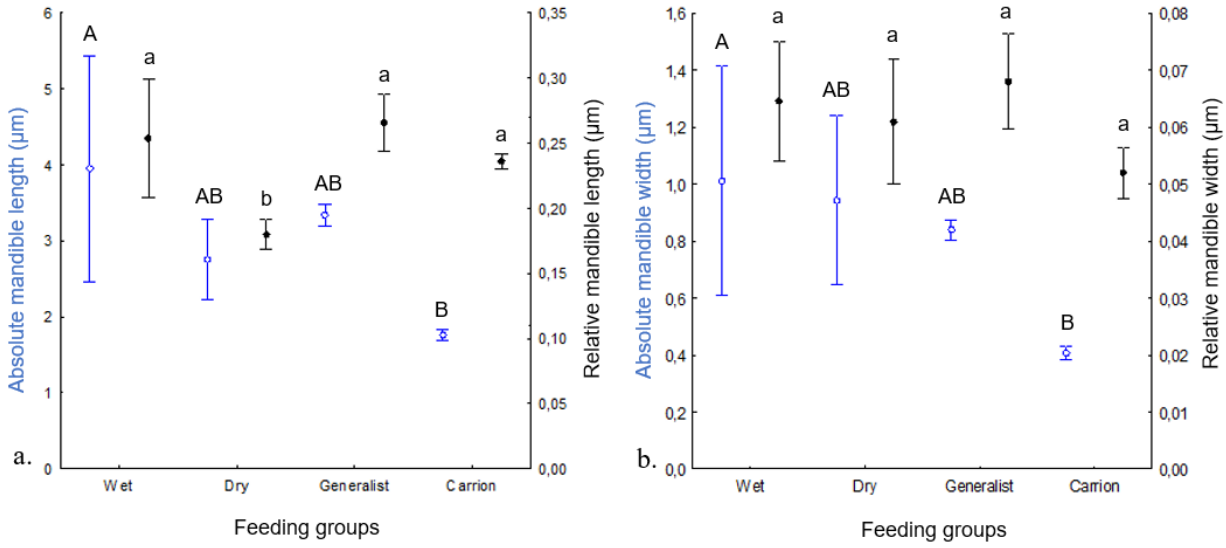


Figure 3.6. Comparison of the mean±SD (box = mean; whisker = SD) mandible dimensions across feeding groups. (a). Absolute and relative measures of the mandible length. Absolute: Kruskal-Wallis test: H (3, N = 40) =13.223; p = 0.0042; relative: Kruskal-Wallis test: H (3, N = 40) = 24.081; p < 0.0001. (b). Absolute and relative measures of the mandible width. Absolute: Kruskal-Wallis test: H (3, N = 40) = 9.842; p = 0.0200; relative: Kruskal-Wallis test: H (3, N = 40) = 8.337; p = 0.05. Relative measurements are those that are divided by PW (pronotum width). Uppercase letters denote statistical significance for absolute measures and lowercase letters denote statistical significance for relative measures.

3.2.2 Components of the incisor lobe

The incisor lobe carries various forms of setae. The prosthecal comb (pc) and distal comb (dc) in particular, are of interest in this study because it is assumed, they are involved in filtering out and excluding large particles of dung. The prosthecal comb of all the feeding groups is made up of dual rows of setae that occur both on the dorsal and ventral side of the innermost border of the incisor lobe. For the generalist, wet dung and carrion feeder, the prosthecal comb is typically recognized by the hook-like extensions or bifurcations that emerge from the ventral surface of the individual bristles (figures 3.7 A, C; 3.8 A, C; 3.9 A, C). The bifurcations of dry feeding species are more linear in shape than hook-like (figures 3.10 A; 3.11 A) (except for *P. hippocrates* in which bifurcations are absent (figure 3.10 C)). Bifurcations make the prosthecal comb of the generalist, wet and carrion feeder distinguishable from their distal comb (except for dry feeders). Only about 10% of the (proximal area) of the pc of dry feeders contains bifurcations.

The shape of these bifurcations varies between species. When these structures are analyzed in their respective feeding groups, the absolute length of the bifurcations do not differ significantly (table 3.3; figure 3.12c). The relative lengths of the bifurcations of carrion feeders are, however, significantly longer than those of the generalist ($p = 0.032$), wet ($p = 0.002$) and dry feeders ($p = 0.0002$) (table 3.3; figure 3.12c).

To determine and compare the length of the prosthecal comb (pcl) across feeding groups, only the length of the comb that contained bifurcations was measured (hence approximately 10% of the prosthecal comb of dry feeders was measured because the remaining prosthecal comb is identical to their dorsal comb). The absolute pcl and the relative pcl of dry feeders is significantly shorter than that of wet feeders ($p < 0.0001$; $p = 0.00002$ respectively) and generalist feeder ($p = 0.028$; $p = 0.0002$ respectively) but not significantly different to that of the carrion feeder (table 3.3; figure 3.13a). The absolute width of the prosthecal comb (pcw) of wet feeders and generalists is significantly greater than the pcw of carrion feeders ($p = 0.005$; $p = 0.010$ respectively) but not significantly different to that of dry feeders whereas the relative pcw of wet feeders and generalist feeder is significantly wider than that of dry feeders ($p = 0.00002$; $p = 0.0002$ respectively) but not significantly different to carrion feeders (table 3.3; figure 3.13b).

The gaps between the bristles of the pc and dc between feeding groups vary significantly. The pc gaps of dry feeders are significantly wider than those of the generalist ($p = 0.0002$), wet (p

= 0.0002) and carrion feeders (p = 0.0002) (table 3.3; figure 3.12a). In addition, the pc gaps of wet feeders are significantly wider than those of carrion feeders (p = 0.003) (table 3.3; figure 3.12a). The relative pc gaps of dry feeders are significantly wider than those of wet feeders (p = 0.0003) and the generalist feeder (p = 0.004) but not carrion feeders (table 3.3; figure 3.12a). The relative pc gaps of carrion feeders are significantly wider than those of wet feeders (p = 0.016) and the generalist feeder (p = 0.04) (table 3.3; figure 3.12a).

The distal comb of wet feeders and the generalist feeder is plumose in nature (figures 3.7 B, D; 3.8 B; 3.9 D) as opposed to that of *S.* (*Sc.*) *damarensis* (figure 3.8 D), dry feeders (figures 3.10 B, D; 3.11 B) and the carrion feeder (figure 3.9 B) which lack branches. The dc gaps of wet feeders and dry feeders are significantly wider than those of the carrion feeder (p = 0.04; p = 0.010 respectively) but not to each other (table 3.3; figure 3.12b). The relative dc gaps for all the feeding groups are not significantly different to each other (table 3.3; figure 3.12b).

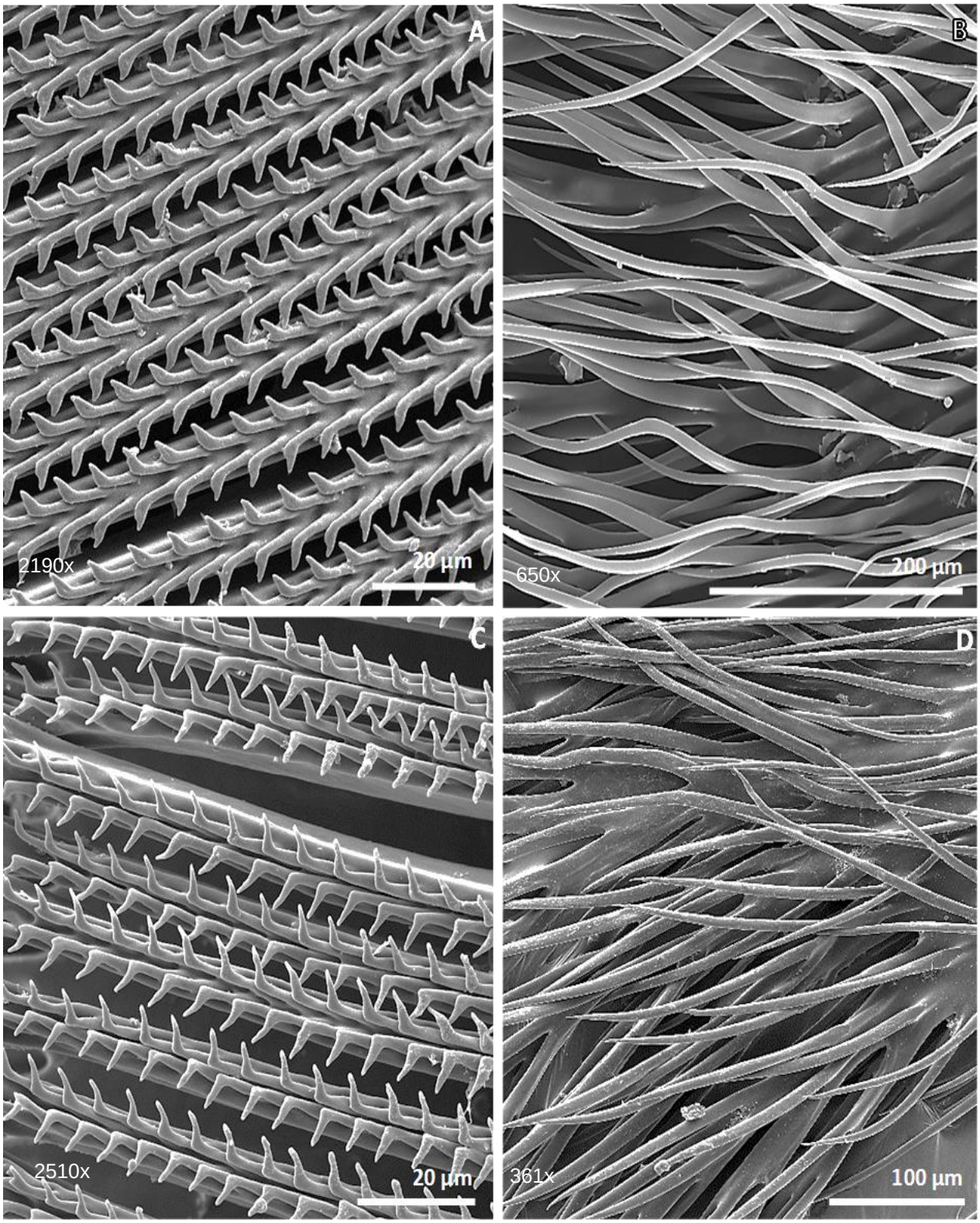


Figure 3.7. Ventral view of the setal composition of the incisor lobes of dung beetle species feeding on a wet dung diet: (A, B) *Kheper lamarcki* and (C, D) *Kheper nigroaeneus.* (A, C) Aspects of the prosthecal comb and its bifurcations. (B, D) Details of the distal comb. Scale as indicated per micrograph.

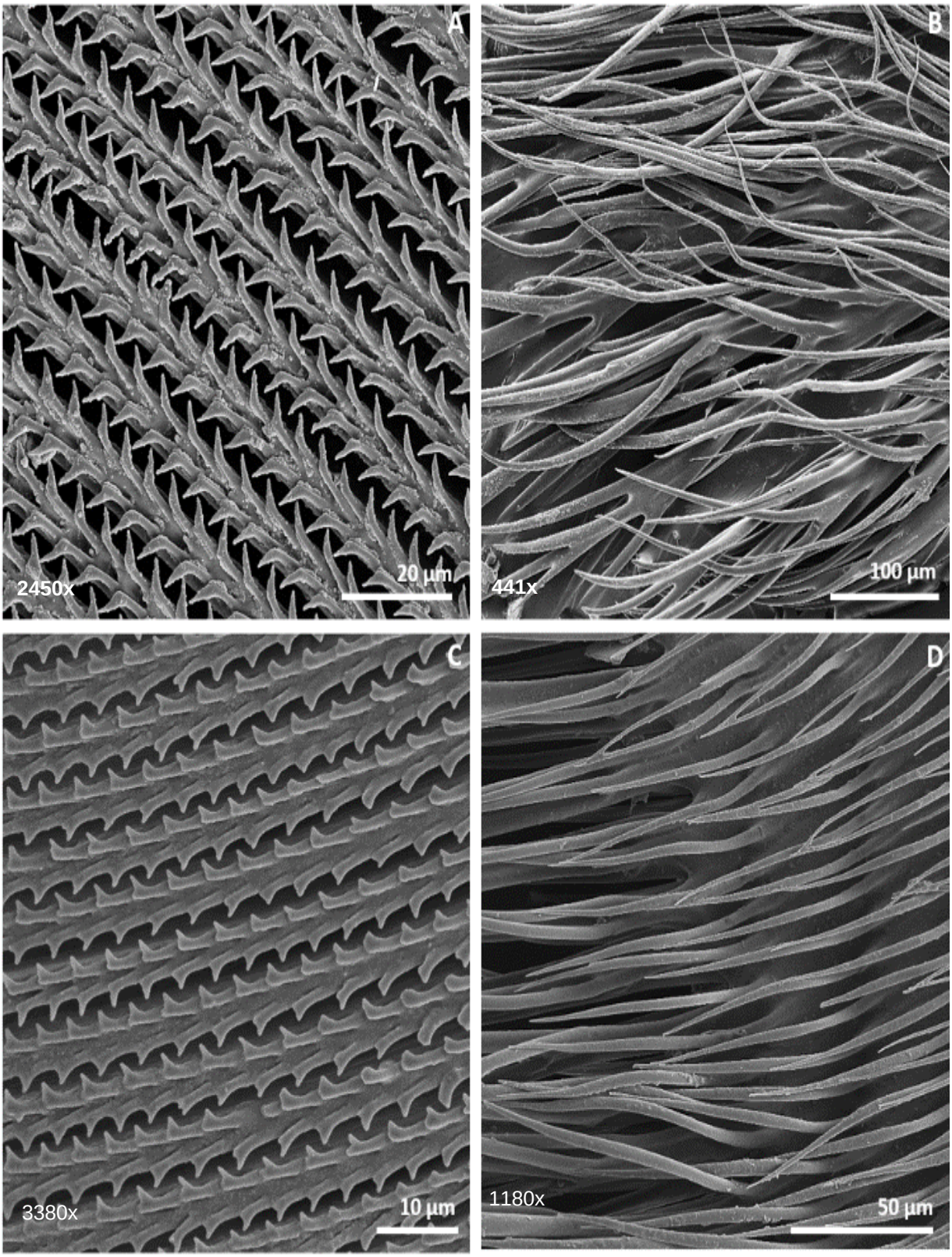


Figure 3.8. Ventral view of the setal composition of the incisor lobes of dung beetle species feeding on a wet dung diet: (A, B) *Scarabaeus zambesianus* and (C, D) *Scarabaeolus damarensis*. (A, C) Aspects of the prosthecal comb and its bifurcations. (B, D) Details of the distal comb. Scale as indicated per micrograph.

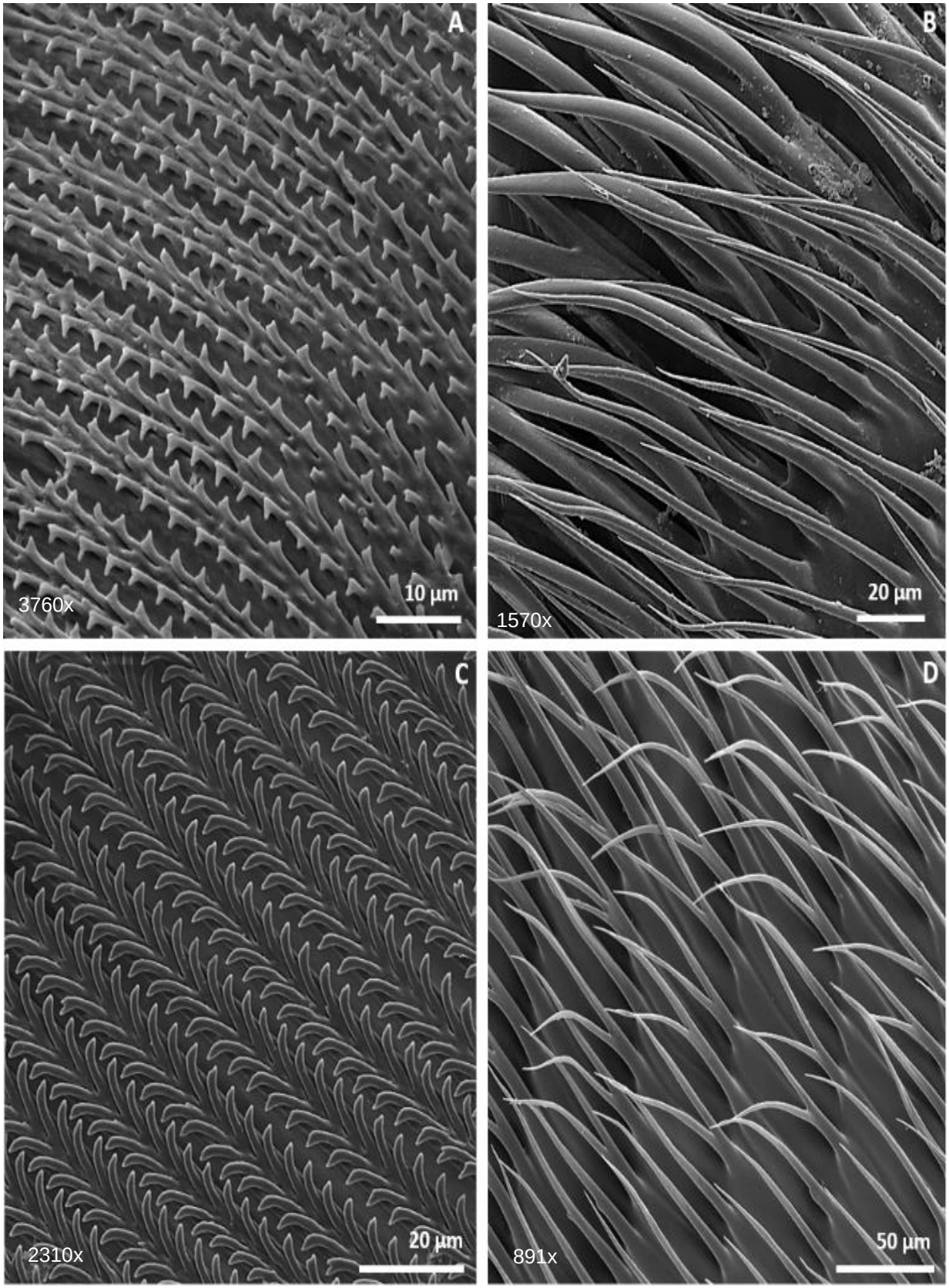


Figure 3.9. Ventral view of the setal composition of the incisor lobes of dung beetle species feeding on a carrion diet (A, B) *Scarabaeolus carniphilus* and a generalist diet (C, D) *Anachalcos convexus*. (A, C) Aspects of the prosthecal comb and its bifurcations. (B, D) Details of the distal comb. Scale as indicated per micrograph.

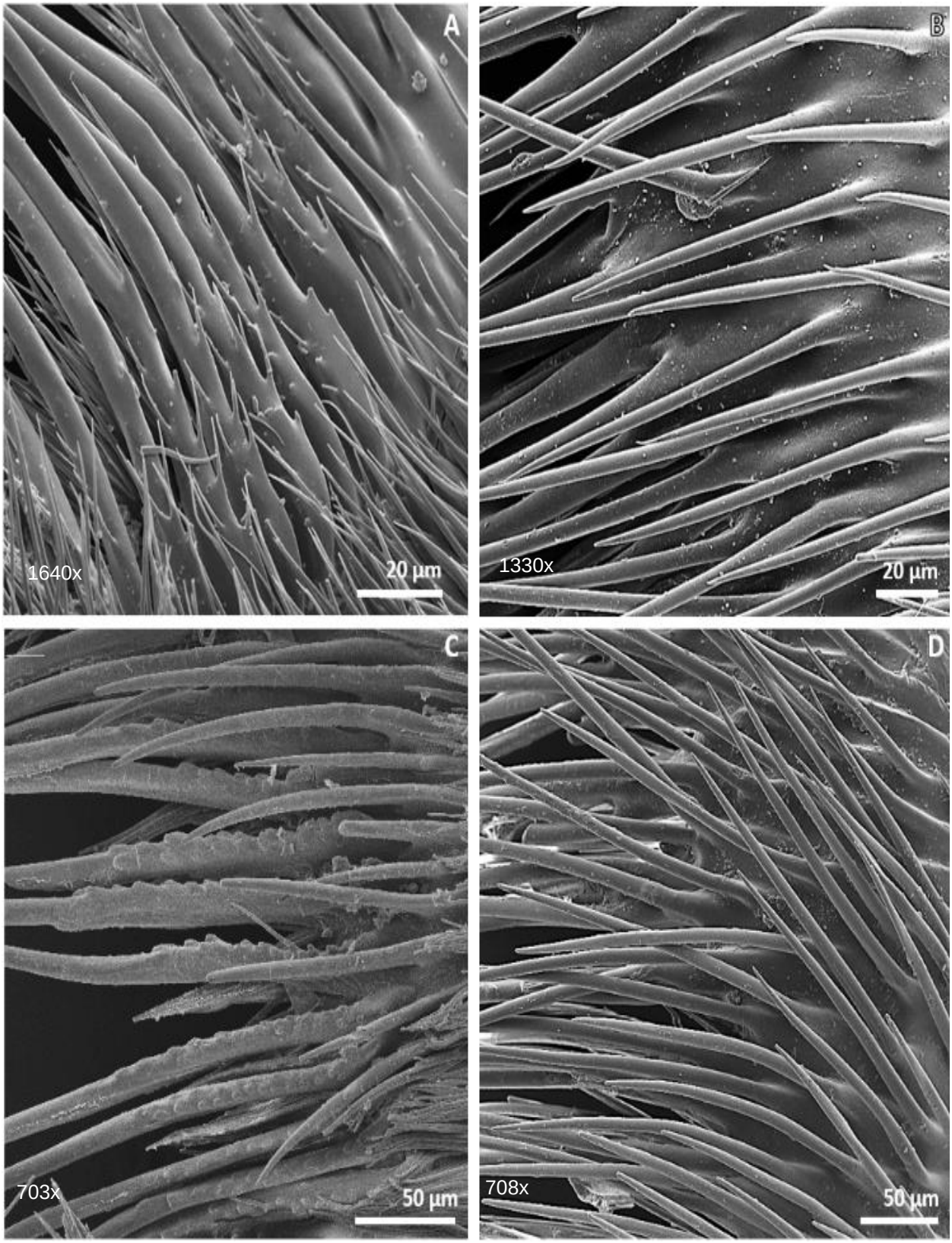


Figure 3.10. Ventral view of the setal composition of the incisor lobes of dung beetle species feeding on a dry dung diet: (A, B) *Pachysoma endroedyi* and (C, D) *Pachysoma hippocrates*. (A, C) Aspects of the prosthecal comb and its bifurcations. (B, D) Details of the distal comb. Scale as indicated per micrograph.

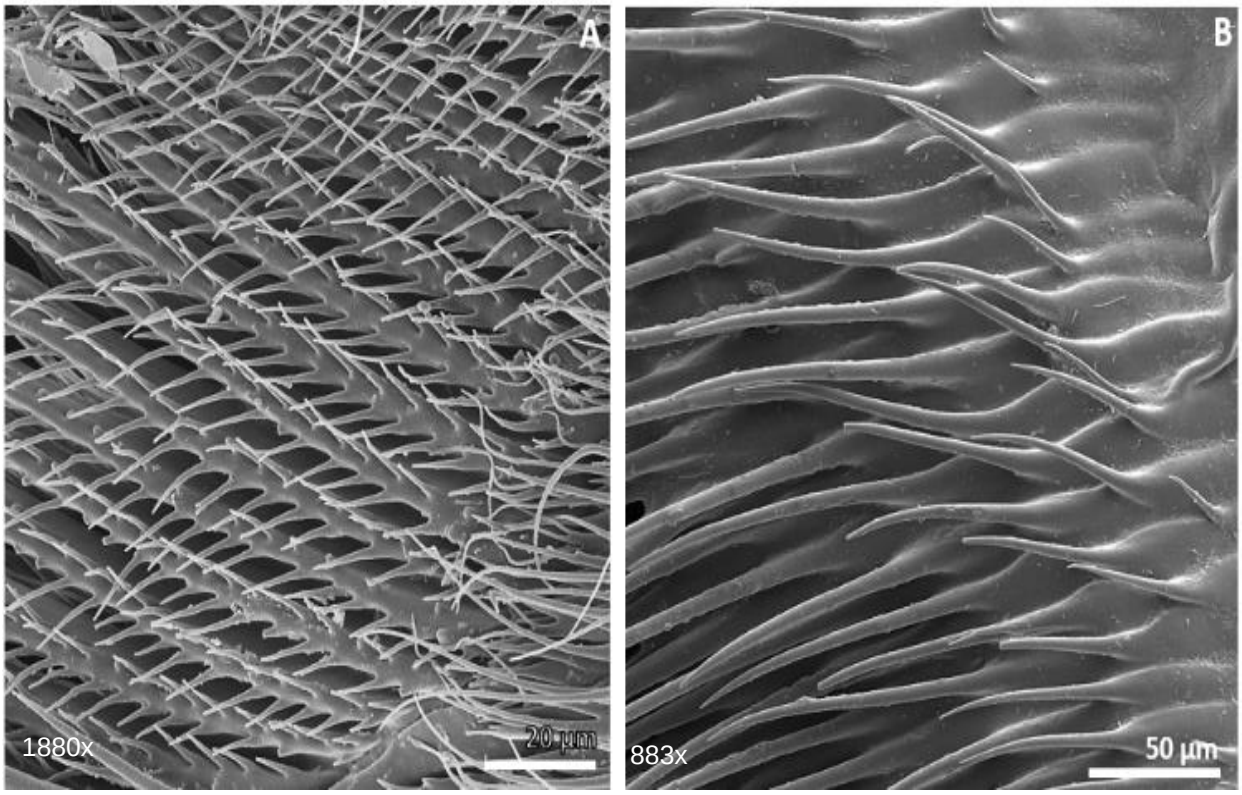


Figure 3.11. Ventral view of the setal composition of the incisor lobes of a dung beetle species feeding on a dry diet: (A, B) *Pachysoma striatum*. (A) aspects of the prosthecal comb and its bifurcations; (B) details of the distal comb. Scale as indicated per micrograph.

Table 3.3. Absolute and relative mean±SD measurements of several dimensions of various setae found on the incisor lobe of dung beetle species from different feeding groups. Relative measurements are those that are divided by pw (pronotum width). Abbreviations: bf, length of the bifurcations; cf, carrion feeder; dc, distal comb gaps; df, dry feeder; gf, generalist feeder; pc, prosthecal comb gaps; pcl, length of the prosthecal comb; pcw, width of the prosthecal comb; wf, wet feeder.

Species	***n***	**Feeding**	**pc**	**pc/pw**	**dc**	**dc/pw**	**bf**	**bf/pw**	**pcl**	**pcl/pw**	**pcw**	**pcw/pw**
Units		**group**	**µm**	**µm**	**µm**	**µm**	**µm**	**µm**	**mm**	**mm**	**mm**	**mm**
1 Kheper lamarcki	5	wf	3.60±0.39	0.18±0.01	14.56±3.69	0.72±0.12	11.60±0.46	0.58±0.07	1.52±0.11	0.08±0.007	0.43±0.04	0.021±0.002
2 Kheper nigroaeneus	5	wf	4.13±0.46	0.24±0.04	11.00±1.22	0.64±0.06	9.31±0.30	0.54±0.04	1.75±0.17	0.10±0.012	0.55±0.02	0.032±0.002
3 Scarabaeus zambesianus	5	wf	3.74±0.34	0.22±0.04	12.17±0.62	0.73±0.10	13.53±1.34	0.80±0.03	1.20±0.10	0.07±0.004	0.36±0.03	0.021±0.001
4 Scarabaeolus damarensis	5	wf	2.53±0.18	0.33±0.03	5.08±0.10	0.66±0.04	9.50±2.98	1.23±0.41	0.52±0.05	0.07±0.006	0.15±0.01	0.020±0.002
5 Scarabaeolus carniphilus	5	cf	2.40±0.22	0.32±0.03	5.99±0.31	0.80±0.06	10.68±0.80	1.43±0.14	0.47±0.02	0.06±0.002	0.15±0.01	0.020±0.001
6 Anachalcos convexus	5	gf	2.97±0.36	0.24±0.03	8.41±0.98	0.67±0.10	10.62±0.62	0.85±0.11	1.07±0.04	0.09±0.009	0.40±0.03	0.032±0.003
7 Pachysoma endroedyi	2	df	4.74±0.24	0.33±0.02	14.06±0.89	0.99±0.08	8.98±2.62	0.63±0.19	0.12±0.00	0.008±0.00	0.22±0.001	0.02±0.0001
8 Pachysoma hippocrates	4	df	5.72±0.11	0.31±0.01	15.79±1.30	0.86±0.08	0.00	0.11±0.02	0.15±0.02	0.008±0.00	0.30±0.01	0.016±0.001
9 Pachysoma striatum	4	df	4.73±0.08	0.36±0.02	8.22±0.45	0.63±0.03	11.54±2.14	0.88±0.11	0.15±0.01	0.01±0.001	0.24±0.02	0.018±0.002

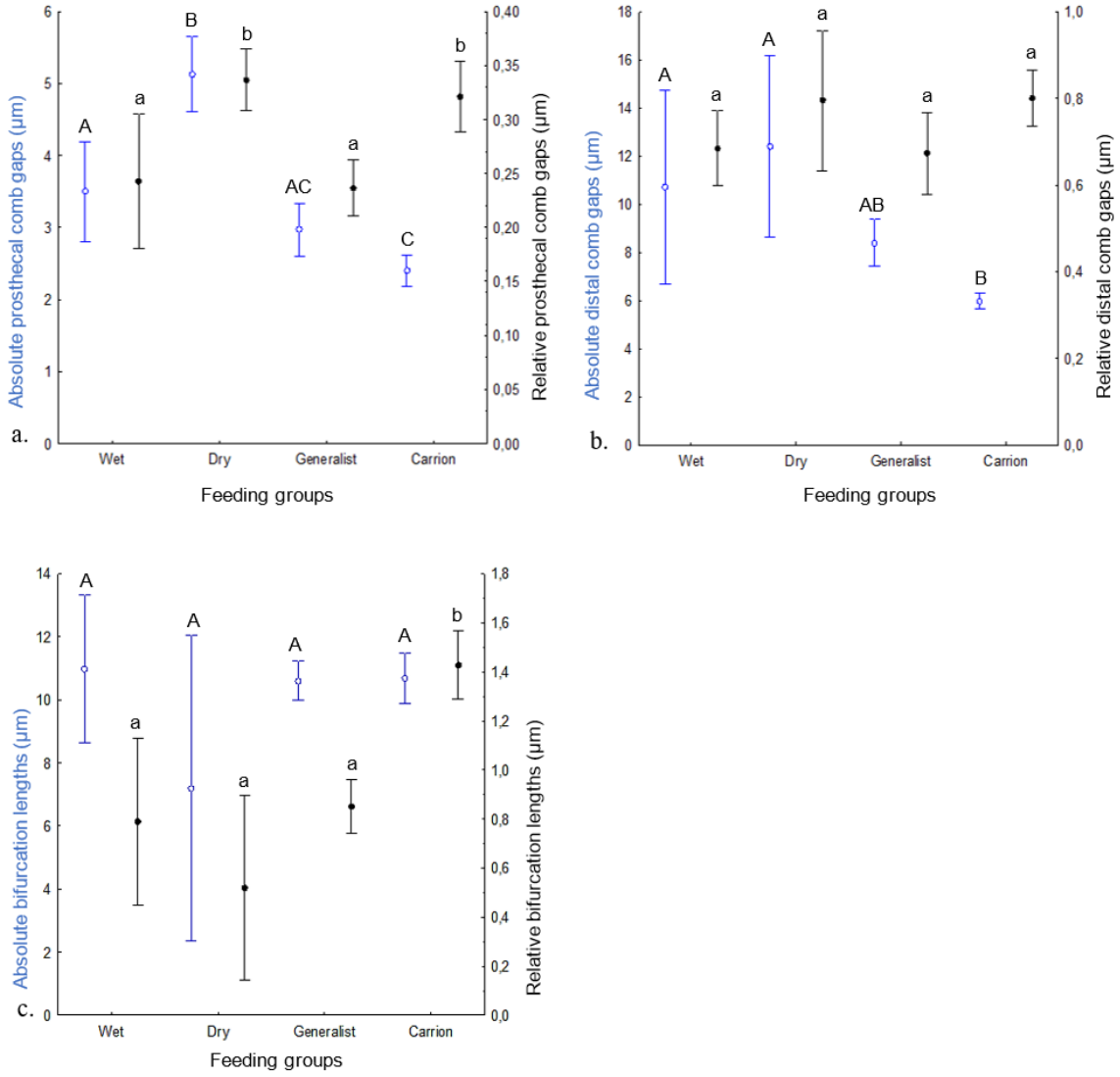


Figure 3.12. Comparison of the mean±SD (box = mean; whisker = SD) dimensions of various setae of the incisor lobe across feeding groups. (a). Absolute and relative measures of the prosthecal comb gaps. Absolute: ANOVA: $F(3, 36) = 31.744$; $p < 0.0001$; relative: ANOVA: $F(3, 36) = 10.504$; $p = 0.00004$. (b) Absolute and relative measure of the distal comb gaps. Absolute: ANOVA: $F(3, 36) = 4.352$; $p = 0.010$; relative: Kruskal-Wallis test: $H (3, N = 40) = 6.374$; $p = 0.095$. (c). Absolute and relative measures of the bifurcation lengths. Absolute: Kruskal-Wallis test: $H (3, N = 40) = 4.561$; $p = 0.2069$; relative: ANOVA: $F(3, 36) = 9.207$; $p < 0.0001$. Relative measurements are those that are divided by PW (pronotum width). Uppercase letters denote statistical significance for absolute measures and lowercase letters denote statistical significance for relative measures.

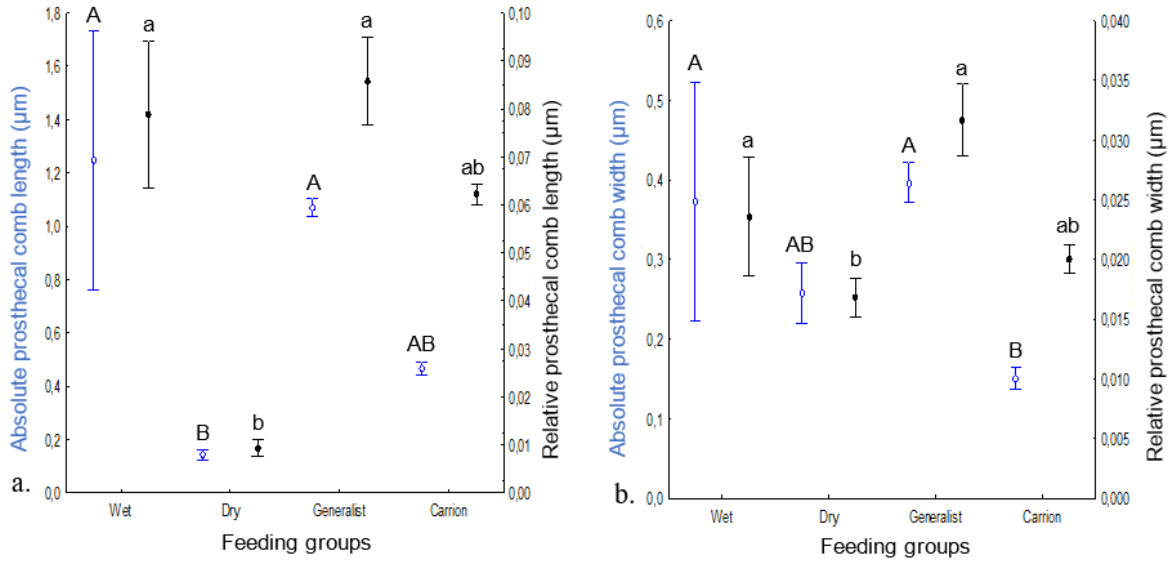


Figure 3.13. Comparison of the mean±SD (box = mean; whisker = SD) dimensions of the prosthecal comb across feeding groups. (a) Absolute and relative measures of the prosthecal comb length. Absolute: Kruskal-Wallis test: H (3, N = 40) = 28.783; $p < 0.0001$; relative: Kruskal-Wallis test: H (3, N = 40) = 28.830; $p < 0.0001$. (b) Absolute and relative measures of the prosthecal comb width. Absolute: Kruskal-Wallis test: H (3, N = 40) =15.446; $p = 0.0015$; relative: Kruskal-Wallis test: H (3, N = 40) = 25.277; $p < 0.0001$. Relative measurements are those that are divided by PW (pronotum width). Uppercase letters denote statistical significance for absolute measures and lowercase letters denote statistical significance for relative measures.

3.2.3 Molar lobe

The molar lobes are asymmetrical with a left concave surface (figures 3.14 A, E; 3.15 A, E; 3.16 A, D; 3.17 A, E; 3.18 A) and a right convex surface (figure 3.19) that are of similar sizes and mesh into each other when dung is being processed. Molar lobes consist of many crosswise molar ridges that are divided by furrows known as filtration channels (figures 3.14 B, F; 3.15 B, F; 3.16 B, E; 3.17 B; 3.18 B). These channels originate from the blind side of the molar, across its surface and lead to the open side. These features are prevalent across all feeding groups.

Pertaining to the molar ridges of wet, carrion and generalist feeder, each molar ridge (for both concave and convex molars) is made up of well-defined paired rows of "tightly packed scalloped blocks" (Hata and Edmonds 1983) or "substructures" (Holter and Scholtz 2011) known as triators that are joined in the middle down the length of the ridge and form a level and relatively smooth surface (figures 3.14 C, G; 3.15 C, G; 3.16 B, F). These types of ridges make up at least 75% of the molar and predominate in the posterior area of the molar. The anterior area of the concave molar is usually wider than its posterior counterpart and contains molar ridges with condensed substructures that are somewhat elevated and curve inwards at the edges (see anterior region of figures 3.14 A, E; 3.15 A, E; 3.16 A, D).

For dry feeding species, the posterior area of the concave molar is marked with molar ridges with substructures that are not as pronounced (figures 3.17 C, 3.18 C) as with the other feeding groups (figures 3.14 C, G; 3.15 C, G; 3.16 B, F). The ridges of the molar surface of *P. endroedyi* (figure 3. 17 B, C) are not deeply divided by filtration channels compared to the molar ridges of *P. striatum* (figure 3.18 D) while the inner molar region of *P. hippocrates* (figure 3.17 E) lacks ridges completely. The anterior area for all dry feeders is wider than the posterior area, and the innermost surface is smooth because it lacks ridges whereas the periphery of the anterior area possesses large "hook-like" (Holter and Scholtz 2011) ridges that curve inwards (figures 3.17 A, E; 3.18 A). The convex molars of all the dry feeding species contain large and uneven ridges that are not fully separated in the anterior region (although open at the edges) and gradually become smaller and divided as they continue into the posterior region (figures 3.19 G, H, I). The ridges of dry feeders are elevated and uneven and thus form a potential grinding surface as opposed to

the other feeding groups with flat surfaces which appear porous. Dry feeders are also found to possess fewer ridges than wet feeders, carrion feeder and generalist feeder (table 3.4).

Measurements of the filtration channel widths and heights of the molar ridges vary considerably across species (table 3.4). The absolute values of the filtration channels widths that were analysed across feeding groups indicate that dry feeders possess the largest filtration channel widths and that the absolute convex anterior (cva) filtration channel widths of dry feeders are significantly larger than those of the wet ($p = 0.0008$), carrion ($p = 0.00008$) and the generalist feeder ($p = 0.008$) (table 3.4, figure 3.20a). The relative convex anterior filtration channels widths of dry feeders are significantly larger than those of the wet feeders ($p < 0.0001$) and the generalist feeder ($p = 0.003$) (table 3.4, figure 3.20a). The absolute convex posterior (cvp) filtration channel widths of dry feeders are significantly larger than those of wet feeders ($p = 0.011$) and the carrion feeder ($p = 0.002$) whereas the cvp filtration channel widths of the generalist feeder are significantly larger than that of the carrion feeder ($p = 0.009$) (table 3.4, figure 3.20b). The relative cvp filtration channel widths of the dry feeders are significantly greater than those of the wet ($p = 0.0002$) but significantly different to the generalist and the carrion feeder while the relative cvp filtration channel widths of the wet feeder are significantly smaller than those of the generalist feeder ($p = 0.016$) (table 3.4, figure 3.20b).

The absolute concave anterior (cca) filtration channel widths of dry feeders is significantly larger than that of the wet ($p = 0.002$), generalist ($p = 0.013$) and the carrion feeder ($p = 0.0001$) while the wet, generalist and the carrion feeder are not significantly different to each other (table 3.4, figure 3.20c). The relative cca filtration channel widths of dry feeders are significantly larger than those of wet feeders ($p = 0.00004$) and the generalist feeder ($p = 0.003$) but not significantly different to the carrion feeder whereas the relative cca filtration channel widths of wet, generalist and carrion feeder are not significantly different to each other (table 3.4, figure 3.20c). The absolute concave posterior (ccp) filtration channel widths of the carrion feeder are significantly smaller than those of the dry feeders ($p = 0.002$) and the generalist feeder ($p = 0.026$) but not significantly different to the wet feeders. The relative ccp filtration channel widths of wet feeders is significantly smaller than that of dry feeders ($p = 0.003$) and the generalist feeder ($p = 0.009$) but is not significantly different to that of the carrion feeder (table 3.4, figure 3.20d).

The absolute height of the convex anterior molar ridges of dry feeders is significantly greater than that of wet feeders ($p = 0.021$), the carrion feeder ($p = 0.003$) and the generalist ($p = 0.007$) (table 3.4, figure 3.21a). The relative convex anterior molar ridge heights of dry feeders are significantly greater than that of wet feeders ($p = 0.002$) and the generalist feeder ($p = 0.006$) but not significantly different to that of the carrion feeders while the relative convex anterior molar ridge heights of the carrion feeder are significantly greater to that of the generalist feeder (table 3.4, figure 3.21a). The absolute convex posterior molar ridge heights of dry feeders is significantly greater than that of wet feeders ($p = 0.001$) and the carrion feeder ($p = 0.002$) but is not significantly different to that of the generalist feeder (table 3.4, figure 3.21b). The convex posterior molar ridge heights of wet, generalist and the carrion feeder are not significantly different to each other (table 3.4, figure 3.21b). The relative convex posterior molar ridge heights of dry feeders is significantly greater than that of wet feeders ($p = 0.0002$) and the generalist ($p = 0.009$) and the convex posterior molar ridge heights of wet feeders is significantly smaller than that of carrion feeders ($p = 0.001$) (table 3.4, figure 3.21b).

The absolute concave anterior molar ridge heights of dry feeders is significantly greater than that of wet ($p = 0.003$), generalist ($p = 0.002$) and the carrion feeder ($p < 0.0001$) while the wet, generalist and the carrion feeder are not significantly different to each other (table 3.4, figure 3.21c). The relative concave anterior molar ridge heights of dry feeders is significantly greater than that of wet feeders ($p = 0.0001$) and the generalist feeder ($p = 0.0001$) but is not significantly different to that of the carrion feeder (table 3.4, figure 3.21c).

The absolute concave posterior molar ridge heights of dry feeders is significantly greater than that of the wet feeders ($p = 0.0003$) and the carrion feeder ($p = 0.0005$) but is not significantly different to the generalist feeder (table 3.4, figure 3.21d). The concave posterior molar ridge heights of wet, generalist and the carrion feeders is not significantly different to each other (table 3.4, figure 3.21d). The relative concave posterior molar ridge heights of dry feeders is significantly greater than that of the wet feeders ($p < 0.0001$) but is not significantly different to the generalist and the carrion feeder (table 3.4, figure 3.21d). The relative concave posterior molar ridge heights of wet, generalist and the carrion feeder are significantly different to each other (table 3.4, figure 3.21d).

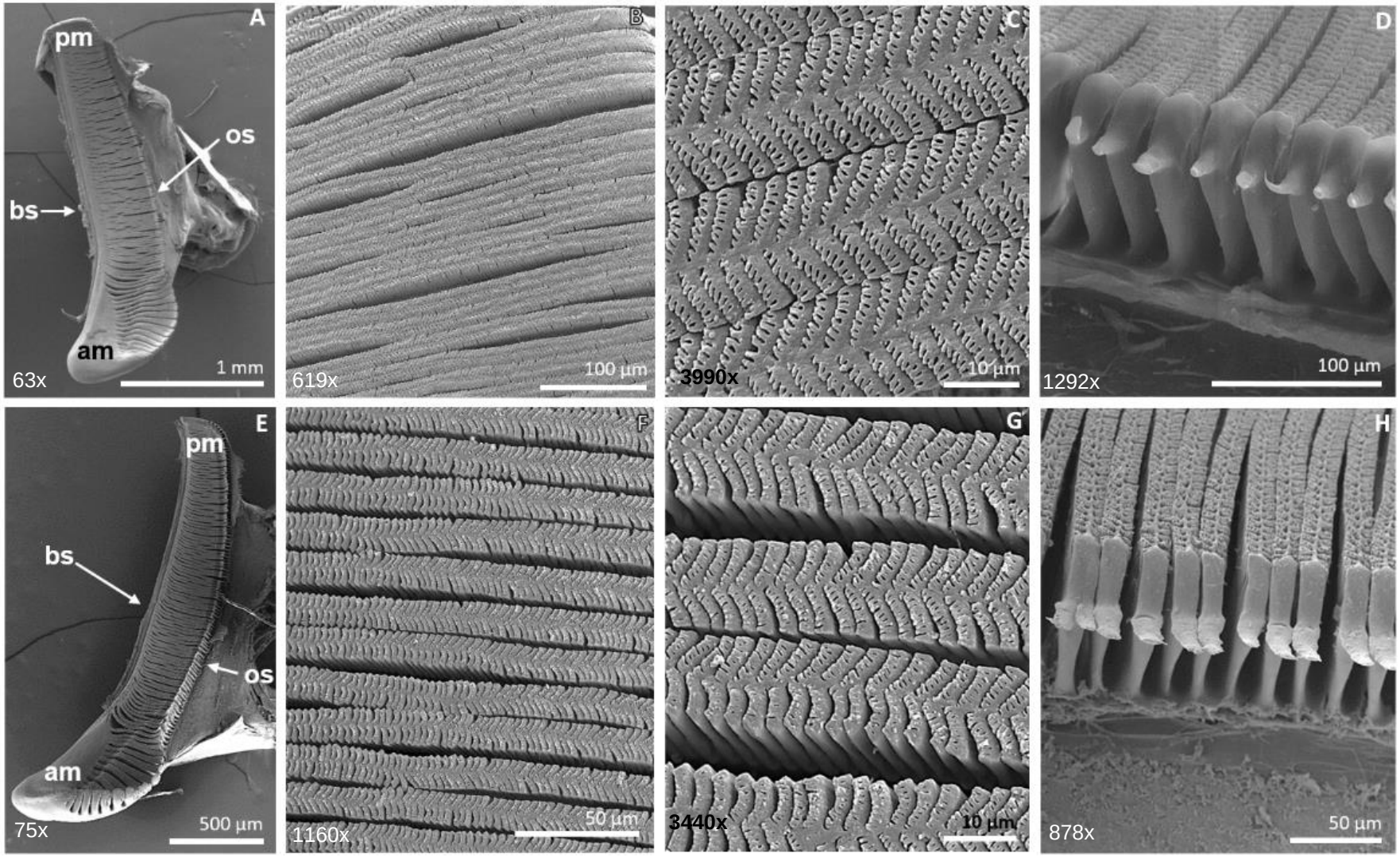


Figure 3.14. Mesal view of various components of the concave molar lobe of dung beetle species feeding on a wet dung diet (A – D) *Kheper lamarcki*, (E – H) *Kheper nigroaeneus.* (A, E) Concave molar with molar tapetum removed; (B, F) Molar ridges; (C, G) Details of molar ridges (triators); (D, H) Details of the open side of the molar, filtration channels. Abbreviations: am, anterior region of molar; bs, blind side/margin of molar; os, open side/margin of molar; pm, posterior region of molar. Scale as indicated per micrograph.

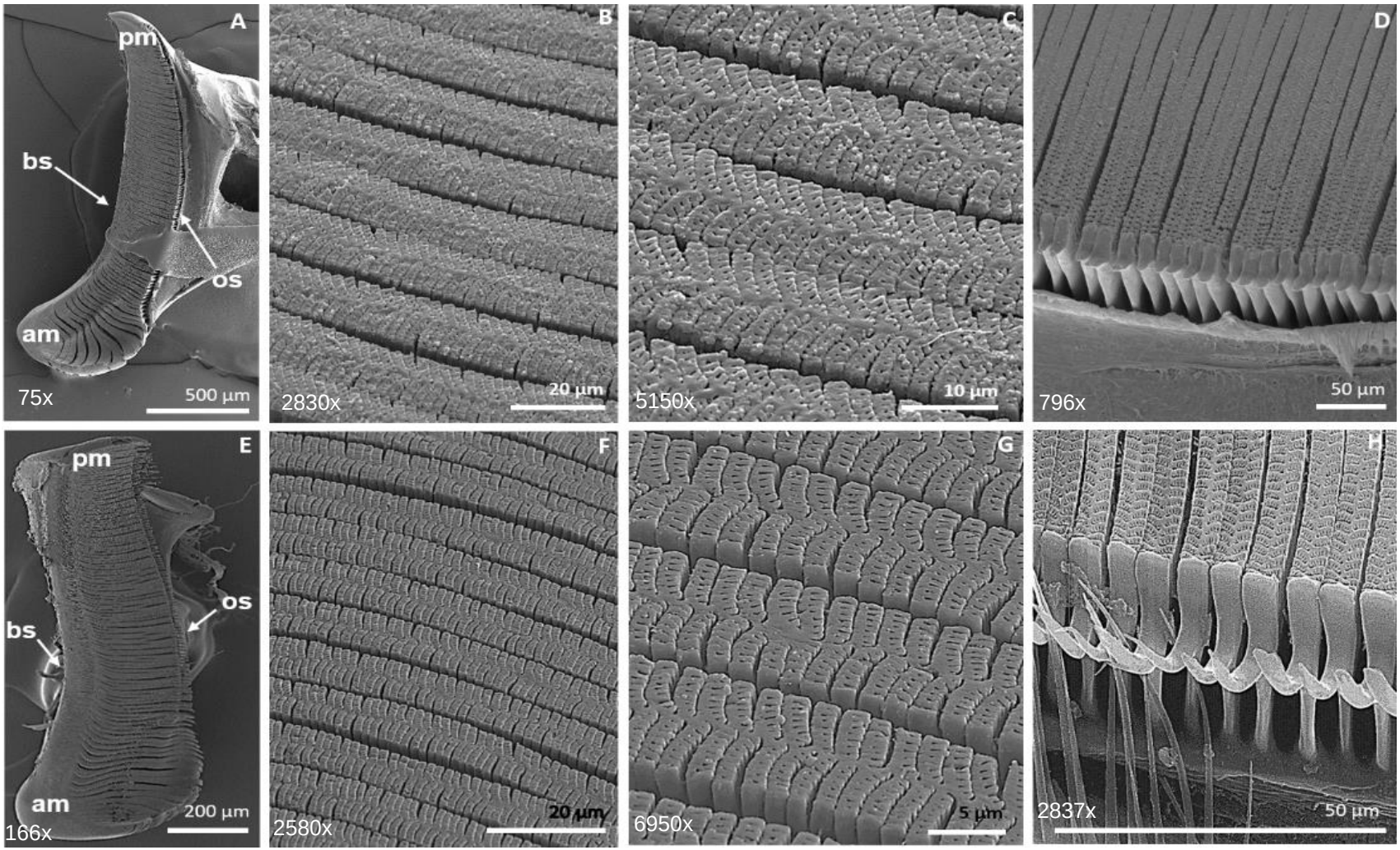


Figure 3.15. Mesal view of various components of the concave molar lobe of dung beetle species feeding on a wet dung diet (A – D) *Scarabaeus zambesianus*. (E – H) *Scarabaeolus damarensis.* (A, E) Concave molar with molar tapetum removed; (B, F) Molar ridges; (C, G) Details of molar ridges (triators); (D, H) Details of the open side of the molar, filtration channels. Abbreviations: am, anterior region of molar; bs, blind side of molar; os, open side of molar; pm, posterior region of molar. Scale as indicated per micrograph.

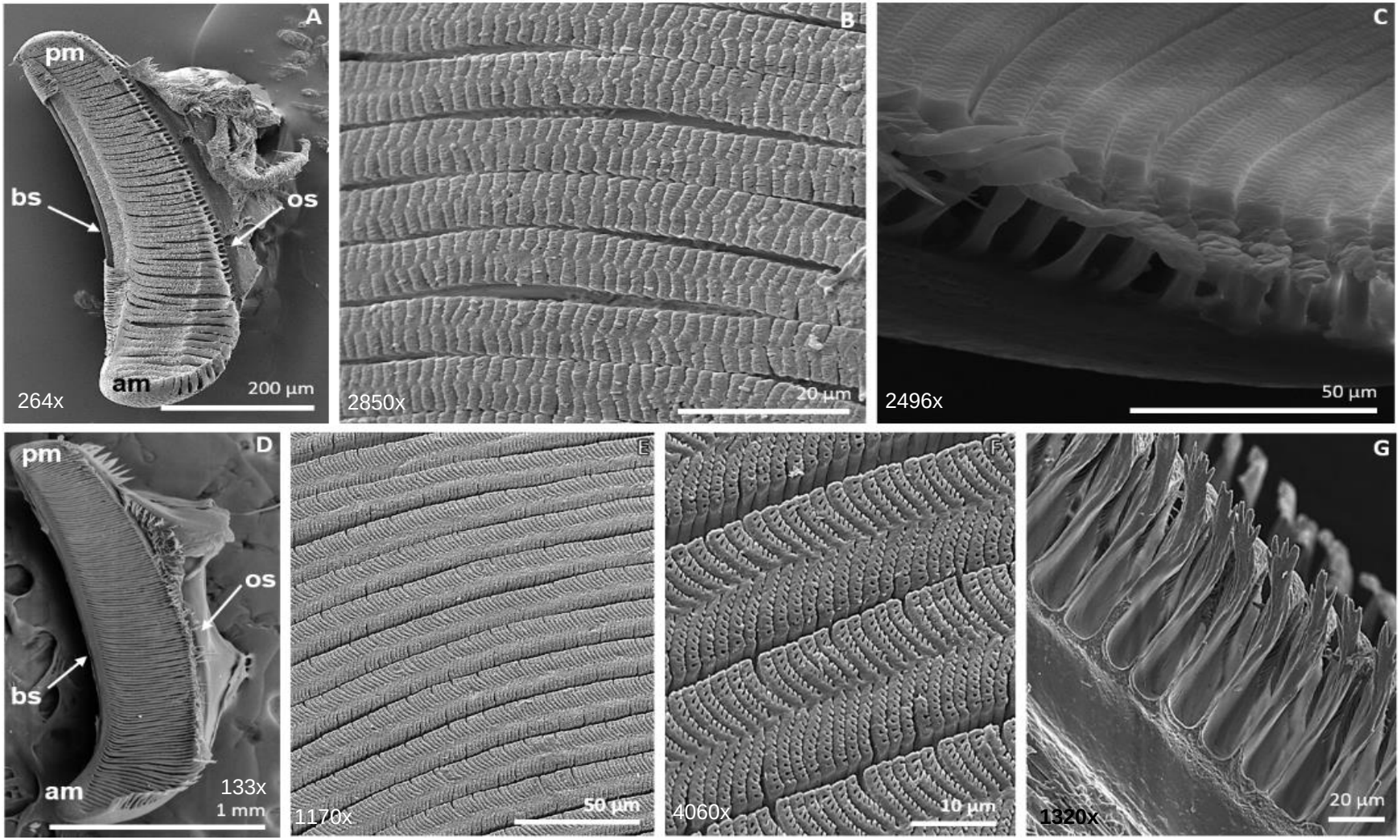


Figure 3.16. Mesal view of various components of the concave molar lobe of dung beetle species feeding on a carrion diet (A - C) *Scarabaeolus carniphilus* and a generalist feeder (D – G) *Anachalcos convexus.* (A, D) Concave molar with molar tapetum removed; (B, E) Molar ridges; (F) Details of molar ridges (triators); (C, G) Details of the open side of the molar, filtration channels. Abbreviations: am, anterior region of molar; bs, blind side of molar; os, open side of molar; pm, posterior region of molar. Scale as indicated per micrograph.

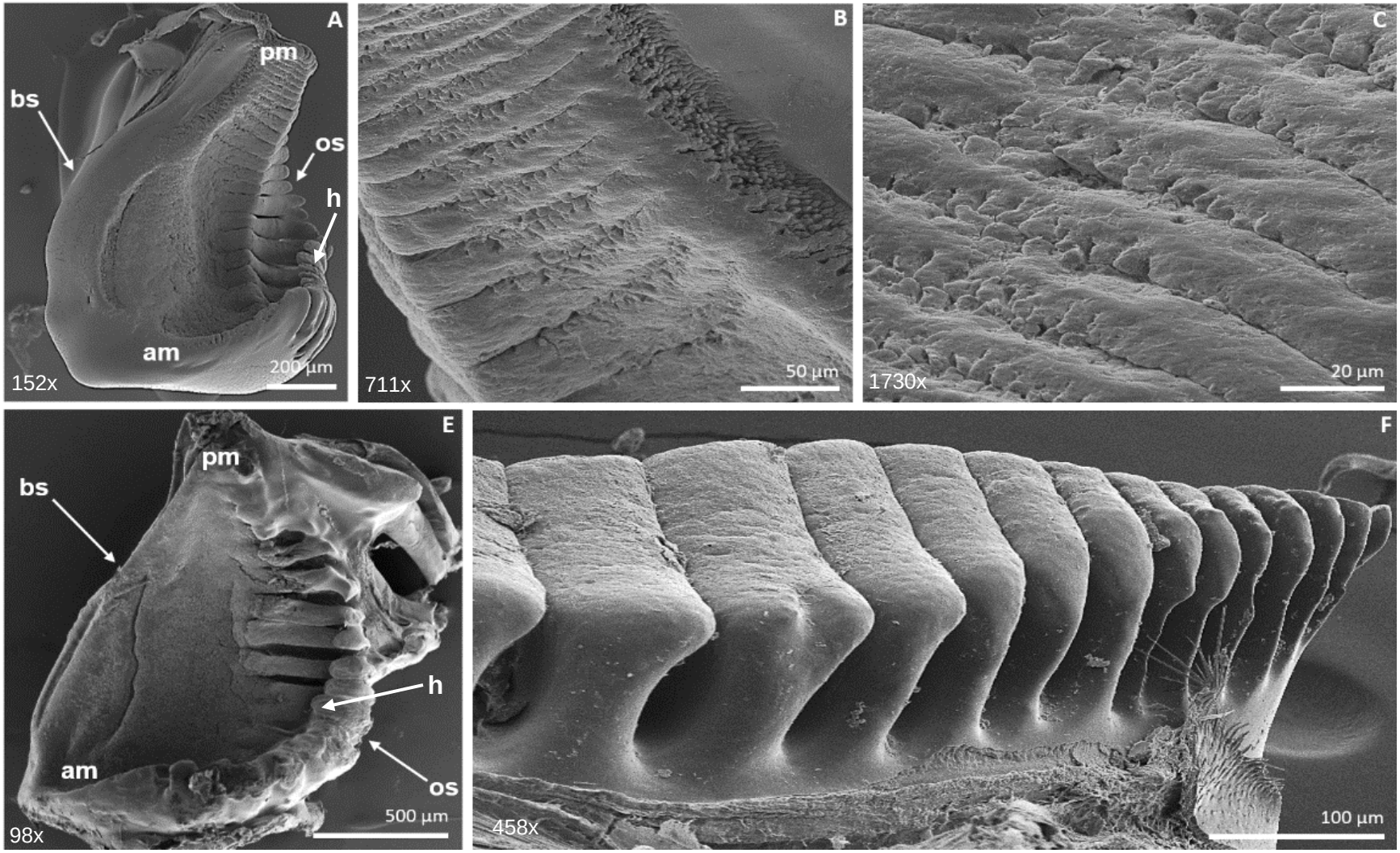


Figure 3.17. Mesal view of various components of the concave molar lobe of dung beetle species feeding on a dry (A - C) *Pachysoma endroedyi.* (E, F) *Pachysoma hippocrates.* (A, E) Concave molar with molar tapetum removed; (B, C) Molar ridges; (F) Details of the open side of the molar, filtration channels. Abbreviations: am, anterior region of molar; bs, blind end of molar; h, hooks; os, open side of molar; pm, posterior region of molar. Scale as indicated per micrograph.

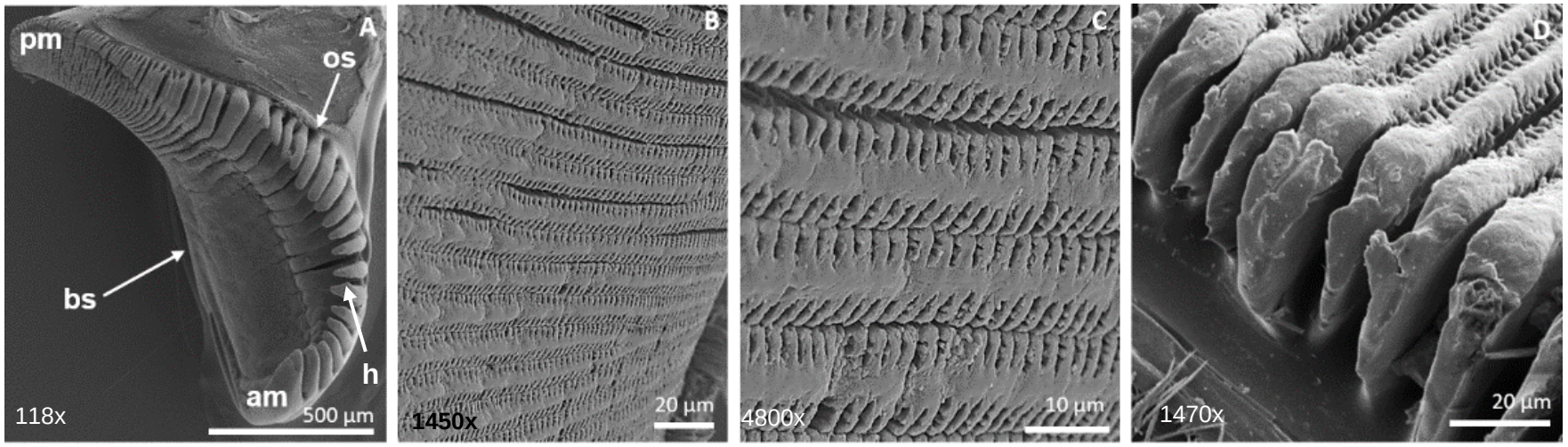


Figure 3.18. Mesal view of various components of the concave molar lobe of dry feeding dung beetle species, *Pachysoma striatum*. (A) Concave molar with molar tapetum removed. (B) Molar ridges. (C) Details of molar ridges (triators). (D) Details of the open side of the molar, filtration channels. Abbreviations: am, anterior region of molar; bs, blind end of molar; h, hooks; os, open side of molar; pm, posterior region of molar. Scale as indicated per micrograph. Scale as indicated per micrograph.

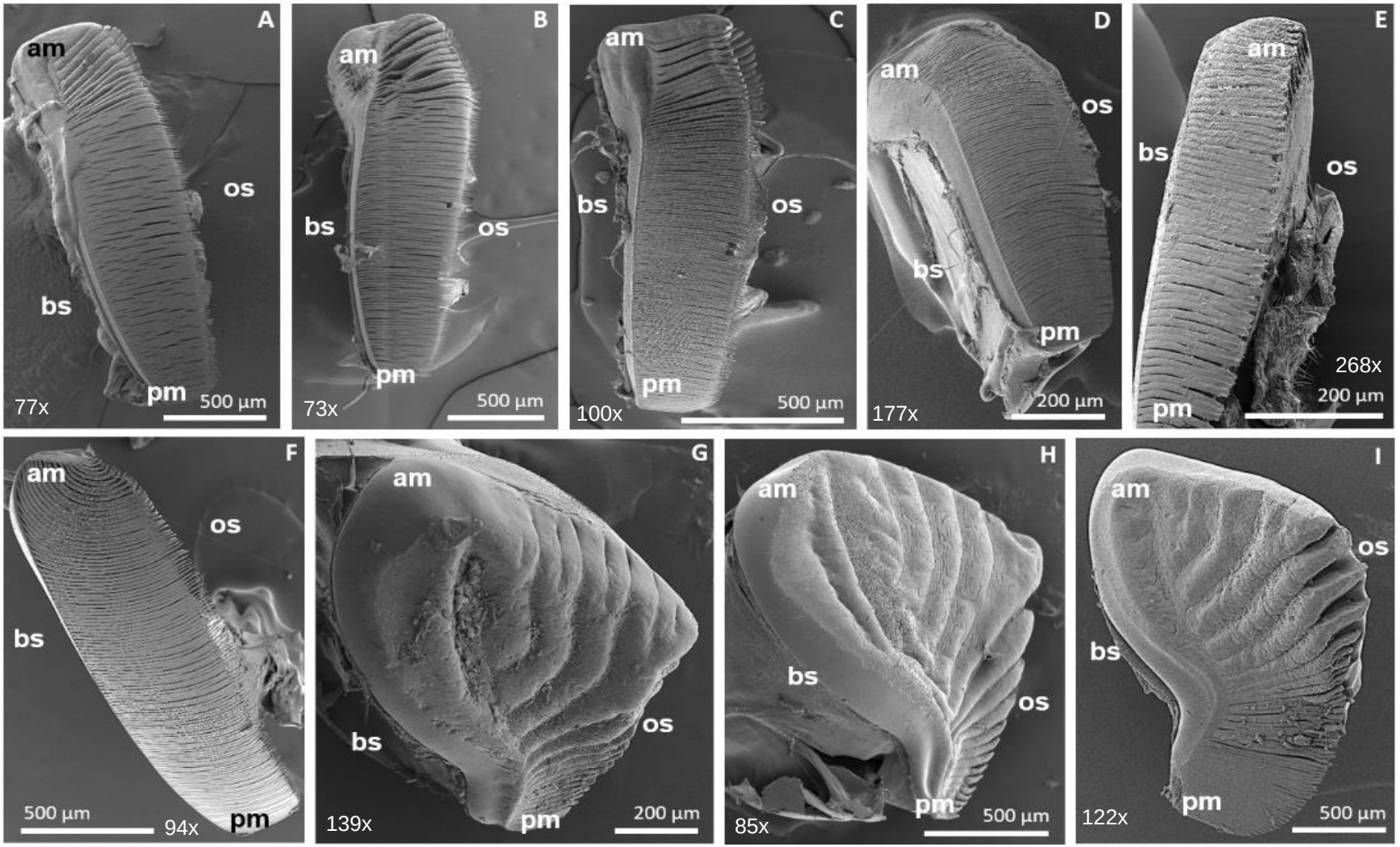


Figure 3.19. Mesal view of convex molar lobes of dung beetle species feeding on a wet diet (A, *Kheper lamarcki*; B, *Kheper nigroaeneus*; C, *Scarabaeus zambesianus*; D, *Scarabaeolus damarensis*), carrion diet (E, *Scarabaeolus carniphilus*), generalist diet (F, *Anachalcos convexus*) and dry diet (G, *Pachysoma endroedyi*; H, *Pachysoma hippocrates*; I, *Pachysoma striatum*). Abbreviations: am, anterior region of molar; bs, blind side of molar; op, open side of molar; pm, posterior region of molar. Scale as indicated per micrograph.

Table 3.4. Absolute and relative mean±SD measurements (in µm) of several dimensions of the concave and convex molars as well as counts of ridges of the concave molar of dung beetle species (cf. figure 2.4). The relative measures were calculated as the ratio between the dimensions measured and the pronotum width. Abbreviations: ccac, concave anterior; ccp, concave posterior; cf, carrion feeder, cva, convex anterior; cvp, convex posterior; df, df feeder; gf, generalist feeder; wf, wet feeder

Species	***n***	**Feeding group**	**cva filtration channel widths**	**cvp filtration channel widths**	**cca filtration channel widths**	**ccp filtration channel widths**	**cva molar ridge height**	**cvp molar ridge height**	**cca molar ridge height**	**ccp molar ridge height**	**Ridge number**
Absolute measures											
1 Kheper lamarcki	4	wf	24.23±3.11	8.16±0.58	21.94±3.15	12.75±1.00	115.19±26.25	75.88±2.74	100.55±19.83	53.16±10.63	±142
2 Kheper nigroaeneus	5	wf	24.01±3.80	8.06±0.75	23.24±2.50	11.55±1.36	101.19±26.71	48.80±13.11	114.11±21.14	57.46±15.90	±116
3 Scarabaeus zambesianus	5	wf	25.96±2.36	7.98±0.86	24.55±2.39	8.27±1.00	132.40±25.94	45.82±6.70	124.00±6.15	42.30±14.65	±131
4 Scarabaeolus damarensis	5	wf	11.82±2.21	4.81±0.28	13.01±1.16	5.63±0.73	64.81±10.56	28.35±3.57	67.54±5.76	29.68±5.63	±122
5 Scarabaeolus carniphilus	5	cf	13.57±1.22	5.33±1.25	14.19±1.83	5.85±0.74	73.21±6.09	38.21±9.07	59.45±7.03	30.39±1.56	±68
6 Anachalcos convexus	4	gf	17.16±1.07	11.44±0.91	17.43±0.33	12.58±0.74	74.77±5.21	54.83±9.13	67.40±1.93	63.84±4.42	±124
7 Pachysoma endroedyi	2	df	40.67±1.61	16.89±2.42	62.97±1.46	19.00±1.81	219.63±45.58	74.32±18.00	277.38±26.47	132.00±7.31	±12
8 Pachysoma hippocrates	4	df	70.10±9.23	32.85±3.43	47.60±8.72	20.52±7.70	242.32±68.54	120.72±14.60	325.62±49.93	168.75±16.72	0
9 Pachysoma striatum	4	df	44.82±4.20	7.98±1.06	30.71±5.74	9.81±1.53	114.05±9.86	75.04±8.77	261.41±80.40	74.95±19.14	±48
Relative measures											
1 Kheper lamarcki	4	wf	1.21±0.06	0.41±0.06	1.11±0.26	0.64±0.08	5.69±0.72	3.80±0.32	5.01±0.79	2.69±0.73	-
2 Kheper nigroaeneus	5	wf	1.40±0.26	0.47±0.02	1.35±0.19	0.67±0.09	5.90±1.72	2.83±0.82	6.61±1.33	3.33±0.96	-
3 Scarabaeus zambesianus	5	wf	1.56±0.28	0.47±0.05	1.46±0.15	0.50±0.10	7.91±1.60	2.72±0.37	7.42±0.88	2.50±0.80	-
4 Scarabaeolus damarensis	5	wf	1.54±0.31	0.62±0.05	1.69±0.16	0.73±0.11	8.43±1.57	3.69±0.58	8.78±1.06	3.86±0.79	-
5 Scarabaeolus carniphilus	5	cf	1.81±0.16	0.71±0.15	1.90±0.31	0.78±0.12	9.78±0.85	5.09±1.10	7.91±0.64	4.06±0.18	-
6 Anachalcos convexus	4	gf	1.34±0.16	0.89±0.05	1.36±0.11	0.99±0.13	5.85±0.72	4.30±0.87	5.26±0.29	4.99±0.48	-
7 Pachysoma endroedyi	2	df	2.86±0.07	1.19±0.15	4.43±0.04	1.34±0.11	15.43±2.98	5.22±1.19	19.50±1.58	9.29±0.65	-
8 Pachysoma hippocrates	4	df	3.84±0.52	1.80±0.22	2.61±0.50	1.12±0.42	13.23±3.57	6.60±0.77	17.83±2.83	9.23±0.88	-
9 Pachysoma striatum	4	df	3.42±0.28	0.61±0.09	2.34±0.40	0.75±0.11	8.75±1.22	5.72±0.59	19.90±5.94	5.73±1.58	-

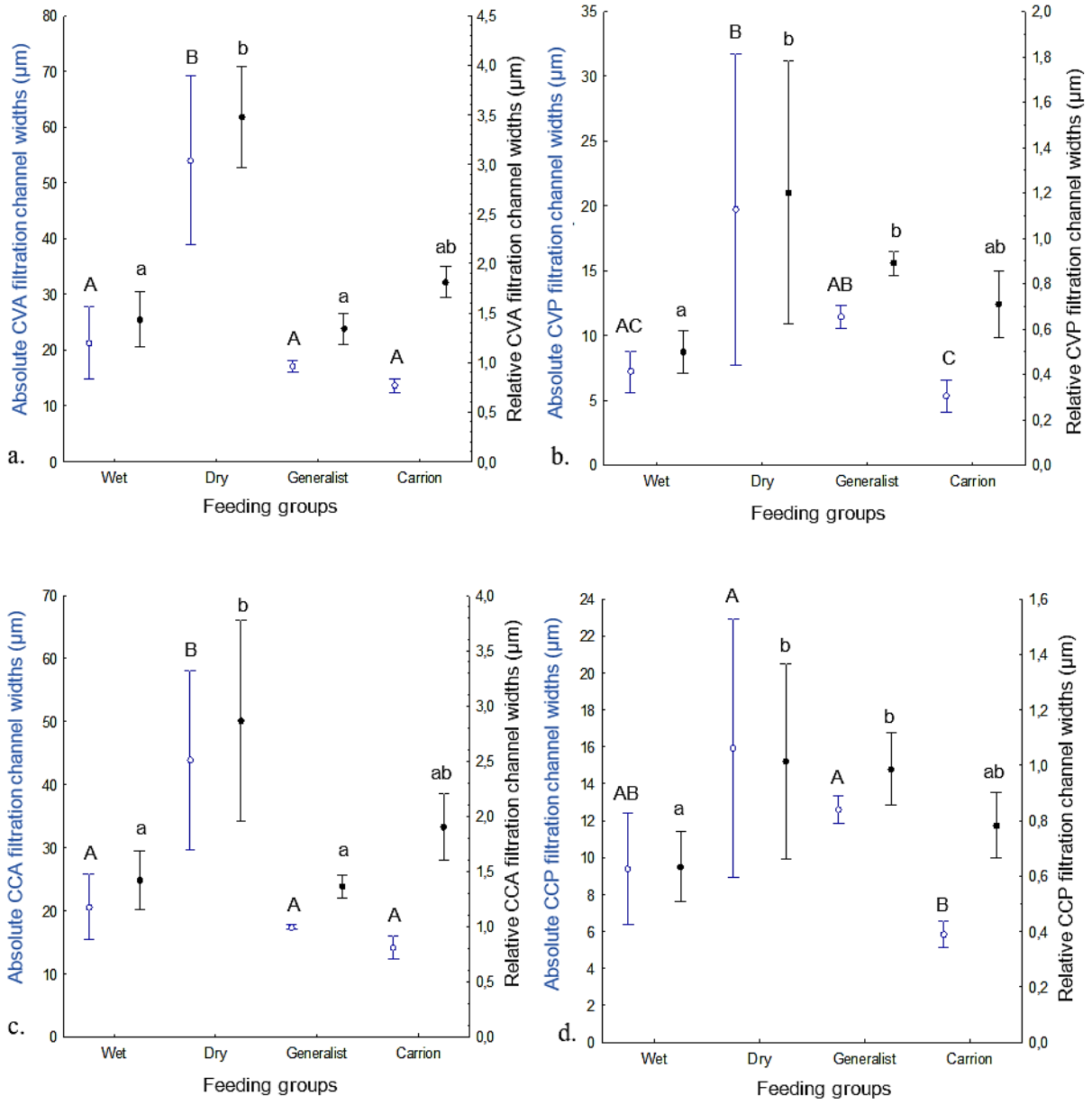


Figure 3.20. Comparison of the mean±SD (box = mean; whisker = SD) of filtration channel widths of concave and convex molar lobes across feeding groups. (a) Absolute and relative convex anterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 24.828; $p < 0.0001$; relative: Kruskal-Wallis test: H (3, N = 38) = 25.918; $p < 0.0001$. (b) Absolute and relative convex posterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 20.186; $p = 0.0002$; relative: Kruskal-Wallis test: H (3, N = 38) = 22.227; $p = 0.0001$. (c) Absolute and relative concave anterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 22.755; $p < 0.0001$; relative: Kruskal-Wallis test: H (3, N = 38) = 24.727; $p < 0.0001$. CVP, convex posterior. (d) Absolute and relative concave posterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 16.539; $p = 0.0009$; relative: Kruskal-Wallis test: H (3, N = 38) = 18.264; $p = 0.0004$. Relative measurements are those that are divided by PW (pronotum width). Uppercase letters denote statistical significance for absolute measures and lowercase letters denote statistical significance for relative measures.

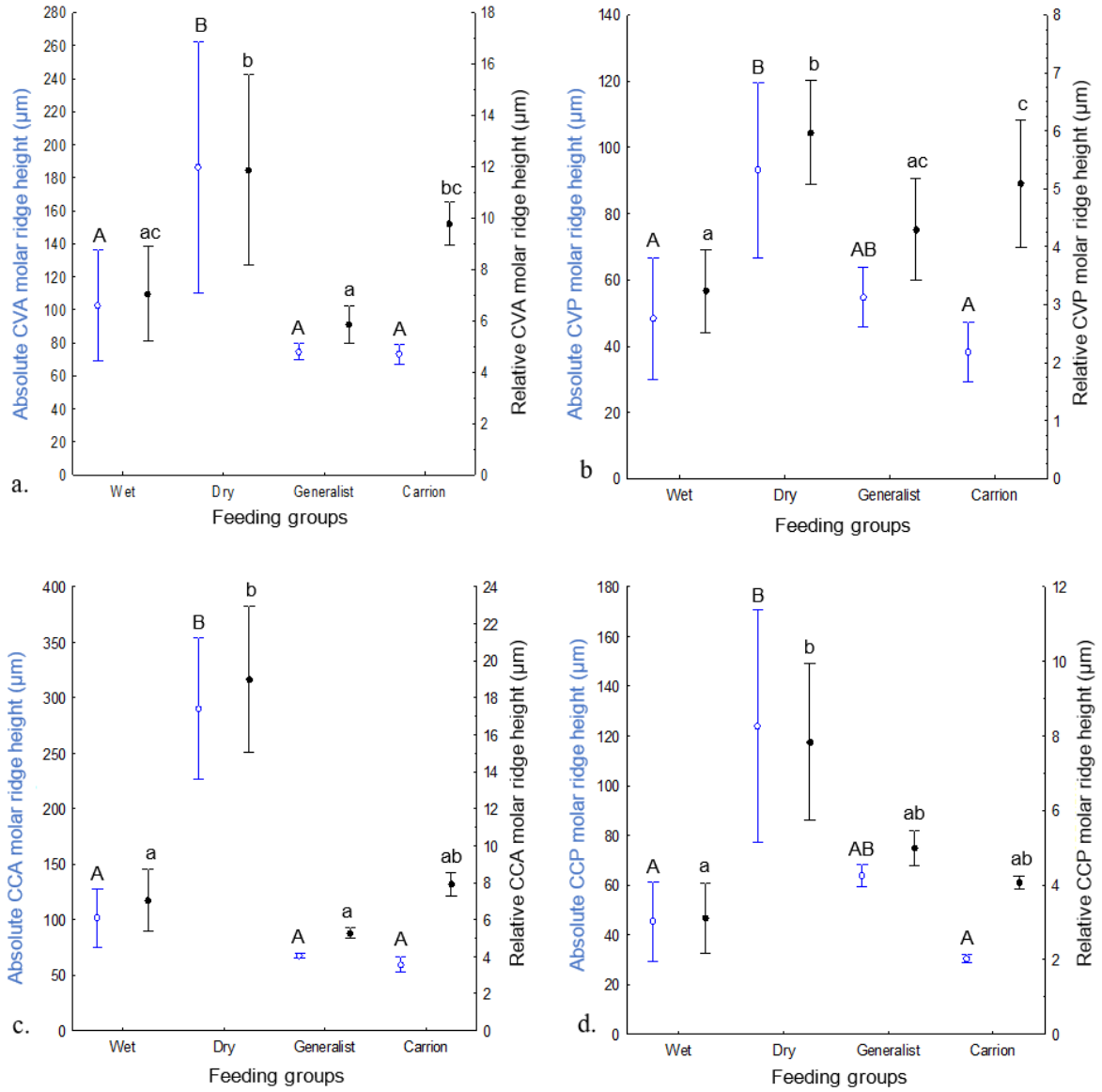


Figure 3.21. Comparison of the mean±SD (box = mean; whisker = SD) of the ridge height of concave and convex molar lobes across feeding groups. (a). Absolute and relative convex anterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 17.986; $p = 0.0004$; relative: Kruskal-Wallis test: H (3, N = 38) = 19.919; $p = 0.0002$. (b). Absolute and relative convex posterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 18.498; $p = 0.0003$; relative: ANOVA: F(3, 34) = 25.237, $p < 0.0001$. (c). Absolute and relative concave anterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 29.052; $p < 0.0001$; relative: Kruskal-Wallis test: H (3, N = 38) = 25.581; $p < 0.0001$. (d). Absolute and relative concave posterior measures. Absolute: Kruskal-Wallis test: H (3, N = 38) = 22.553; $p = 0,0001$; relative: Kruskal-Wallis test: H (3, N = 38) = 25.967; $p < 0.0001$. Relative measurements are those that are divided by PW (pronotum width). Uppercase letters denote statistical significance for absolute measures and lowercase letters denote statistical significance for relative measures.

3.2.4 Epipharynx

Like most members of Scarabaeinae, the wet, dry, carrion and generalist feeding species in this study possess a membranous epipharynx that is partitioned by the clypeo-labral suture (cls) into a distal and proximal section (figures: 3.22 A-F, 3.23 G-I). The epipharynx is located horizontally beneath the clypeus and houses setae of different sizes and arrangements across its surface.

The shape of the apical margin (apm) of the epipharynx typically ranges in shape from being square or rounded to being faintly notched, or bilobed (Nel and Scholtz, 1990). For wet and carrion feeders (figures 3.22 A-E) it is roughly square and straight across the distal edge although for *K. lamarcki* (figures 3.22 A; 3.24 A) it is slightly pointed mesally. In dry feeders, there is a strong indentation in the middle region of the apm and is slightly rounded (figures 3.23 G-I), whereas the generalist feeder *Anachalcos convexus* has a partly bilobed apm (figure 3.22 F).

The apical margin of the epipharynx of wet and carrion feeders is lined with long and narrow setae, known as the acropariae (apf) that are inclined forward for *S. damarensis* (figure 3.22 D) and *S. carniphilus* (figure 3.22 E). The dry feeder's apical fringe is also long and thin (figures 3.23 G-I), except for *P. striatum* (figure 3.23 I) which has thick setae whereas the middle region of the apical margin of the generalist feeder consists of hairs that are short and fine but longer and thicker in the lateral regions (figure 3.22 F).

In the medio-anterior region of the epipharynx, a thick brush of setae known as the median brush (mb) is present in wet feeders (figure 3.24 C, F, I; 3.25 C) (although for *S. zambesianus* part of the brush is extended on a short stalk), the carrion feeders (figure 3. 25 F) and the generalist feeder (figure 3.25 I). The medio-anterior region of dry feeders instead holds a sclerotized epipharyngeal tooth (et) that is presumably a substitute for the median brush (Holter and Scholtz, 2011) found in the other feeding groups. This epipharyngeal tooth assumes a triangular prism shape for *P. endroedyi* (figure 3.26 C) and *P. hippocrates* (figure 3.26 F) while for *P. striatum* (figure 3.26 I) the tooth is long and thin with a wide base and blunt tip.

The epipharyngeal tooth is assumed to work in conjunction with the median thorn (mt) (figure 3.27 G, H, I) located in the middle region of the clypeal scraper (cs) (figure 3.27 G, H, I) (the ridge found beneath the border of the clypeus) (Harrison and Philips, 2003) when incising and breaking down large pieces of dry food into smaller ones. This structure is only present in all three dry feeding species (figure 3.27 G, H, I). Furthermore, on the epipharynx posteriorly

extending from the tooth is another sclerotized structure known as the anterior median process (amp) (figure 3.26 C, F, I). This sclerotized part is not as conspicuous in the wet, carrion and generalist groups as it is with dry feeders. The anterior median process in all feeding groups is surrounded by a mass of compact, small and thin spines of setae that progress into the posterior median tormal process (pmp) (figures 3.24 – 3.26).

The lateral regions of the epipharynx of wet (figures 3.22 A, B, C, D; 3.24 B, E, H; 3.25 U) and carrion feeders (figures 3.22 E, 3.25 E) consists of two rows of setae making up the chaetopariae/lateral comb (lc) where one row consists of regular, narrow hairs that start long in the distal part of the epipharynx and become shorter along the length of the proximal part of the epipharynx (lc_1). The inner, second row of hairs forms an irregular row of setae that is usually shorter and thicker than the first row (lc_2). Unlike the wet feeders, dry feeders only possess a single row (lc_1) of the lateral hairs that are long and thin and in fact larger (figures 3.23; 3.26 B, E, H) than those of the wet feeders. The second row (lc_2) is absent in *P. hippocrates* (figure 3.26 B) and *P. endroedyi* (figure 3.26 E) but short, thick and irregular for *P. striatum* (figure 3.26 H) even though it is highly reduced although not visible on figure 3.23 I.

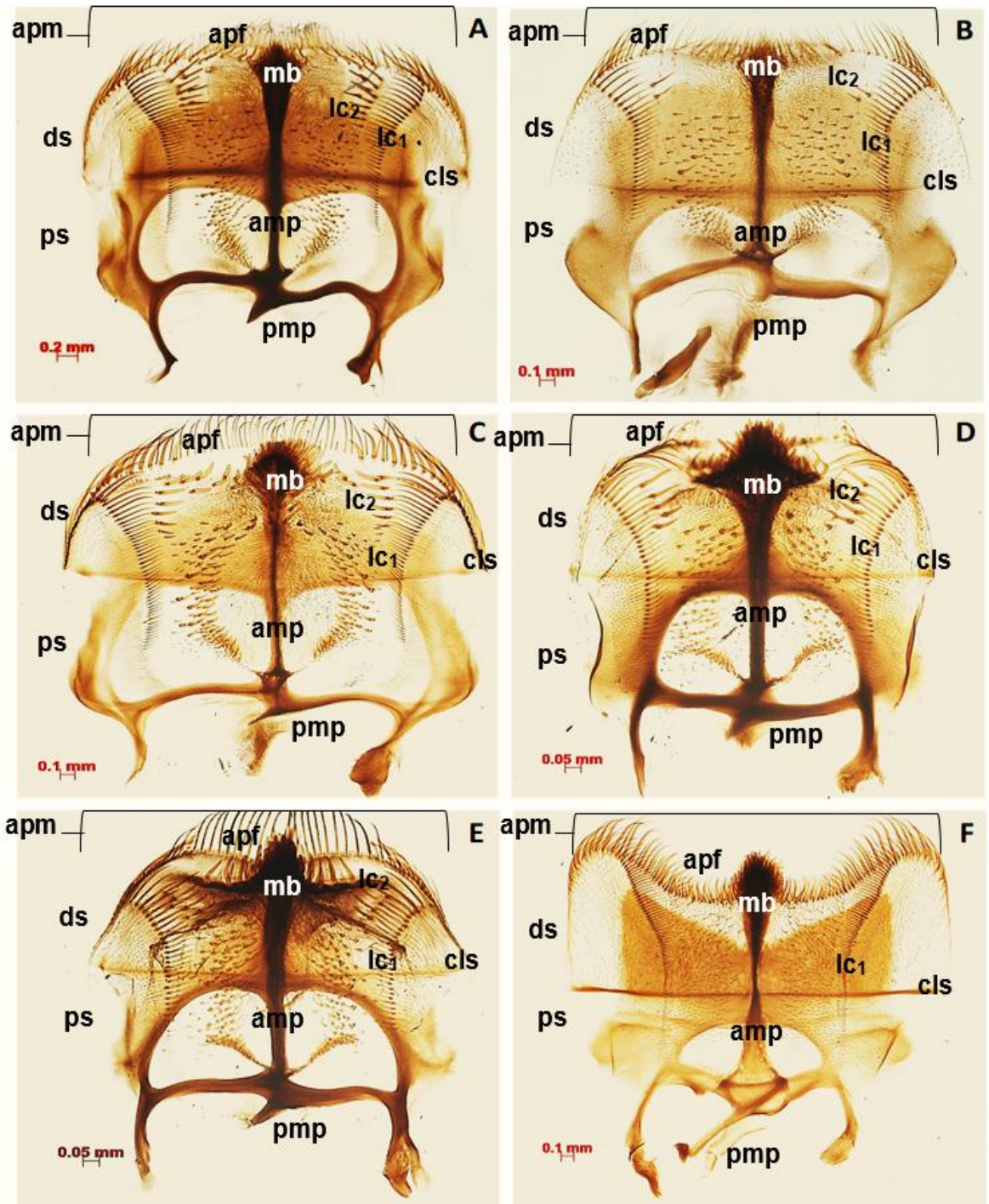


Figure 3.22. Light micrographs of the ventral view of the epipharynx of wet feeders (A, *Kheper lamarcki* at 23x; B, *Kheper nigroaeneus* at 24x; C, *Scarabaeus zambesianus* at 29x; D, *Scarabaeolus damarensis* 71x), carrion feeder (E, *Scarabaeolus carniphilus* at 70x) and generalist feeder (F, *Anachalcos convexus* at 31.5x) dung beetle species. Abbreviations: amp, anterior median process; apf, apical fringe; apm, apical margin; cls, clypeo-labral suture; ds, distal section; lc_1, first row of the lateral comb; lc_2, second row of lateral comb; mb, median brush; pmp, posterior median tormal process; ps, proximal section. Scale as indicated per micrograph.

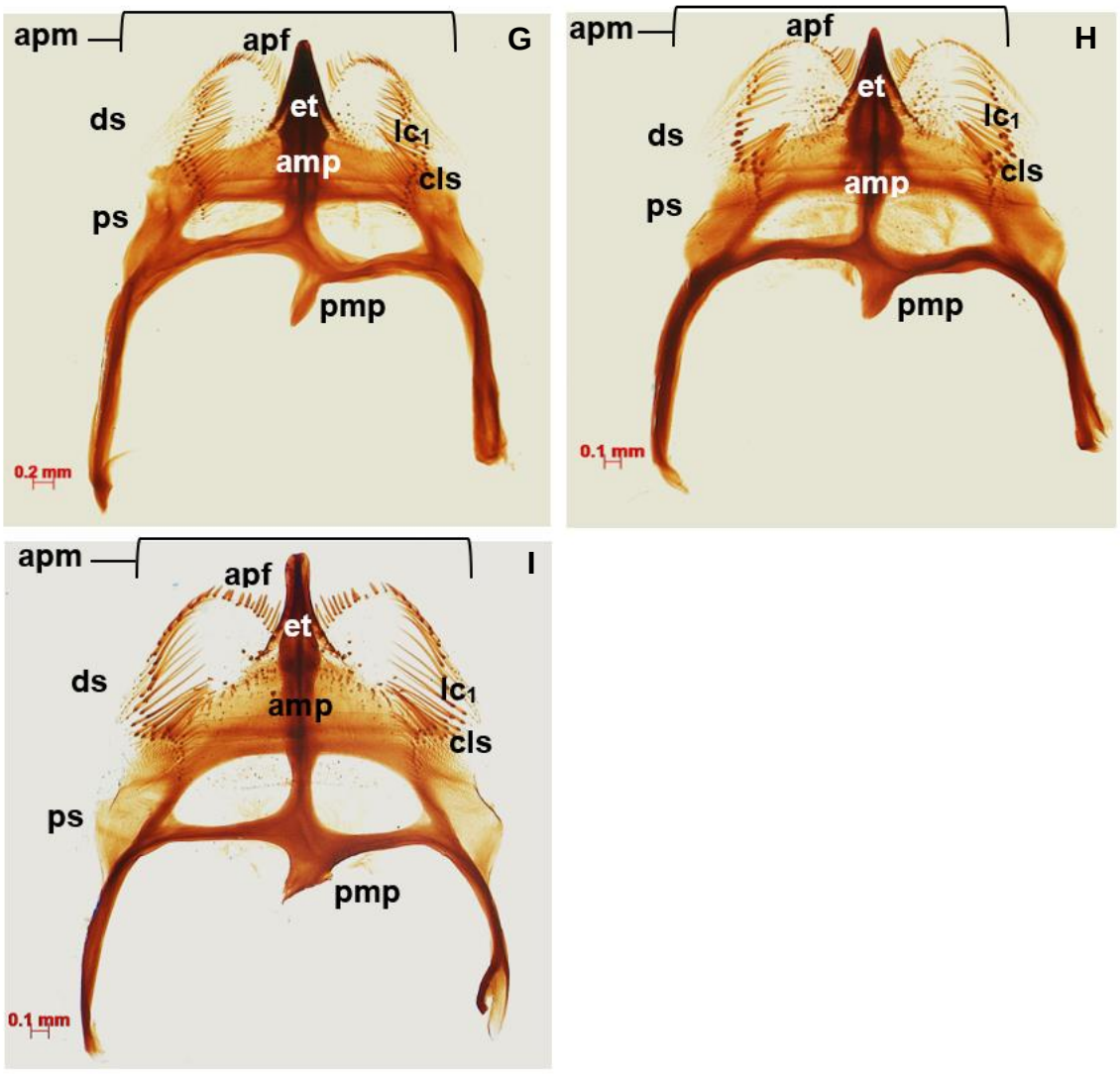


Figure 3.23. Light micrographs of the ventral view of the epipharynx of dry feeding dung beetle species (G, *Pachysoma endroedyi* at 32x; H, *Pachysoma hippocrates* at 22x; I, *Pachysoma striatum* at 34.5x). Abbreviations: amp, anterior median process; apf, apical fringe; apm, apical margin; cls, clypeo-labral suture; ds, distal section; et, epipharyngeal tooth; lc_1, first row of the lateral comb; pmp, posterior median tormal process ps, proximal section. Scale as indicated per micrograph.

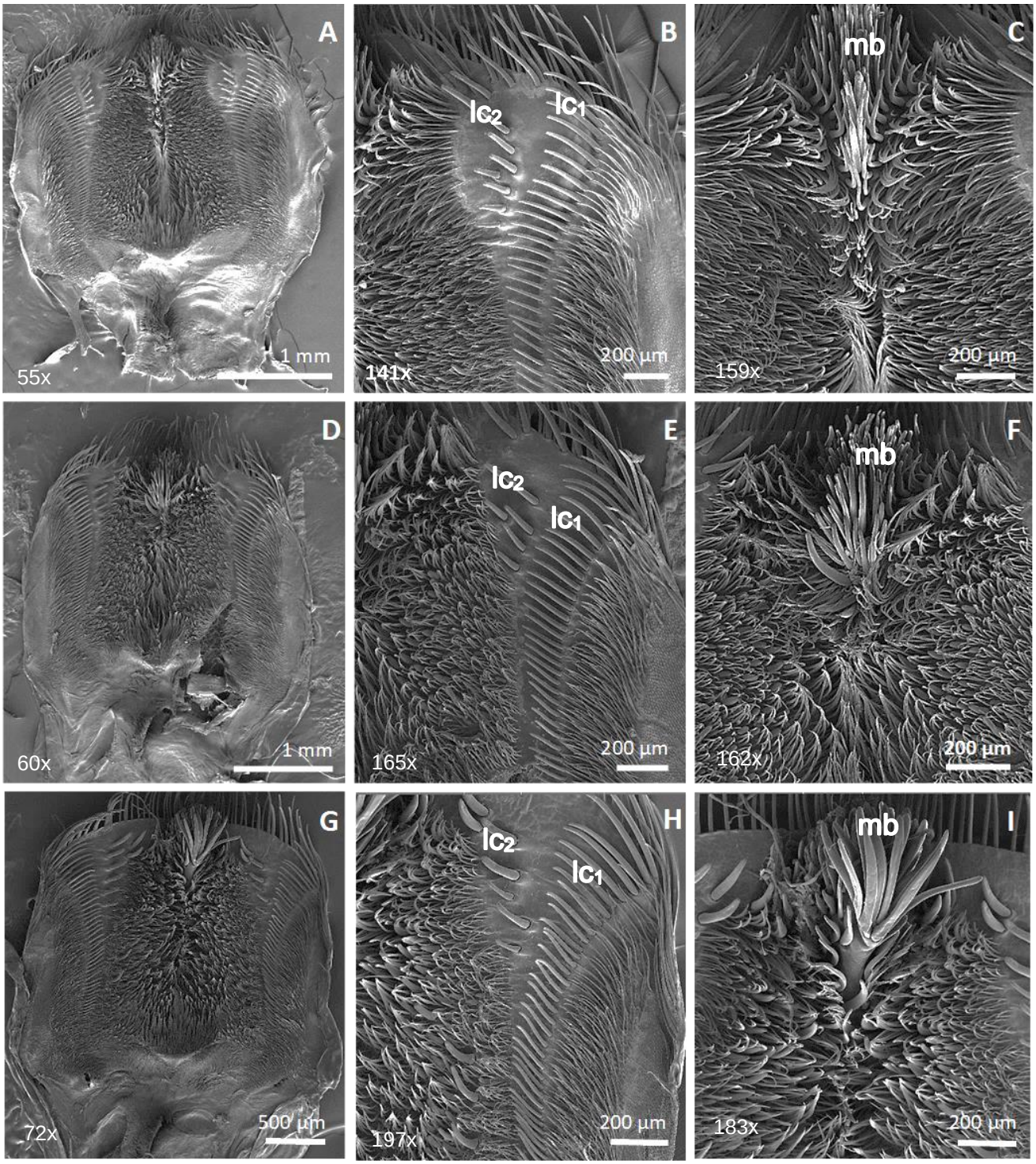


Figure 3.24. Components of the epipharynx of wet feeding dung beetle species. (A-C) *Kheper lamarcki*. (D-F) *Kheper nigroaeneus*. (G-I) *Scarabaeus zambesianus*. (A, D, G) Ventral view of the epipharynx. (B, E, H) Lateral aspects of the setal variation and arrangements. (C, F, I) Medio-anterior area. Abbreviations: lc_1, first row of the lateral comb; lc_2, second row of lateral comb; mb, median brush. Scale as indicated per micrograph.

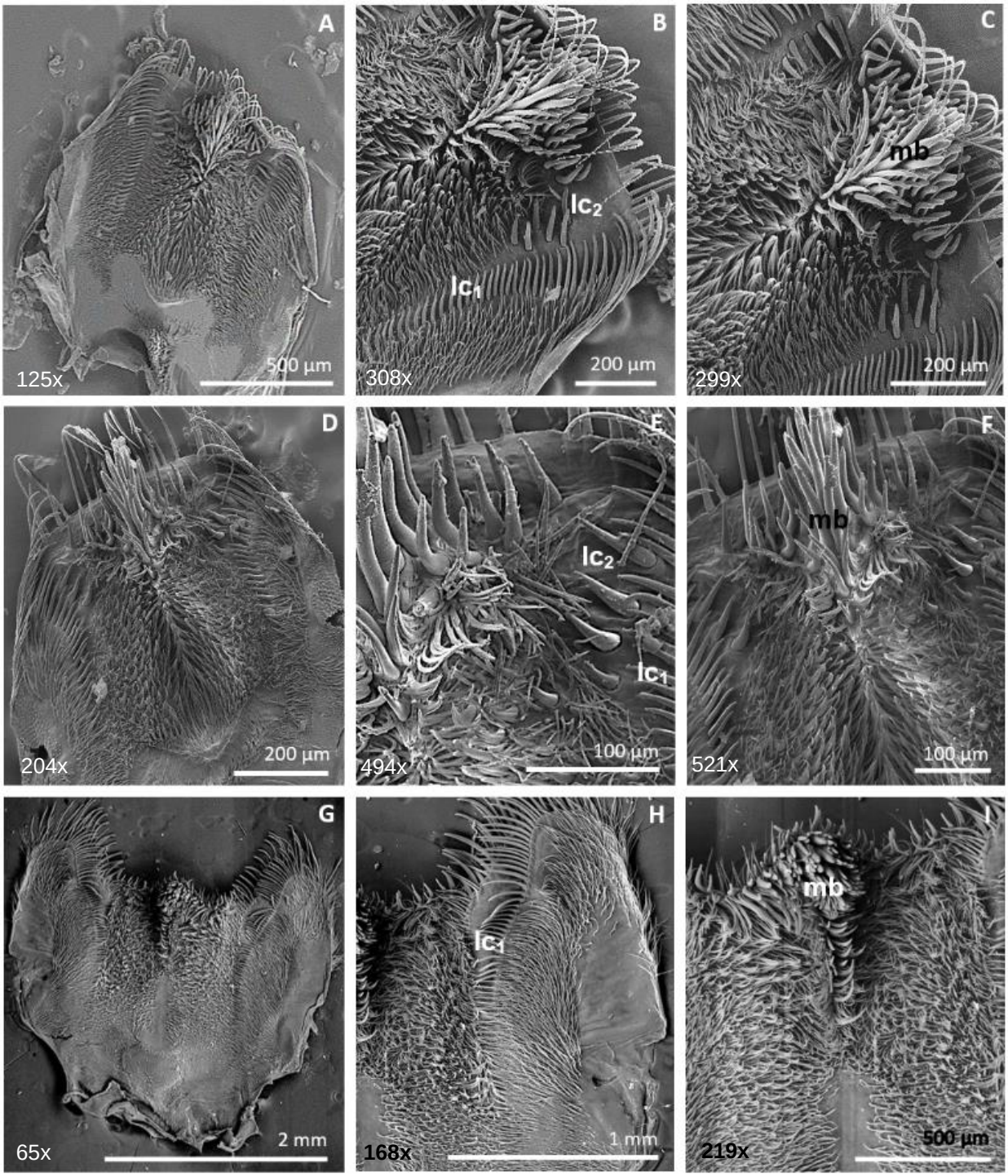


Figure 3.25. Components of the epipharynx of wet feeder (A-C) *Scarabaeolus damarensis*, carrion feeder *Scarabaeolus carniphilus* (D-F) and (G-I) generalist feeder *Anachalcos convexus*. (A, D, G) Ventral view of the epipharynx. (B, E, H) Lateral aspects of the setal variation and arrangements. (C, F, I) Medio-anterior area. Abbreviations: lc_1, first row of the lateral comb; lc_2, second row of lateral comb; mb, median brush. Scale as indicated per micrograph.

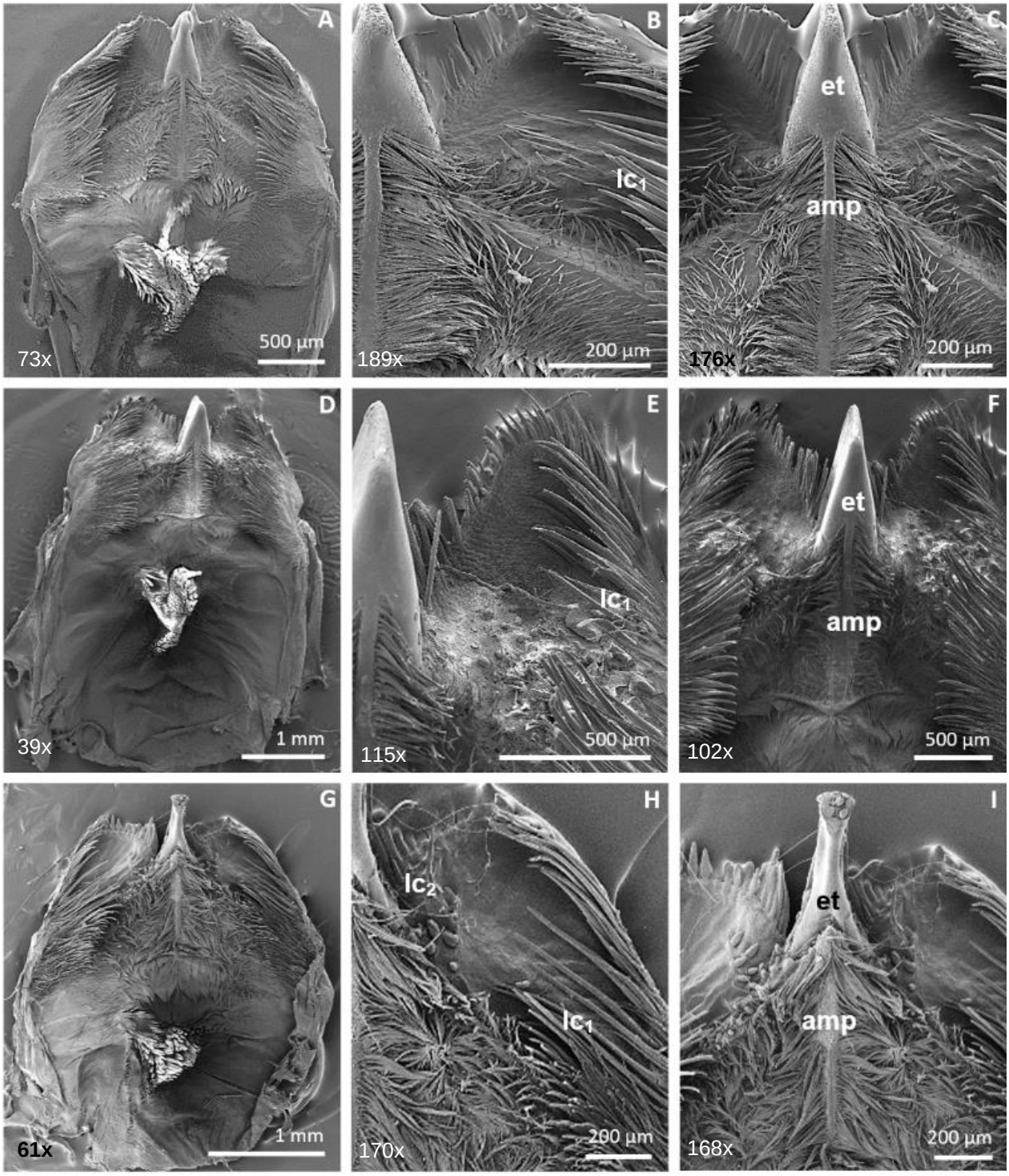


Figure 3.26. Components of the epipharynx of dry feeding dung beetle species. (A-C) *Pachysoma endroedyi*. (D-F) *Pachysoma hippocrates*. (G-I) *Pachysoma striatum*. (A, D, G) Ventral view of the epipharynx. (B, E, H) Lateral aspects of the setal variation and arrangements. (C, F, I) Medio-anterior area. Abbreviations: amp, anterior median process; et, epipharyngeal tooth; lc_1, first row of the lateral comb; lc_2, second row of lateral comb. Scale as indicated per micrograph.

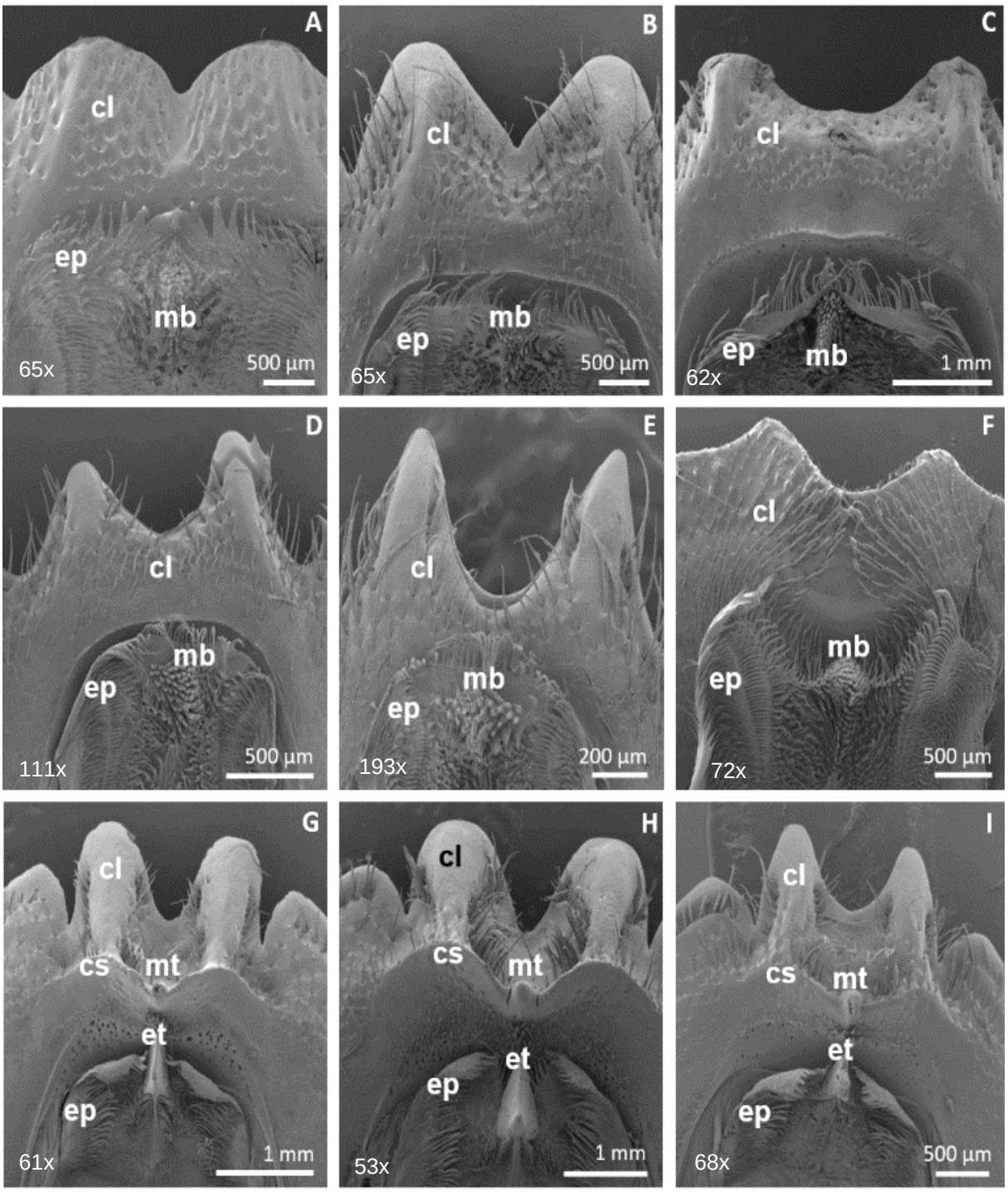


Figure 3.27. Ventral view of the clypeus and the distal part of the epipharynx of wet feeders (A, *Kheper lamarcki*; B, *Kheper nigroaeneus*; C, *Scarabaeus zambesianus*; D, *Scarabaeolus damarensis*), carrion feeder (E, *Scarabaeolus carniphilus*), generalist feeder (F, *A.convexus*) and dry feeders (G, *Pachysoma endroedyi*; H, *Pachysoma hippocrates*; I, *Pachysoma striatum*), Abbreviations: cl, clypeus; cs, clypeal scraper; ep, epipharynx; et, epipharyngeal tooth mb, median brush; mt, median thorn. Scale as indicated per micrograph.

3.3. Epipharynx shape analysis

Geometric morphometric analysis was used to analyse differences in the shape of the epipharynx between species. The tangent vs Procrustes distance analysis indicates a substantial approximation of the linear tangent space to the non-linear shape space (slope: 0.996, correlation: 0.999998, root of MS error: 0.000078). This confirms that the geometrical heterogeneity in shape (i.e., the shape variation of the epipharynx) in the data set is small enough to continue with subsequent geometric morphometric analyses across species.

The scatterplot of the first two principal component analyses depicts four separate clusters (generalists, dry feeders and two distinct wet feeding groups) (figure 3.28). The cumulative shape variation of the epipharynx that is accounted for by the first two principal components is 66.24%. The occurrence of two distinct sets of wet feeding groups suggests that the grouping is based on traits that these wet feeding species share because of their taxonomic relationships rather than morphological adaptation to a particular diet because these wet feeding groups belong to different tribes despite feeding on a very similar diet. Nonetheless, the epipharynx of the wet feeders overall is noticeably different to that of the dry feeders and generalist feeders but overlaps with the carrion feeders (probably because they belong to the same genus).

The outcome of the Discriminant Function analysis, particularly the significant Procrustes *p* – values (which compares the mean shape of each feeding group with other feeding groups) and the significant Mahalanobis *p* – value (which determines the covariance and distance between points in a multivariate space) confirmed a significant epipharyngeal shape difference between the carrion and dry feeders clusters, a difference between the dry feeders and generalist cluster, a difference between the dry and wet feeders and a difference between the generalist and wet feeders (table 3.5).

For the carrion and generalist feeder's clusters, the Mahalanobis *p* – value is not significant due to the low level of covariance. This could be due to the fact that the carrion and generalist feeding groups only consist of one species each with very few individuals compared to other feeding groups. However, the mean shape of the epipharynx between the carrion and generalist feeders is significantly different as shown by the Procrustes *p* – value (table 3.5). The analysis between the carrion and wet feeders' clusters showed a Mahalanobis *p* – value that is significant, however the mean epipharyngeal shape between these clusters are not significantly different as the Procrustes *p* – value is not significant (table 3.5). The mean

shape differences between feeding groups which correlate with the Procrustes *p* – values are also demonstrated by the transformation grids shown in figure 3.29.

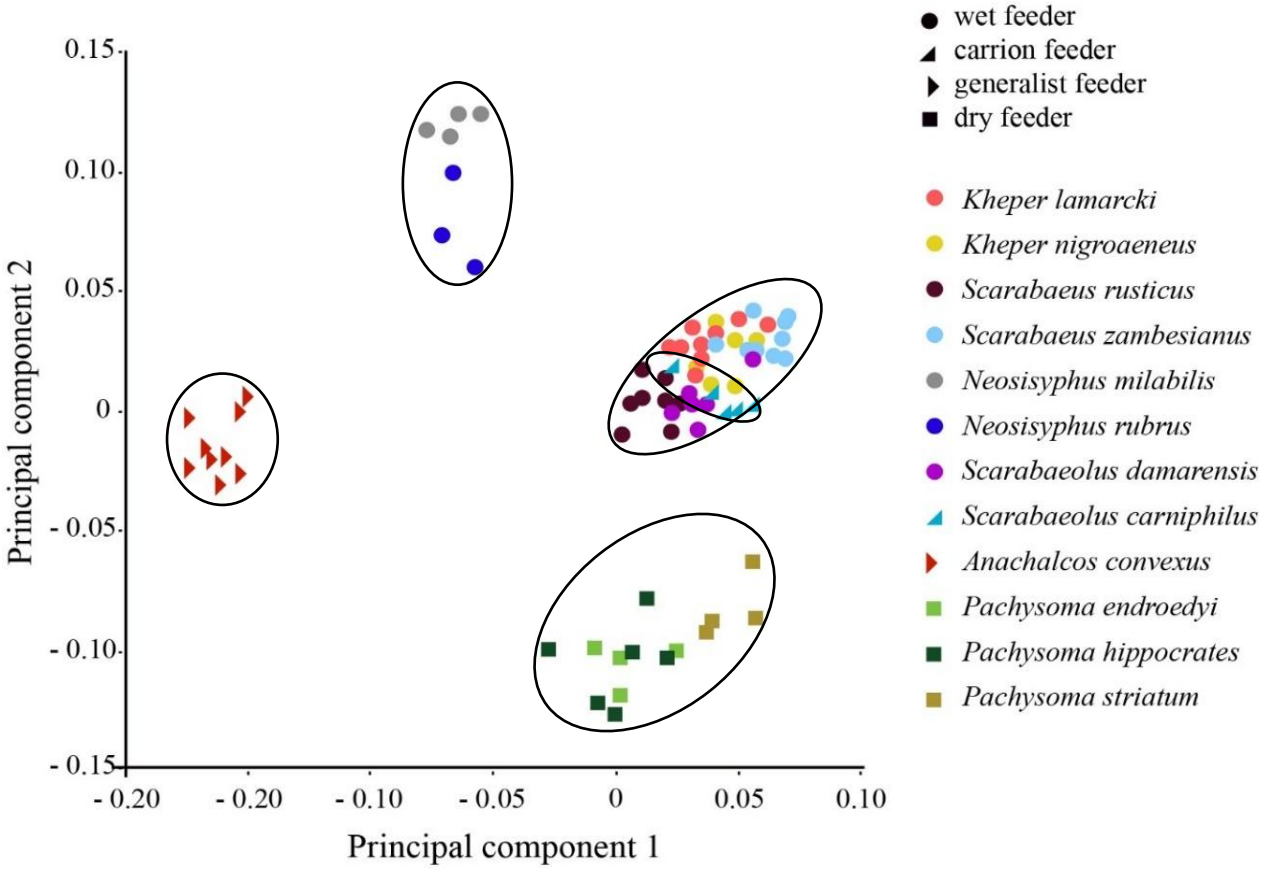


Figure 3.28. Scatterplots of the first two principal component analysis (PCA) scores depicting the morphological variation of the epipharynx of 12 species belonging to the tribes Scarabaeini, Canthonini and Sisyphini (Coleoptera, Scarabaeinae). PC 1 accounts for 40.84% of the variance while PC 2 accounts for 25.40% of the variance.

Table 3.5 Discriminant Function outputs across the three feeding groups. Abbreviations: cf, carrion feeders; df, dry feeders; gf, generalist feeders; wf, wet feeders.

	cf – df	**cf – gf**	**cf – wf**	**df – gf**	**df – wf**	**gf – wf**
Mahalanobis distance	54.4625	62.6999	4.2703	71.2402	17.8239	19.7443
Mahalanobis distance *p* – value	0<.0001	0.0887	0<.0001	0<.0001	0<.0001	0<.0001
Procrustes distances	0.1406	0.2109	0.0579	0.2207	0.1409	0.1976
Procrustes distances *p* – value	0<.0001	0.0004	0.0759	0<.0001	0<.0001	0<.0001

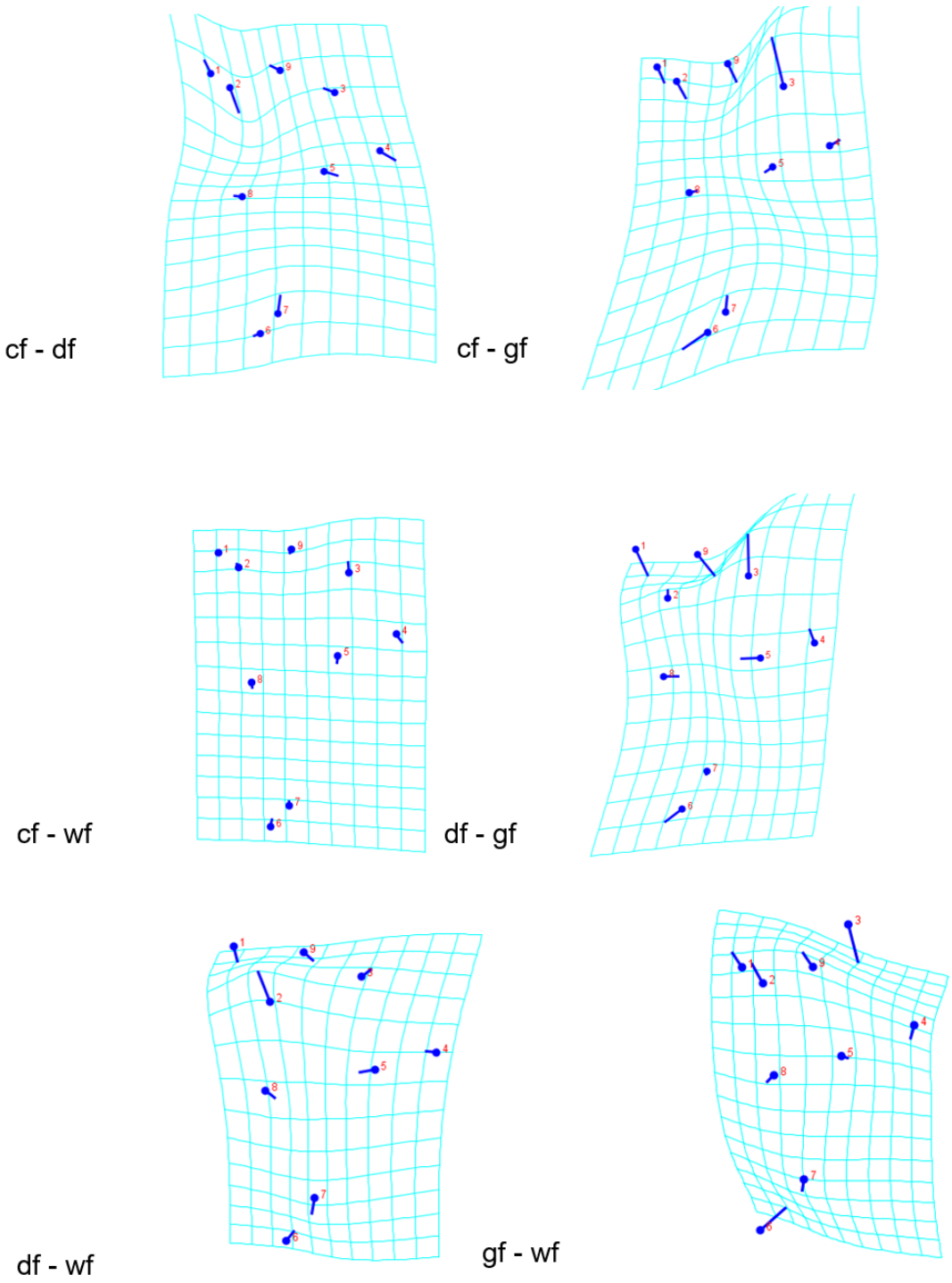


Figure 3.29. Transformation grid depicting differences between various feeding goups. See their level of significance from the Discriminant Function outcome (table 3.5). Abbreviations: cf, carrion feeders; df, dry feeders; gf, generalist feeders; wf, wet feeders.

3.4 Literature cited

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Chapter IV: Discussion

The overarching purpose of this study is to understand how dung beetles subsist on the faeces of mammalian herbivores. By analysing the morphology of dung beetle mouthparts, Miller (1961) and Hata and Edmonds (1983) established that dung beetles possess a set of specialized and complex mouthparts that enable them to filter a food source that is fundamentally made up of nutrient-poor food items. However, the mode of operation of these mouth parts and their role in particle feeding has not yet been fully determined. Thus, this study investigated the morphology of dung beetle mouthparts both qualitatively and quantitatively to understand the role that various components of the mouth play in the process of particle feeding.

4.1 Mandibles

Dissection of the mandibles showed that all the feeding groups have soft and membranous, dorsoventrally flattened incisor lobes and sclerotised molar lobes. Although the dry feeders examined in this study were mainly pellet and detritus feeders, they appear to have retained the membranous incisor lobes and therefore lack the ability to use their incisor lobes to cut through food (Holter and Scholtz, 2011).

The length and the width of the mandibles were measured across feeding groups (table 3.2). These dimensions were similar across all feeding groups except the mandible length and width of wet feeders which were significantly greater than those of carrion feeders. This could be as a result of body size differences because the wet feeders have larger body sizes than the carrion feeders; in addition, the relative mandible length of wet feeders was not significantly different to that of carrion feeder (Table 3.1). Furthermore, the relative mandible widths were similar across feeding groups, however, the relative mandible length of dry feeders were significantly shorter than all the feeding groups. Although this study has highlighted the variations in these dimensions, it is not known how these size differences affect the particle feeding process.

Fitted between the molar lobe and the incisor lobe is the conjunctivuse. The conjunctivuse for wet, carrion and the generalist feeders, was larger than the one found in dry feeders where it was seen to be very reduced in *P. striatum* (figure 3.5) and *P. hippocrates* (figure 3.4) and

almost absent in *P. endroedyi* (figure 3.4). According to Holter (2004), the presence of the conjunctivuse is more prevalent in all adult dung beetles feeding on wet dung than other types of beetles. This study has shown that this structure is important in carrion and generalist feeders. The function of this structure is not known (Holter and Scholtz, 2011); however, a morphological study of the mouth parts of *Canthon pilularius* (Linnaeus) by Hata and Edmonds (1983) suggests that the conjunctivuse might be involved in resisting the backflow of liquid dung when it is being squeezed between the molar surfaces considering how the shoulders of the hypopharynx fit right into the conjunctivuse (Hata and Edmonds 1983).

Hata and Edmonds (1983) indicated that the molar tapetum covers about 20% of the convex molar and approximately 50% of the concave molar. This observation was likewise observed (but not measured in this study) in the wet, carrion and generalist feeders examined in this study. Despite being present in dry feeders, the molar tapetum is reduced to a point where it does not cover the molar surface. The significance of this reduction might be caused by the use of the molar surface for grinding rather than squeezing dung. Covering the molar surface for dry feeders would probably interfere with the grinding process and would result in the tapetum getting damaged in the process.

Given that the molar tapetum originates from the blind side of both the concave and the convex molars (figures 3.14 A, E; 3.15 A, E; 3.16 A, D; 3.17 A, E; 3.18 A), Hata and Edmonds (1983) suggest that the tapetum could act as a seal that occludes the escape of liquid from the mouth when dung is being pressed between the molars. I believe this to true for wet, carrion and generalist feeder.

4.2 Incisor lobe components

Prior to ingestion, it is believed setae lining the incisor lobe are largely involved in removing fibrous plant material (Madle, 1934; Hata and Edmonds, 1983). Lining the inner margin of the incisor lobe is the prosthecal comb. This comb is easily recognised in wet, carrion and generalist feeders by the hook-like bifurcations emerging from the ventral surface of each individual seta. However, in dry feeders, the few bifurcations on the prosthecal comb are more linear in shape than hooked and tend to be shorter in length. The exact function of these bifurcations has not really been determined, however, because the wet, carrion and generalist feeders examined in this study generally exploit wet dung, the hook-like shape could allow

for long, thin and flat fibrous material to be captured and entangled during the filtration process.

Since dry feeders have few bristles that contain bifurcations, the length of their prosthecal comb is reduced. This reduction in dry feeders could be linked to the fact that dry feeders do not need to eliminate the plant material found in dung because they possess a grinding apparatus that enables them to utilise large plant material pieces (Holter and Scholtz, 2011). I therefore suspect that the prosthecal comb in dry feeders could be vestigial. Its presence probably aids in the movement of food from the distal part of the mouth to the molar lobes where it will be ground up but is not involved in filtering food. Moreover, the gaps between the prosthecal comb of dry feeders are significantly wider than those of wet feeders and generalists, making the passage of large particles possible compared to the other feeding groups that restrict ingestion of food to small particles. The distal comb found at the tip of the incisor lobe is plumose in nature for wet feeders (except for *Scarabaeolus damarensis*) and the generalist feeding group and lacks bifurcations. This study suggests that the distal comb alongside the apical fringe is involved in transporting dung from the sclerotised and external mouthparts (maxilla, maxillary palps, galea etc) to the inner part of the mouth and are also involved in collecting plant fragments ready to be discarded from the prosthecal comb out the mouth.

The size ranges of the gaps between the prosthecal comb and the distal comb are between 2.40 – 5.72 µm and 5.08 – 15.79 µm (table 3.2) respectively. In light of this, Holter et al., (2002), Holter (2004), Holter and Scholtz (2005, 2007) found ingested dung particle size ranges between 2 – 5 µm and 130 µm for various adult dung beetles species whereas Madzhive et al., 2020 found ingested dung particle size range between 1.4 and 2000 µm for *Scarabaeus goryi* which suggests that despite the prosthecal and distal combs having narrower gap size ranges, dung particles larger the these comb gaps are able to bypass it. This study therefore proposes a rake-like action by the prosthecal comb and its bifurcations. Like a rake, it operates by retaining minute and nutritious dung particles that fall within the range of the prosthecal comb gaps but its structural configuration does not allow it to be as restrictive as a typical sieve or filter with a mesh would be, hence large particles that could not get entangled by the bifurcations are able to bypass the “rake mechanism” and are then transferred from the distal part of the mouth to the inner part of the mouth and later ingested. Although some particles bypass the prosthecal comb, the elimination of the first batch of

indigestible plant fragments with a high C/N would have contributed to the initial step of concentrating the food and thus elevating nutrient levels (Holter and Scholtz, 2007; Madzivhe et al., 2020).

4.3 Molar lobes and filtration channel sizes in relation to particle size and Holter's (2000) hypothesis

To date, the role of the molar lobe's filtration channels in particle feeding has not been entirely understood. Madle (1934) suggested that the collected dung gets squeezed between the molar lobes, resulting in a fine particulate mixture of all the nutrient rich food items passing though the filtration channels and subsequently pouring into the pharynx. For these channels to function as a true filter typically would, Madle (1934) further proposed that the size of dung particles passing through these channels cannot exceed the width of the channels, implying that the size of dung particles in the gut is determined by the width of the channels. However, observations made on *Aphodius fimetarius* and *A. fossor* by Holter (2000) show that despite the presence of a supposed filtering apparatus, the midgut content of these dung beetles contained particles larger than the widths of their filtration channels.

The discrepancy between the ingested dung particle size range and filtration channel widths was first explained by Holter (2000) where he suggested that the morphology of the molar surfaces indicates that dung beetles squeeze the collected dung between the molar lobes as Madle (1934) suggested, followed by excess liquid being extracted from the dung through the filtration channels and concentrating the food in the process (Holter, 2000). He further explains that the liquid flows away from the pharynx and is eliminated out of the mouth (Holter, 2000) because superfluous water would force the gut to have to deal with great volumes of water (Holter, 2016). However, the principal findings of this study have brought to light additional factors that questions this basic premise.

The quantitative analysis of the filtration channel widths of both molars (both anterior and posterior) (table 3.4) show that the channels are incapable of only eliminating superfluous liquid because the widths of these channels are not small enough to impede the passage of dung particles. Holter (2016) states that there is no experimental evidence supporting the loss of good quality dung particles when the liquid is being eliminated, however, figure 4.1 shows the filtration channels of the carrion feeder *Scarabaeolus carniphilus* with a paste-like consistency of dung wedged within the channels after a meal. This image provides some

perspective on why a pasty consistency of small dung particles is typically found in dung beetles' gut (Holter, 2000). Had these channels been solely used for eliminating excess water, perhaps they would be in a much cleaner state than they are shown in (figure 4.1).

Scattered between the fibrous plant material and water in dung are minute particles of living and non-living microbial matter that contribute between 50 – 60% of the faecal nitrogen (Holter, 2016). Lohnis and Fred (1923) indicate that bacteria contributes almost 10 – 20% of the nitrogen in dung (dry weight measure). Depicted in figure 4.2 are unidentified worm-like organisms that are covered in what resembles rod-shaped gut bacteria (with a body size length that is less than 1.5 µm). With the filtration channels widths ranging between 4 µm (smallest dung beetle studied) and 25 µm (largest dung beetle studied) (table 3.3) for the wet, carrion and generalist feeder, it is quite probable that large masses of bacteria would easily pass through the channels and be discarded when the water is being squeezed out considering that bacteria, on average have body size between 0.5 - 5 µm. Moreover, Aschenborn et al. (1989) and Edwards (1991) found that the liquid component of dung with minute particles suspended in it is the main food supply for dung beetles, so the elimination of water would carry with it lots of valuable food.

Edwards and Aschenborn (1988) observed *Kheper nigroaeneus* exploiting dung of varying water contents from wet, cow and horse dung to drier, impala and giraffe dung. The drier impala and giraffe dung have a higher nitrogen content (Paetel, 2002) than wet cow dung. However, wet feeders such as *Kheper nigroaeneus* choose wet dung over dry dung despite the lower nitrogen content of wet dung (Paetel, 2002). Moreover, a study on *Euoniticellus intermedius* by Edwards (1991) demonstrated that *Euoniticellus intermedius* is more likely to breed and feed on dung with a 65 – 78% water content and will not breed or feed in dung with a water content below 65%. It is therefore counterintuitive for dung beetles to prefer dung that has a higher water content, to later use their filtration channels to get rid of it. Furthermore, an image of *Circellium bacchus* (figure 4.3) feeding on wet cow dung shows no indication of excess liquid being eliminated during the feeding process. Instead, a pile of fibrous plant material has been filtered out of the wet dung.

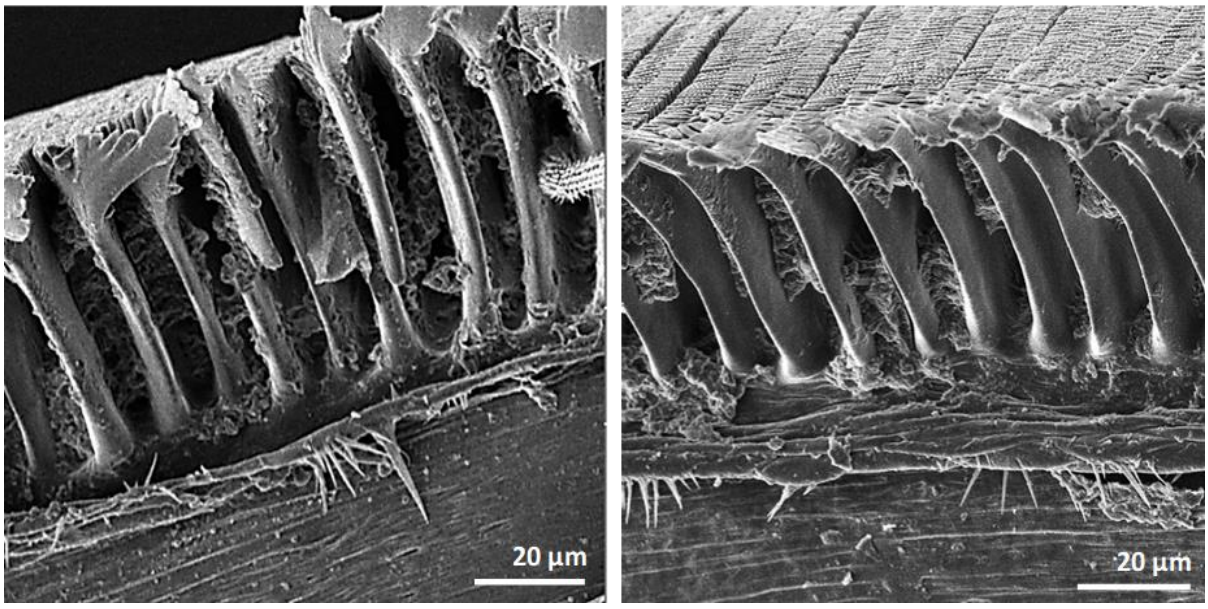


Figure 4.1. The condition of the filtration channels of the carrion feeder *Scarabaeolus carniphilus* following a cow dung meal. Dung paste consisting of minute particles and other foreign particles are caught within the filtration channels. Although it feeds on carrion, it was attracted to cow dung and was found feeding on it in the field.

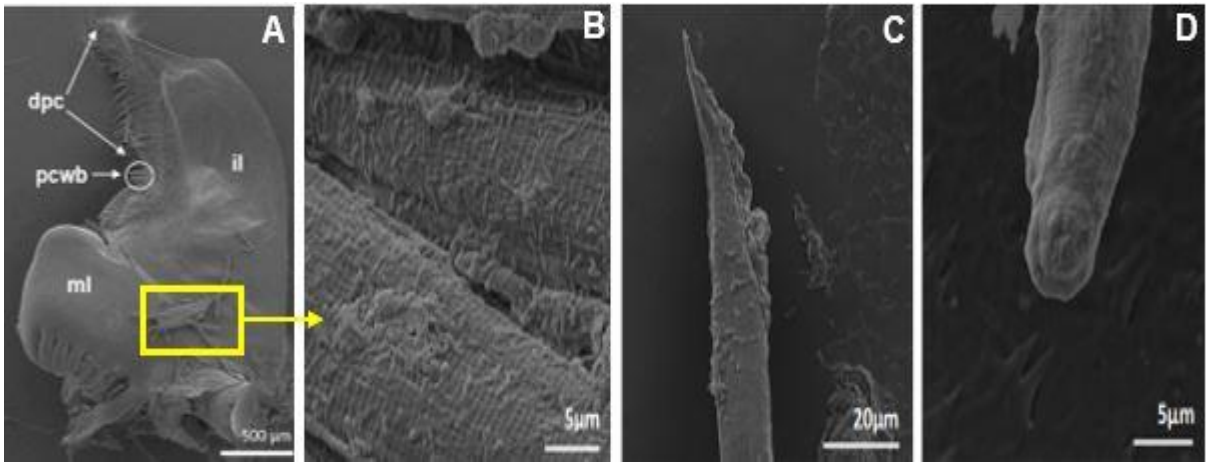


Figure 4.2. Unidentified worm-like organisms covered in what resemble rod-shaped bacteria. These organisms were found in the pre-oral cavity of the dry feeding *Pachysoma endroedyi*. (a). Worm – like structure on the mandible of *Pachysoma endroedyi*. (b). Body of the worm – like organism. (c). Posterior region of the organism. (d). Anterior region of the organism.



Figure 4.3. *Circellium bacchus* Fabricius, (Coleoptera: Scarabaeidae). Dung beetle using its mouth parts to filter out indigestible plant fragments while feeding on a pile of wet cattle dung. (Source: Holter, 2016. Photographer: L. Stenseng)

The morphological assessment of the molar region shows that the molar lobes of wet, carrion and the generalist feeder contain molar ridges with pronounced microstructures that are divided by narrow and deep filtration channels (figures 3.14 D, H; 3.15 D, H; 3.16 C, G). The anterior filtration channel widths of species in these feeding groups are significantly larger than their posterior filtration channel widths for both concave and convex molars (table 3.4). The reason for the size disparity between the anterior and posterior channel widths is unclear, nevertheless, the same observation has been made in dry feeding species (table 3.4) despite their filtration channels of *P. endroedyi* and *P. hippocrates* only occurring on the margin of their molar lobes (figures 3.17 A, E; 3.18 A).

Compared to the wet, carrion and generalist feeder, the inner surface of the anterior region of the concave molar of dry feeders has been replaced by a smooth surface (figure 3.17 A, E; 3.18 A). Whereas the anterior region of the convex molar has been replaced by an uneven and rough surface created by molar ridges that are fused together (figure 3.19), thus creating a surface conducive for the grinding of food (Holter and Scholtz, 2011). The height of the anterior ridges of dry feeders is significantly greater than those of the wet, carrion and generalist feeder (figure 3.21; table 3.4). The flexibility of these ridges and their hook-like design is probably what enables the concave molar to hook/attach itself to the convex molar during grinding to prevent slippage of the molars and promote efficient grinding. Dry feeders are hypothesized to have evolved from a wet dung feeding lineage, thus they may have gained cutting and grinding structures as a result of adaptations to environmental changes that confined them to arid habitats (Scholtz et al., 2009).

The posterior region of the concave molar of dry feeders, except for *P. hippocrates*, contains ridges with less pronounced microstructures. They appear to be fused in *P. endroedyi* (figure 3.17) and deeply divided by filtration channels in *P. striatum* (figures 3.18) whereas the posterior ridges of the convex molar of *P. endroedyi* and *P. hippocrates* (figure 3.17) are fused but deeply divided by filtration channels in *P. striatum.* Furthermore, the posterior filtration channel widths of both concave and convex molars of *P. striatum* are relatively similar in size to those of wet feeders (table 3.4).

Observations on *P. striatum* by Scholtz (1989) have found that *P. striatum* predominantly feeds on dry sheep pellets that are stored in underground chambers that are 45 – 60 cm deep. The rehydration of food substrates by *Pachysoma* species has been a contentious issue following findings by Holter et al. (2009) which showed that the detritus collected and buried by the detritivore *P. glentoni* does not absorb moisture from underground chambers. *Pachysoma striatum* usually burrows into xeric sands of dunes, riverbanks, hillocks and coastal hummocks (Scholtz 1989; Harrison et al., 2003). During the wet season, because of the inability of sandy soil to retain large volumes of water, the water usually drains away from these sandy chambers. However, dung masses buried in these chambers have been found to absorb and hold water because of the “capillary suction” (Scholtz, 1989; Harrison et al., 2003). In light of this, the presence of filtration channels in the posterior region of the molar lobe of *P. striatum* and the lack thereof in the anterior region has led me to suggest a dual function of its molar.

Firstly, the rehydration of food substrates in *Pachysoma* species has only been disproved in *P. glentoni* and not with *P. striatum.* Therefore, I hypothesize that in the chambers, the water capillary suction allows for the rehydration of sheep pellets to an extent where *P. striatum* can process the pellets the same way that wet feeders do through squeezing. I propose that the function of the filtration channels is to facilitate the movement of food from the molar lobes to the pharynx to be ingested. Unlike the incisor lobe, the molar lobe lacks setae to move food around the mouth. Once the dung has been squeezed and transported to the pharynx, the second function of the molar surface is to mechanically grind the plant fragments. Given that *Pachysoma* species derive nourishment from such fragments, large plant fragments are ingested unlike the wet feeders which would filter them out.

Apart from dung beetles, the occurrence of filtration channels has been observed in the mandibles of the African leaf chafer *Asthenopholis adspersa* Brenske 1898 (Harrison, 2009). Harrison (2009) describes the mandibles of *A. adspersa* as heavily sclerotised, with asymmetrical molar lobes containing rough ridges (figure 4.4) and indicates that they are adapted to plant feeding. The manner in which *A. adspersa* uses its mandibles to process plant material is not explained by Harrison (2009). However, from personal observation of the micrographs, it appears that the molar lobes cut and grind the plant material (possibly with a high-water content). The ground up material could be collected by these channels and is then transported to the pharynx for ingestion.

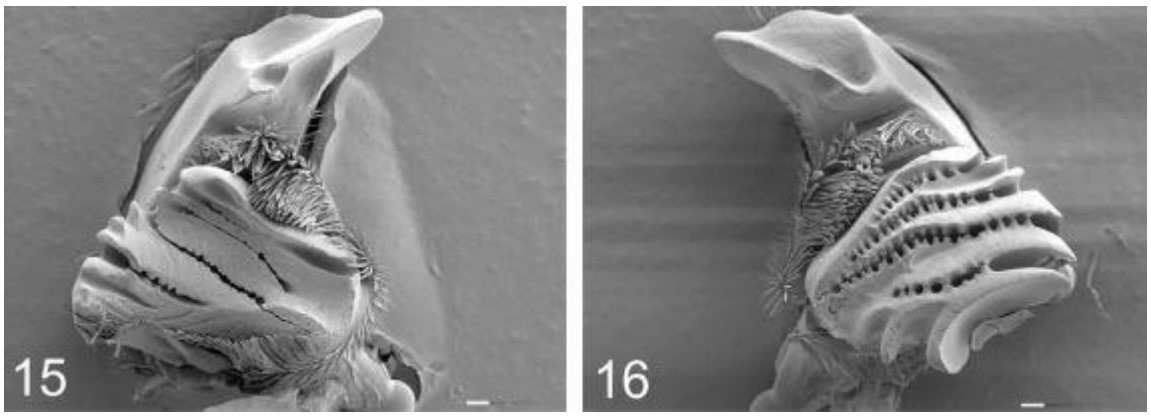


Figure 4.4. Lateral view of the left (concave) mandible and right (convex) mandible of *Asthenopholis adspersa*. Source: Harrison (2009).

It is apparent, that in both dung beetles and leaf chafers, the filtration channels are associated with food substrates that have a certain amount of water. Considering that detritus is drier than dung pellets, there is no need for filtration channels along the surface of the molar hence the ridges of the detritus feeding *P. hippocrates* and mixed feeder *P. endroedyi* are either absent or fused to instead create a grinding surface suitable for food much harder and drier than dry dung pellets. The occurrence of the filtration channels only on the margin of the molar lobes of *P. hippocrates* and *P. endroedyi* might suggest that perhaps after grinding, the ground up food also gets transported from the molar lobes to the pharynx for ingestion via these channels.

Dry feeders typically inhabit arid environments and consume food with a low water content (Holter et al., 2009). During the day when they are active above the ground, there is no liquid water available to them and like other organisms inhabiting dry environments, they spent most of their time underground (Seely and Mitchell, 1987) at temperatures around 20 °C

therefore, it is unlikely that they could be using filtration channels to get rid of water. Despite coexisting with other detritivores in this arid environment, dry feeders have not been observed acquiring water from sources besides the food they eat (Holter et al., 2009), unlike their detritivorous counterparts (from the Tenebrionidae) seen fog-basking (Seely, 1979; Nørgaard, T., and Dacke, 2010) and constructing trenches near the fog winds as strategies of collecting water to meet their water demands (Seely and Hamilton, 1976).

This study therefore proposes a revised version of the hypothesis by Holter (2000). I suggest that the dung gets collected by the sclerotised external components of the mouth (maxilla, maxillary palps, galea, etc) (Madle, 1934; Holter, 2000). Once the dung is inside the mouth, the interaction between the setae of the epipharynx and the incisor lobe (Nel and Scholtz, 1990; cf. Holter and Scholtz, 2011 for image) filters out the larger fibrous plant material (Holter, 2000). The remaining smaller particles are transferred to the molar region (by the incisor lobe setae) and subsequently squeezed between the opposing molar surfaces (Holter, 2000).

During squeezing, a pasty dung mixture passes through the filtration channels, flows out of the molars via the open side of the molar and is subsequently ingested. Hata and Edmonds (1983) have produced an illustration of the relative position of the molar in the head (figure 4.4). This drawing shows the open side of the molar facing the pharynx and the blind side facing the mouth opening. It is not known why the molar lobes are situated in this position. However, I suspect that the role of the blind side is to prevent the escape of the dung paste out the mouth forwards during the squeezing process. Following the squeezing, I believe remnants of fibrous material on the molar lobes is transported out the mouth via the interaction between the setae of the epipharynx and the incisor lobe. The left-over residue of dung particles on the molar lobes following the filtering (be it bacteria, fungi or large fragments that could still not be filtered out) (figure 4.5) are also somewhat ingested. This probably explains why Madzivhe *et al.* (2020) found a particle size range of dung particles between 1.4 µm and 2000 µm in the foregut of the wet feeder *Scarabaeus goryi* despite the wet feeders in this study (of similar body sizes to *S. goryi*) having a channel width range between 4 and 25 µm.

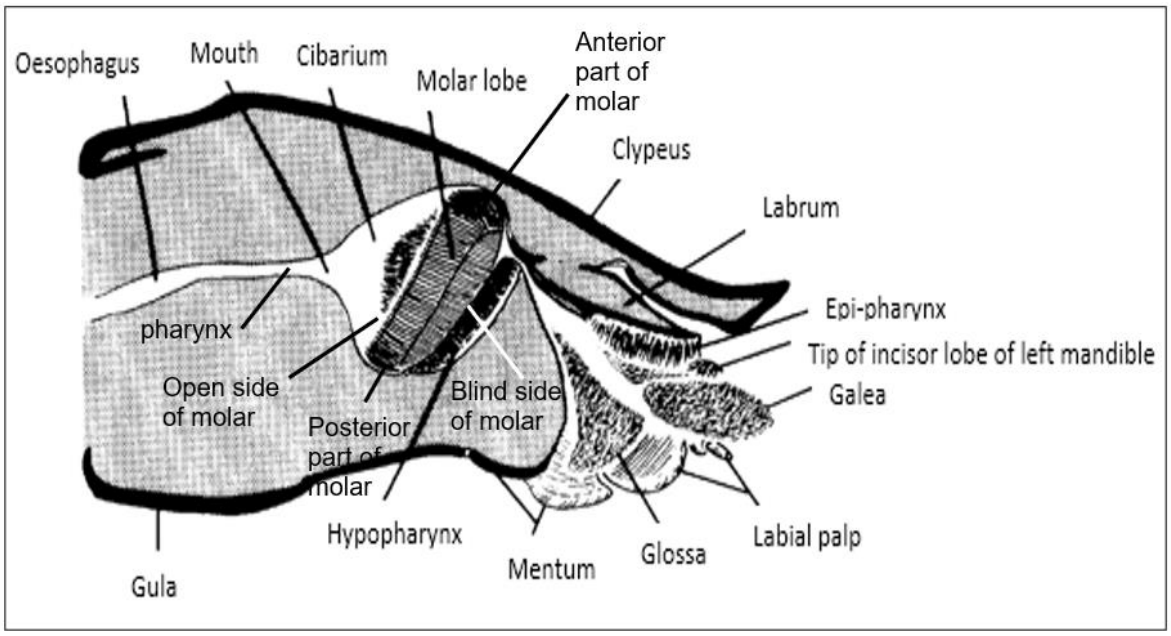


Figure 4.5. *Canthon pilularius.* Adapted semi-diagrammatic sagittal section through head. Source: Hata and Edmonds (1983).

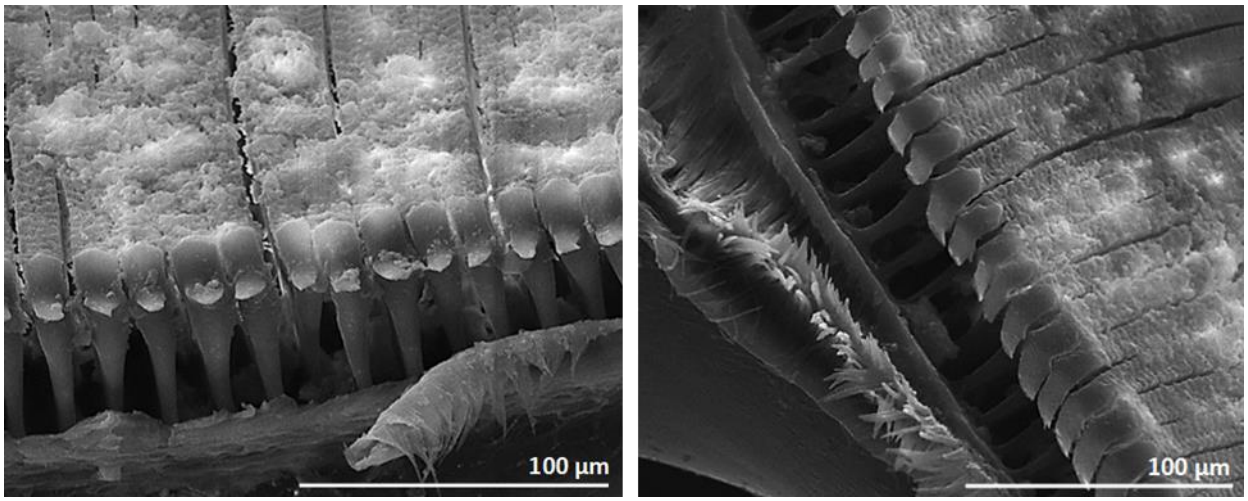


Figure 4.6. Mesal view of the surface of the concave molar of wet-dung feeding *Scarabaeus zambesianus* following a cow dung meal. The molar surface appears to be covered in dung.

4.4 Epipharynx

Following the examination of the taxa used in this study, the epipharynx of wet, carrion and the generalist feeder was found to have more setae in the anterior region than the epipharynx of dry feeders. Following the divergent evolution of dry feeders from wet feeders, dry feeders have evolved an epipharyngeal tooth that is presumed to have developed from the fusion and sclerotization of the median brush found in the wet, carrion and generalist feeder (Holter and Scholtz, 2011). Dry feeders also possess a clypeal scraper and median thorn that work in

synergy with the epipharyngeal tooth when cutting and breaking up large pieces of dry food into smaller ones (Holter and Scholtz, 2011).

Having to adapt to a dry diet such as dry dung pellets and detritus due to severe environmental constraints (Scholtz et al., 2009) made the presence of filtering setae on the epipharynx of dry feeders less important as they were no longer filtering food. However, the setae that remained on the epipharynx of dry feeders is suggested to be largely involved in facilitating the movement of food from the mouth to the gut (Palestrini et al., 2020). In addition, considering how the distal comb occurring on the tip of the ventral side of the incisor lobe alongside the ventral row of the prosthecal comb of the mandible interacts directly with the dorsal side of the epipharynx (figure 1.6) (Nel and Scholtz, 1990, Holter and Scholtz, 2011), I believe that this interaction makes that movement of food particles possible although this is also applicable to the other feeding groups as well.

The presence of cutting structures on the epipharynx and their functions are not only unique to the *Pachysoma* genus but have been observed in the Eucraniini tribe (Palestrini,et al., 2020). Dung beetles in the Eucraniini are endemic to Argentina and like *Pachysoma* they typically inhabit arid environments with scant vegetation and are adapted to feed on dry dung pellets of small mammals (Ocampo, 2005), as do some *Pachysoma* species. According to Palestrini et al. (2020), the morphological modifications that insect mouthparts experience are dictated to by food preferences and feeding habits and is a view shared by several authors (Forsythe, 1983; Hörnschemeyer et al., 2013; Bai et al, 2015; Karolyi et al., 2016). Based on this narrative, one could also conclude that perhaps the similar anatomical characters of the epipharynx that *Pachysoma* species share with the Eucraniini species could be attributed to adaptation to their food type preference as a consequence of inhabiting xeric habitats; however, the geometric morphometric outcome from this study suggests otherwise.

Geometric morphometric analysis was used to depict the shape variations of the epipharynx across dung beetle species belonging to different feeding groups and tribes. The PCA showed how the dung beetle species were grouped into four distinct clusters of feeding groups. The *Scarabaeus* (and their *Scarabaeolus* sub – genus) were clustered together to form the wet feeders, the single *Scarabaeolus* species formed the carrion feeder group which overlapped with that of wet feeders (probably because they both feed on liquid-based diets), the *Pachysoma* species formed the dry feeding group whereas the single *Anachalcos* species

formed the generalist group. Because of this observation, it is quite evident that the dung beetle species grouped themselves according to shared structural similarities based on feeding habits and food preference. A consecutive PCA was subsequently run. This time it included wet – dung feeding beetle species *Neosisyphus mirabilis* and *Neosisyphus rubus* belonging to the tribe Sisyphini. Instead of forming part of the already existing wet feeders cluster, *Neosisyphus mirabilis* and *Neosisyphus rubus* formed a second wet feeder's cluster.

From these observations, it appears as though phylogeny emerges as a strong and primary element that dictates the evolution of mouthparts compared to food preference and feeding habits. However, the epipharynx is generally used as a diagnostic character by taxonomists to identify species and subsequently classify them (Pizzo et al., 2009) because it is taxonomically sensitive. Carrying out a geometric morphometric analysis on the mandibles of these dung beetles, instead of their epipharynx (such as the one conducted by Bai et al., 2015), might be informative to the feeding question (being whether mouth morphology is determined by feeding habits or phylogeny?) and show that mouth morphology is determined by feeding habits and food preference.

4.5 Conclusion

Dry feeders are not exactly particle feeders but considering that they have evolved from a wet-dung feeding ancestor, comparing the morphology of their mouth parts to that of generalist, carrion and wet feeders provided insight on the likely function of the various components of dung beetle mouthparts and their role in particle feeding. This has enabled me to establish that the generalist, carrion and wet feeders may not be eliminating excess water as it has been previously assumed. My study has suggested that the channels could instead be mandibular structures that aid in facilitating the movement of digestible food after initially being filtered by the filtering setae of the incisor lobe and the epipharynx. I have hypothesized the function of some of the components of the mouth (such as the molar tapetum, bifurcations of the prosthecal comb, filtration channels) but l would still need to verify some functions (e.g., the role of the conjunctivuse) with the use of other imaging techniques such as micro computed tomography.

Although it has not been experimentally tested, the morphological evidence and interpretation from this study has provided a reasonable and practical way of looking at how these mouthparts operate and has enabled me to provide a revision of Holter's hypothesis of the

probable mode of action of the mouthparts during particle feeding. The mouthpart dimensions measured by me in relation to the particle size range determined by Holter and Madzivhe et al., (2020) have also shown that the mouthparts do not function as a filter in the strictest sense as it was previously proposed by Holter (2000) and I have thus proposed a rake – like mechanism. My study of the mouthpart morphology has also shown that there is convergence between the mouthparts of wet feeders and carrion feeders. However, there is divergence between the mouthparts of the dry feeders and those of wet feeders. Furthermore, *P. striatum* (belonging to the basal taxonomic group) was found to possess structural components of its molar that are similar to those of wet, carrion, generalist and dry feeders. A limitation in my study was the use of only one species for the generalist and carrion feeding groups due to the fact that I could not source the desirable number of species due to drought conditions in South Africa during the sampling phase of this study. Thus, l recommend broadening the generalist and the carrion feeding groups' taxa for future studies, having equal sample sizes across species, having larger sample sizes and accounting for phylogenetic non-independence in order to obtain a more comprehensive understanding of the dynamics of mouthpart morphology and drivers of particle feeding.

4.6 Literature cited

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