

Re-establishment of biting mouthparts in dung beetles (Scarabaeidae: Scarabaeinae) feeding on plant litter - old structures re-acquired or new ones evolved?

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ABSTRACT

Evolution of dung beetle (Scarabaeidae: Scarabaeinae) mouthparts for eating moist, fresh dung has led to a loss of any ability to chew. However, the desert-living genus *Pachysoma*, probably evolved from a wet-dung feeding, *Scarabaeus*-like ancestor, has switched to dry faecal pellets (of rodents or small ruminants) and plant litter that might require re-establishment of chewing. Indeed, gut contents of a litter-feeding *Pachysoma* species indicate efficient food comminution prior to ingestion. Cutting and grinding mouthpart structures in six species, of two lineages and with different food preferences, are described and compared with homologous structures in wet-dung feeding *Scarabaeus* species. In *Pachysoma*, cutting and breaking of large food items is performed by a clypeal scraper, a prominent epipharyngeal tooth and large maxillary galeal hooks. Further comminution is achieved by a large, grinding area evolved on the mandibular

molae. Interspecific differences and the probable function and evolution of these structures are discussed. Particularly the unique tools for cutting/breaking are novel structures and not re-acquired normal biting mouthparts.

ADDITIONAL KEYWORDS: desert adaptations – detritus feeding – dung feeding – evolution – mouthpart morphology – *Pachysoma*

INTRODUCTION

In most species within the large scarabaeid subfamily Scarabaeinae, adult beetles eat fresh, wet dung of large mammalian herbivores and omnivores by filtering out coarse particles, mainly indigestible plant fragments, and only ingesting very small particles. The latter include excellent food items such as bacteria and dead gut epithelial cells. This unusual feeding, recently reviewed by Scholtz (2009), is achieved by specialised mouthparts (e.g. Miller, 1961; Edmonds, 1972; Hata & Edmonds, 1983; Nel & Scholtz, 1990; Holter, 2004; Verdú & Galante 2004) that are quite different from the normal biting type in most Coleoptera (Crowson, 1981; Lawrence & Britton, 1991), including many Scarabaeidae. Thus, the distal mandibular lobes, i.e. the sclerotised, cutting ‘incisor lobes’ of most beetles, have become membraneous and soft in scarabaeine dung feeders. Also, the normally grinding mandibular molae have been modified into structures that seem unable to perform any grinding (Holter, 2000; Holter et al., 2002). In the absence of e.g. a cutting/grinding gizzard it must be concluded that any ability to masticate food has been lost during the specialisation of scarabaeines for wet-dung feeding.

However, some scarabaeines have adopted other kinds of food than fresh, soft dung (Larsen, Lopera & Forsyth, 2006; Davis, Frolov & Scholtz, 2008; Scholtz, 2009). One example is the genus *Pachysoma* MacLeay (Fig. 1), earlier considered a subgenus of *Scarabaeus* and probably evolved from a *Scarabaeus*-like ancestor (Sole, Scholtz & Bastos, 2005; Forgie et al., 2006; Sole, Bastos & Scholtz, 2007). Typically, *Scarabaeus* beetles are winged ball rollers feeding on fresh dung in the usual scarabaeine way. In contrast, the 13 known *Pachysoma* species (Harrison, Scholtz & Chown, 2003) are flightless and have switched to drier diets (dry faecal pellets, plant detritus or a mixture of both, cf. Fig. 1) available in their arid, sandy habitats along the west coast of southern

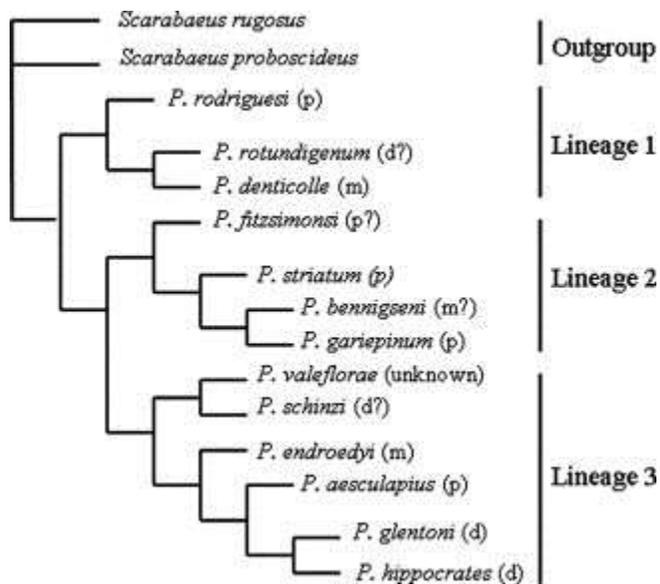


Figure 1. Phylogram of the known *Pachysoma* species, based on Sole et al. (2005) and Davis, Frolov & Scholtz (2008), with information (bracketed) on their preferred food according to Harrison et al. (2003) and, for *P. glentoni*, Holter et al. (2009). Abbreviations: d, plant detritus (litter); p, dry dung pellets of rodents, hares or small ruminants; m, mixture of detritus and pellets; ?, food choice somewhat uncertain, mostly inferred indirectly from e.g. hindleg morphology or observations of habitats.

Africa. Food is collected on the sand surface and dragged, held by the hind legs, into a previously dug burrow where it is stored and eaten (Scholtz, 1989; Holter, Scholtz & Stenseng, 2009).

As far as we are aware, some *Pachysoma* species, such as *P. hippocrates* MacLeay and *P. glentoni* (Harrison, Scholtz & Chown), are the only scarabaeines known to subsist solely on dry plant litter. Studying the nutritional ecology of *P. glentoni*, Holter et al. (2009) hypothesised that the beetles might cope with this presumably difficult substrate by feeding on soft fungi growing on the litter in the underground storage chamber. However, the results did not indicate any such fungal growth and suggested that the beetles were simply eating the plant litter itself. Furthermore, digestion was remarkably efficient judging from a measured assimilation efficiency of about 60 %.

Irrespective of the unknown details of this digestion, efficient chemical breakdown of cell walls in dead plants is likely to require some initial crushing and comminution of the material, i.e. chewing (e.g. Barbehenn, 2005). Indeed, observations of midgut contents in *P. glentoni*, to be detailed later, indicate extensive comminution of all larger pieces of litter. Hence, the lost capability of mastication seems to have been regained during the likely evolution from a wet-dung feeder to *Pachysoma*, which raises an interesting question. Were normal biting mouthparts simply re-acquired, or was chewing re-established via an evolution of novel structures? The first possibility might violate Dollo's Law (the idea that evolution is irreversible, cf. Collin & Miglietta (2008)) and support the somewhat controversial concept of 'reverse' evolution (e.g. Teotónio & Rose, 2001; Porter & Crandall, 2003; Desai, 2009), whereas the second possibility would be in keeping with more conventional wisdom.

In addition to observations on the particle size of midgut contents in *P. glentoni*, the present work explores the mouthparts of six *Pachysoma* species, representing all known diet choices and two out of three intrageneric lineages (Fig. 1), in order to identify and describe structures likely to cut and grind. For comparison, relevant features of mouthpart morphology in three *Scarabaeus* species eating soft dung are also described. Results are discussed in relation to the food of the *Pachysoma* species, their phylogenetic affiliations within the genus and the possible ways of mouthpart evolution outlined above.

MATERIAL AND METHODS

Specimens of *Scarabaeus ebenus* (Klug) were caught (by S. Tind Nielsen) at Majawanga Village, Tanzania (S6.083°, E36.833°). The other species were collected in western South Africa: *Pachysoma glentoni*, *P. aesculapius* (Olivier) and *Scarabaeus rugosus* (Hausmann) near Lamberts Bay (S32.21842°, E18.43522°); *P. striatum* Castelnau, *P. endroedyi* (Harrison, Scholtz & Chown), *S. rugosus* and *S. (Kheper) bonellii* (MacLeay) at Kommandokraal Farm (S31.50312°, E18.20929°); *P. hippocrates* MacLeay at Noup (S30.13910°, E17.20262°); and *P. garipepinum* Ferreira at Holgat River near Port Nolloth (S28.92998°, E16.77575°). *Pachysoma* species were mostly collected by excavation of burrows, whereas the *Scarabaeus* species were caught on fresh dung of cattle (*S. ebenus*) or pig (*S. rugosus* and *S. bonellii*).

Whole individuals of *P. glentoni* and heads of the other species were preserved in 70 % ethanol. Samples of midgut contents were taken from six preserved *P. glentoni*, each sample being gently dispersed in a thin layer of 80 % glycerol and 20 % water in a Petri dish. The high viscosity of the fluid prevented undesirable movement of the particles

once they had been dispersed. Dishes were placed on graph paper with 1 mm squares and examined at $35\times$ magnification. In each sample, lengths of about 600 particles ≥ 0.1 mm and identifiable as plant fragments were recorded as 0.1-1 mm, 1-5 mm, or > 5 mm.

For examination of mouthparts, heads were dissected in 70% ethanol. After dehydration in an ethanol series, dried, platin-coated mouthparts were studied in a JEOL JSM-6335F field emission scanning electron microscope. Generally, we have adopted the terminology used by Nel & Scholtz (1990) in the mouthpart descriptions.

RESULTS

PARTICLE SIZE IN BEETLE-COLLECTED LITTER AND GUT CONTENTS

As the lengths of most plant litter pieces collected by *P. glentoni* were 5-30 mm (Holter, Scholtz & Stenseng, unpubl. field observations), dry-sieving through a 1 mm mesh to get rid of sand (Holter et al., 2009) retained practically the entire organic material. In contrast, an average (\pm S.E.) of 97.6 (± 0.67) % of 3631 measured plant fragments in the six samples of midgut content had lengths of 0.1-1 mm, 81 were 1-5 mm, and only five longer than 5 mm. By far the largest fragment observed was about 1×13 mm.

MOUTHPARTS OF *SCARABAEUS*

Mouthparts of wet-dung feeders have already been described by several authors (cf. Introduction). This section, therefore, deals only with features of special interest in a comparison with *Pachysoma*. Mouthparts of the three *Scarabaeus* species are very similar, and the following brief description, based on *S. rugosus*, is representative of the other species as well.

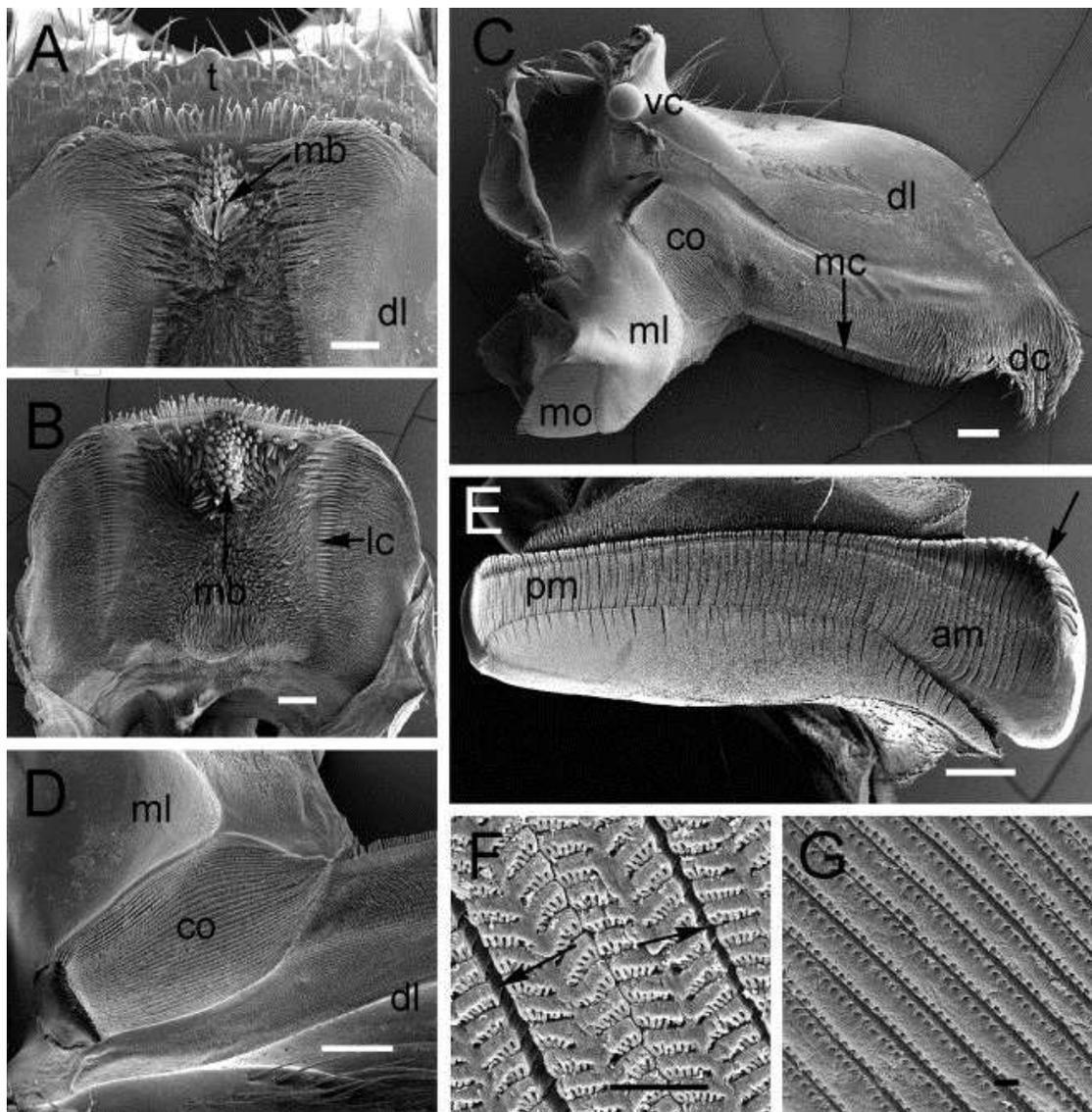


Figure 2. Mouthparts of *Scarabaeus rugosus* (food: wet dung). A-D are ventral, E-G medial views. A, median tooth on clypeal ridge, median brush on epipharynx and distal end of mandibles (maxillae and labium removed). B, epipharynx. C, right mandible with convex mola (only ventro-posterior part visible). D, close-up of conjunctive (left mandible). E, right mola (concave); arrow points at hook-like prolongations of anterior ridges. F, surface substructures in posterior three-quarters of mola (gaps indicated by arrows are shrinkage artefacts). G, surface structures in anterior part of mola. Abbreviations: am, anterior part of mola; co, conjunctive; dc, distal comb; dl, distal lobe; lc, lateral comb; mb, median brush; mc, mesal comb; ml, molar lobe; mo, mola; pm, posterior part of mola; t, median tooth on clypeal ridge; vc, ventral condyle. Scale bars: 200 μm (A-E) and 10 μm (F-G).

Under the medio-anterior edge of the clypeus there is a (sometimes rather weakly defined) horizontal ridge with a small, rounded median tooth (Fig. 2A). The soft, hairy and membranous distal part of the epipharynx (Fig. 2B) bears two distinct lateral rows of large setae, the lateral combs or (e.g. Dellacasa, Bordat & Dellacasa, 2000) chaetopariae. There is also a conspicuous medio-anterior pile of coarse, inflexible setae, the median brush or (Dellacasa et al., 2000) corypha.

Each of the mandibles (Fig. 2C) has a membranous distal lobe (apicalis, distalis or – in normal biting mandibles – ‘incisor lobe’) and a basal, mesally protruding molar lobe. Distally, the soft, medial edge of the apicalis consists of long, more or less plumose setae (distal comb) which proximally pass into a row of finer and shorter simple setae, the mesal comb. Wedged in ventrally between distal and molar lobe is a well-developed structure, the conjunctive (conjunctivus) (Fig. 2C and D), consisting of flat ridges/lamellae that converge towards the point where the mesal edge of the apicalis meets the molar lobe. These lamellae are composed of tightly adhering setae.

The medial surfaces of the molar lobes, the molae or molar surfaces, are asymmetrical, the left concave and the right convex, fit exactly into each other and are hence of equal length. Their longitudinal axis slopes relative to that of the animal, the posterior end being more ventral than the anterior. The right mola is 4-5 times longer than wide, with roughly parallel sides. In the left mola (Fig. 2E), the posterior two-thirds are only about half as wide as the corresponding part of the right. The molar surfaces (Fig. 2E) consist of tightly packed, transverse ‘ridges’. Being almost flat in the posterior three-quarters of the molae, these ridges seem to form a smooth surface, the width of which in the left mola is only about 50 % of that of the right. High magnification reveals

complicated comb-like substructures in each of these ridges, with numerous tiny (diameters around 1 μm) holes (Fig. 2F). In the anterior molar quarter, the ridges become slightly more raised and the substructures are reduced, first to rows of simple holes (Fig. 2G) and at last disappearing completely towards the anterior end. Particularly in the left mola, the coarse, anterior ridges are prolonged towards their periphery into curved, hook-like structures (Fig. 2E).

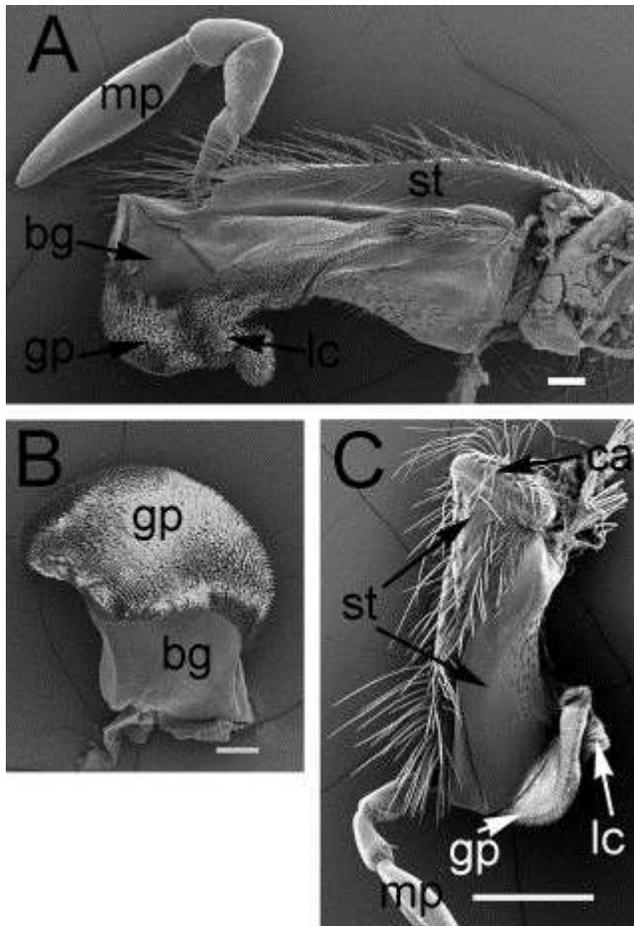


Fig. 3. Maxillae of *Scarabaeus rugosus*. A, right maxilla, dorsal view. B, galea (removed from stipes) with basal sclerite and hairy pad, dorsal view. C, left maxilla, ventral view. Abbreviations: bg, basal galeal sclerite; ca, cardo; gp, galeal pad; lc, lacinal pad; mp, maxillary palp; st, stipes. Scale bars: 200 μm (A-B) and 1 mm (C) .

Most of each maxilla consists of the stipes, connected to the head capsule via a basal segment, the cardo (Fig. 3A & C). The stipes bears two mesally protruding structures, the small lacinia and the larger, more distal galea. Pad-like, distal lobes of both lacinia and galea are covered dorsally by hooked setae, the galeal pad being especially large. The basal part of the galea (subgalea) is a rather thin-walled cuff-like sclerite (Fig. 3B). Some protraction of the stipes with galea and lacinia is probably possible by extension of the maxilla in the ventral ‘hinge’ between cardo and stipes (Fig. 3C).

MOUTHPARTS OF *PACHYSOMA* SPECIES, LINEAGE 2

Both species studied (*P. striatum* and *P. gariepinum*) feed on dry dung pellets, and their very similar mouthparts will be described together.

Under the medio-anterior clypeal edge there is a vertical ridge – more pronounced than the more horizontal one in *Scarabaeus* – with a distinct, median spine which may be strongly worn in old individuals. This structure is the ‘clypeal scraper’ (Harrison et al., 2003) (Figs 4A, 6F). The epipharynx (Fig. 4B) bears the usual lateral combs, their setae being even larger than in the *Scarabaeus* species. Moreover, a big, sclerotised antero-median tooth (Fig. 4C) pointing forward-downward replaces the median brush of wet-dung feeders such as *Scarabaeus*. Ventro-posteriorly, the edge forms a protuberance, which gives the tooth some resemblance of a blunt spine. Whether the tooth is in a completely fixed position or can be moved slightly up and down is not known.

As in the other *Pachysoma* species studied, the distal mandibular lobes (Fig. 4D) are thin and flexible (like those in *Scarabaeus*) and there is no clear distinction between

mesal and distal comb. The conjunctive (Fig. 4D) in the lineage-2 species is reduced compared to that of *Scarabaeus* (see Fig. 2D), but still clearly visible.

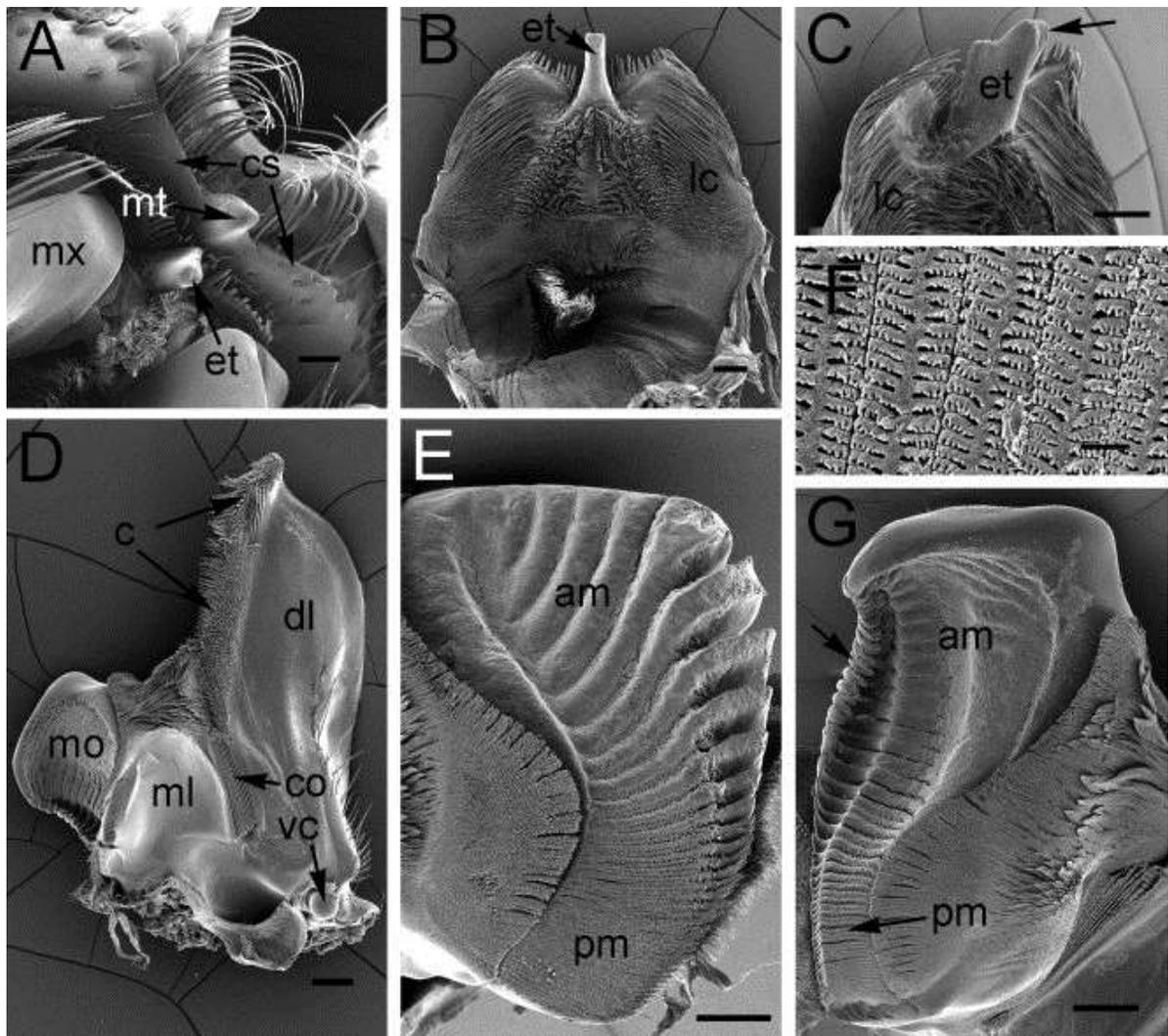


Fig. 4. Mouthparts of *Pachysoma gariepinum* (A, E-G) and *P. striatum* (B-D). Latero-ventral (A, C), ventral (B,D) and medial (E-G) views. A, epipharyngeal tooth and clypeal scraper with median tooth (in-situ view, labium removed). B, epipharynx. C, distal end of epipharynx with tooth; arrow indicates ventro-posterior protuberance. D, left mandible with concave mola. E, right mola. F, microstructures in posterior part of mola. G, left mola with hook-like prolongations of anterior ridges (arrow). Abbreviations: am, anterior part of mola; c, distal + mesal comb; co, conjunctive; cs, clypeal scraper; dl, distal lobe; et, epipharyngeal tooth; lc, lateral comb; ml, molar lobe; mo, mola; mt, median thorn on clypeal scraper; mx, maxilla; vc, ventral condyle. Scale bars: 10 µm (F); 200 µm (others).

The longitudinal axis of the asymmetrical *Pachysoma*-molae slopes more steeply than in *Scarabaeus*, but their dorso-anterior and ventro-posterior ends will just be called anterior and posterior to retain the *Scarabaeus*-terminology. The right mola is covered by flat ridges, very coarse in the anterior half but getting increasingly fine posteriorly and at last disappearing. In the middle third, the ridges are prolonged into flaps along the edge (Fig. 4E). Although the anterior end lacks any microstructures, these turn up in the posterior direction: first rows of simple holes along the ridges and then increasingly elaborate surface structures similar to those in *Scarabaeus* (Fig. 4F). These microstructures are also found in the posterior end of the concave left mola (Fig. 4G). The inner half of its anterior surface is relatively smooth, whereas the flat ridges covering the other half are prolonged into large, inwardly bent hooks, replaced posteriorly by triangular flaps. Hooks and flaps form the medio-dorsal edge of the mola.

In the maxillae (Fig. 5A), the distal pads of hooked setae on galea and lacinia are strongly reduced, particularly the galeal pad (the larger by far in *Scarabaeus*) which is only a small flap in *Pachysoma*. On the other hand, the basal sclerite of galea – quite simple in *Scarabaeus* – has been transformed into a conspicuous, strongly sclerotised hook-like structure, a ‘galeal hook’ (Figs 5A, B), with a peripheral edge around a central, roughly circular depression. The edge is incised immediately below the galeal pad, especially in *P. gariepinum*. The medial side of the sclerite – and thereby the hook apex – can be drawn backwards by a large flexor muscle inserted in the basal medial edge and originating in the stipes. Apparently, this inward ‘nodding’ can be supplemented by a similar movement of the entire stipes, effected by strong muscles arising in the head capsule and attached to the basal medial edge of the stipes and the adjacent edge of the

cardo. Contrary to conditions in *Scarabaeus*, ethanol-preserved *Pachysoma* maxillae did not show any other mobility in the stipes/cardo and/or cardo/head-capsule hinges. Hence there may not be a potential for protraction of the stipes.

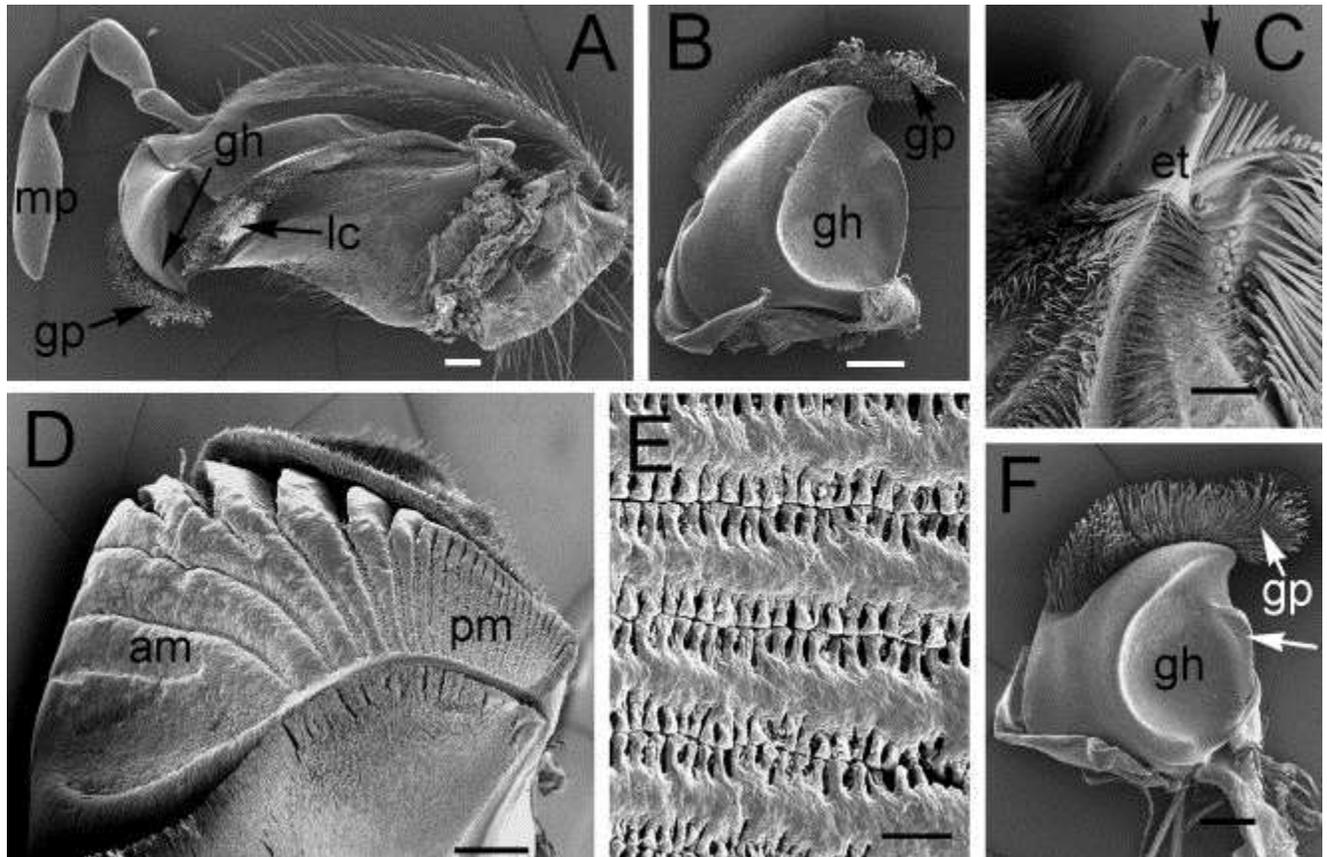


Fig. 5. Mouthparts of *Pachysoma gariepinum* (A-B) and *P. aesculapius* (C-F). Dorsal (A) edio-dorsal (B, F), latero-ventral (C) and medial (D-E) views. A, right maxilla. B, left galeal hook (removed from stipes) and pad. Note the incision in the edge immediately below the pad. C, distal end of epipharynx with tooth; arrow indicates ventro-posterior protuberance. D, right mola. E, fine structure in posterior part of mola. F, left galeal hook (removed from stipes) with ridge (arrow) and pad. Abbreviations: am, anterior part of mola; et, epipharyngeal tooth; gh, galeal hook; gp, galeal pad; lc, lacinial pad; mp, maxillary palp; pm, posterior part of mola. Scale bars: 200 μm (A-D, F) and 20 μm (E).

MOUTHPARTS OF *PACHYSOMA* SPECIES, LINEAGE 3

All examined species have a very prominent clypeal scraper (Fig. 6F). *P. aesculapius* eats dung pellets, and its mouthparts will be described first. According to Harrison et al. (2003), *P. endroedyi* is a mixed feeder (cf. Fig. 1) which eats detritus if necessary. This agrees with two food stores excavated by us: one was mixed and one contained solely plant litter. As *P. endroedyi* mouthparts are quite similar to those of the ‘genuine’ detritus feeders *P. glentoni* and *P. hippocrates*, the mouthparts of these three species are described together.

In *P. aesculapius*, the narrow part of the epipharyngeal tooth’s edge is longer than in the lineage 2-species, and the blunt ventro-posterior protuberance is somewhat smaller but still distinct (Fig. 5C). Like in the detritus feeders, the mandibular conjunctive is strongly reduced (cf. Fig. 6A). The wide anterior part of each mola (Fig. 5D) makes up a larger proportion of the entire surface than in lineage 2, and the fine structures in the narrow posterior part are reduced to a row of elongated holes in or invaginations of each side of the ridges (Fig. 5E). The galeal hooks have a gap between the edge below the small pad of hooked setae and a ridge perpendicular to that edge (Fig. 5F).

In the detritus feeders, the large epipharyngeal tooth terminates in a long, thin edge, more or less incised in the middle, and the blunt posterior protusion seen in dung pellet feeders is clearly reduced (Fig 6F). The molae (Figs 6B, C) are even more dominated by their anterior part than in *P. aesculapius*, and the hooks along the edge of the left mola are massive. In *P. endroedyi*, the short posterior transverse ridges bear a row of holes in each side, like in *P. aesculapius*. No distinct holes were found in the molae of *P. glentoni* and *P. hippocrates*.

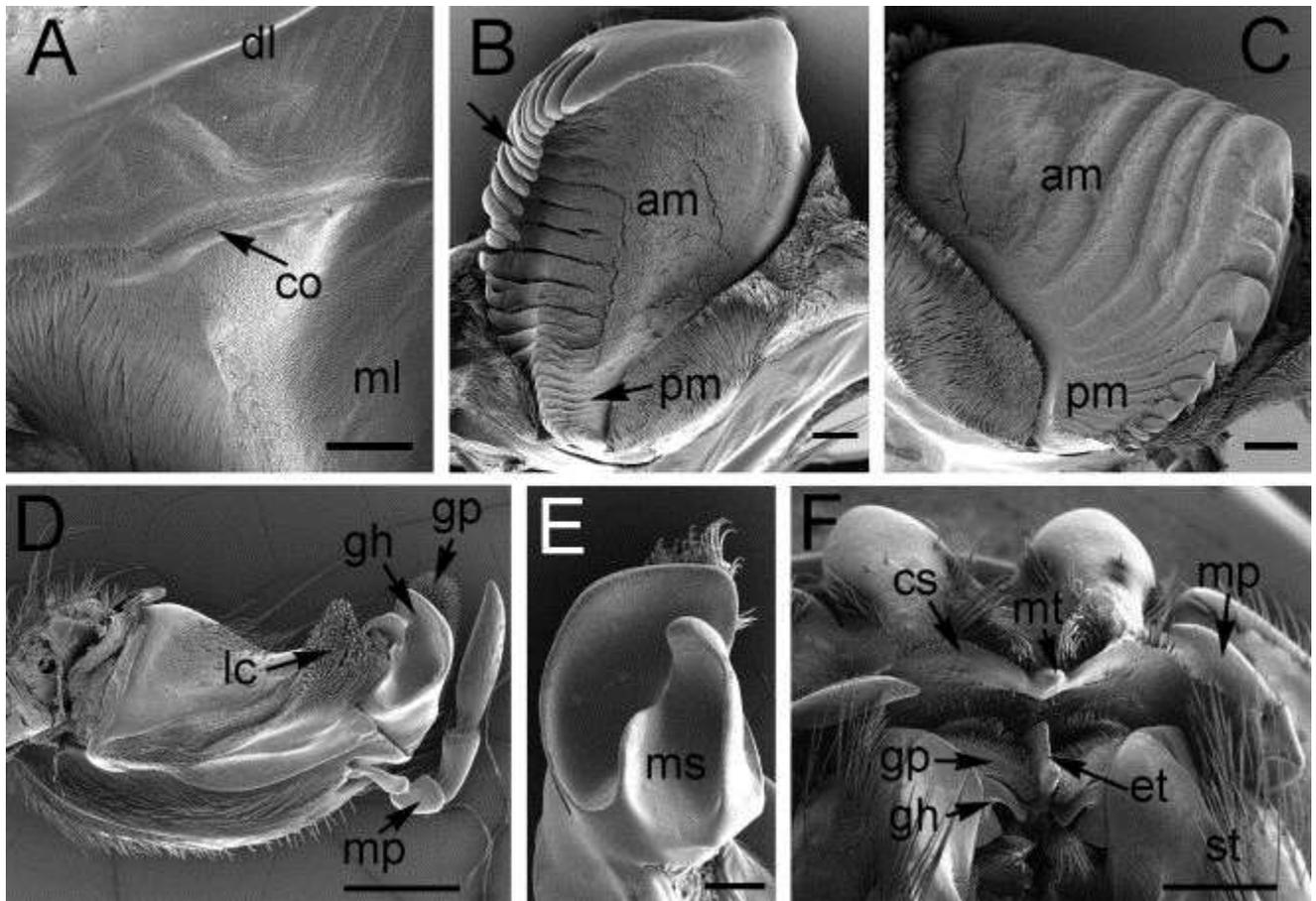


Fig. 6. Mouthparts of *Pachysoma glentoni* (A, C-F) and *P. endroedyi* (B). Ventral (A, F), medial (B-C, E) and medio-dorsal (D) views. A, part of mandible showing strongly reduced conjunctive. B, left mola with very long anterior, peripheral hooks (arrow). C, right mola. D, right maxilla. E, left galeal hook with medially protruding structure. F, in-situ view (labium removed) of anterior, presumably cutting/shredding mouthparts including galeal hooks, epipharyngeal tooth and clypeal scraper. Abbreviations: am, anterior part of mola; co, conjunctive; cs, clypeal scraper; dl, distal lobe; et, epipharyngeal tooth; gh, galeal hook; gp, galeal pad; lc, lacinial pad; ml, molar lobe; ms, medial protruding structure on galeal hook; mt, median thorn on clypeal scraper; pm, posterior part of mola; st, stipes. Scale bars: 200 μ m (A-C, E); 1 mm (D, F).

In the bulky maxillae (Fig. 6D), the galeal hooks are especially elaborate (Fig. 6E). The simple straight ridge perpendicular to the edge below the ‘hairy flap’ (Fig. 5F) in *P. aesculapius* has become a more complex and very prominent structure (Figs 6E, F)

arising from the medio-basal part. As in the other *Pachysoma* species, the hooks can be turned backwards, the position seen in Fig. 6F. The flexor muscle involved in this movement fills most of the considerable volume within the stipes. Like the examined lineage 2 species, those of lineage 3 may be unable to stretch the maxillae forward.

DISCUSSION

Since the labium and hypopharynx are without cutting or grinding structures in both *Scarabaeus* and *Pachysoma*, they have been disregarded in this study. As to the other mouthparts, those of the *Scarabaeus* species are typical of scarabaeine wet dung feeders (Miller, 1961; Edmonds, 1972; Halfpter & Edmonds, 1982; Hatta & Edmonds 1983; Nel & Scholtz 1990). The most important features are: (1) a small horizontal ridge on the underside of the clypeus with a rather weakly defined and blunt median tooth (Fig. 2A); (2) a very distinct median brush (Figs 2A, B) on the epipharynx; (3) thin, flexible mandibular distal lobes without the ability to cut (Fig. 2C); (4) non-grinding, smooth molae with elaborate microstructures on most of their surface (Figs 2E, F); (5) a well-developed conjunctive (Figs 2C, D); (6) maxillary galeae each consisting of a large pad of hooked setae on a simple basal sclerite (Figs 3A, B). Some forward extension of the entire stipes with attached structures is probably possible.

In *Pachysoma* the flexible, distal mandibular lobes have been retained, and so the mandibles are still incapable of cutting. By contrast, the other structures have undergone important changes to be summarised as follows, with a brief discussion of the likely functional consequences.

CLYPEUS AND EPIPHARYNX

In *Pachysoma* the weak clypeal ridge of *Scarabaeus* has become more vertical and much more prominent, with a distinct median spine. The entire structure is the ‘clypeal scraper’ (Figs 4A, 6F), characteristic of the genus (Harrison et al., 2003) and probably suitable for cutting and tearing, including breaking up hard dung pellets.

On the epipharynx, the median brush has been transformed - presumably by fusion of its thick setae - into a large tooth. Particularly in the species feeding on dung pellets, the distal posterior corner of this tooth is drawn out into a blunt protrusion (Figs 4C, 5C) which - like the clypeal scraper - may be useful in breaking up the pellets. In the plant detritus feeders the structure looks more like a ‘normal’ tooth (Fig. 6F), with a relatively long and sharp distal edge that may be suitable for cutting large plant fragments, perhaps aided by the galeal hooks (cf. section on maxillae below). We are not aware of any other Scarabaeinae with an epipharyngeal tooth instead of a median brush with more or less massive setae. There is, however, some resemblance between the tooth and the possibly homologous spatula-shaped epizygum found in some species of *Aphodius* (Scarabaeidae: Aphodiinae) feeding on dry rabbit dung (Verdú & Galante, 2004).

MANDIBLES

In the order *Scarabaeus* (Figs 2E, F) → dung pellets feeders of lineage 2 (Figs 4E-G) → the pellet feeding *P. aesculapius* of lineage 3 (Figs 5D, E) → the detritus feeders of lineage 3 (Figs 6B, C), the initially large posterior part of the molae with an apparently smooth surface consisting of elaborate microstructures is gradually reduced (Fig. 6C). In the detritus feeders, this part takes up only about 10 % of the mola, and the

microstructures have more or less disappeared. The function of these remarkable surfaces in wet-dung feeders is uncertain, but experimental evidence (Holter, 2000; Holter et al., 2002) has not confirmed an earlier idea that larger dung particles are triturated by the microstructures. Instead, it was suggested (Holter, 2000) that superfluous fluid may be squeezed out of the food by the mandibles, escaping through all the tiny holes between the comb-like microstructures, before ingestion. This concentration of very moist food would clearly be useful in wet-dung feeders (cf. Holter & Scholtz, 2007) but useless in species with dry food. Whether the more (Figs 4E-G) or less (Fig. 5D, E) well-developed microstructures on the posterior molar surfaces in pellet feeders still have any important function is unknown, but seems doubtful.

Corresponding to the reduction of the posterior part, the anterior part with coarse ridges (in *Scarabaeus* only about 20 % of the molar length, cf. Fig. 2E) has grown to roughly 90 % of the molar area in the detritus feeders. To digest their intractable food these beetles need particularly efficient grinding of material not sufficiently comminuted by the cutting mouthparts (Barbehenn, 2005). The massive anterior parts of the molae seem suitable for such grinding (cf. Scholtz, 1989).

The large conjunctive of the wet-dung feeding *Scarabaeus* species (Figs 2C, D) is much reduced in the pellet feeders of lineage 2 (Fig. 4D) and has almost disappeared in lineage 3 (Fig. 6A). To our knowledge, a similar reduction of the conjunctive has not been described in any other scarabaeines. The function of this remarkable structure remains unknown, but a conjunctive seems to be present in all adult beetles feeding on fresh, moist dung (Holter, 2004). In the superfamily Scarabaeoidea, these include most Scarabaeinae and Aphodiinae within the Scarabaeidae, and the Taurocerastinae and most

Geotrupinae within the Geotrupidae. Moreover, a similar structure has been found outside the Scarabaeoidea in the fresh-dung feeding genus *Sphaeridium* (Hydrophiloidea, Hydrophilidae) (Holter, 2004). Hence, the conjunctive must somehow be essential for wet-dung feeders but apparently not for other beetles. Its near-absence in the dry-pellet feeding *P. aesculapius* of lineage 3 does not indicate that the reduced, albeit still distinct conjunctive (Fig. 4D) in lineage 2 species with the same food has any important function.

MAXILLAE

The large galeal distal pads of soft-dung feeders are reduced to small flaps in *Pachysoma*, whereas a new prominent structure, the ‘galeal hook’, has appeared medially on the initially (in *Scarabaeus*) simple basal sclerite in each galea. This seems to be a unique structure, not described for other scarabaeids. The hooks of the detritus feeders (Fig. 6E) are somewhat larger, and clearly more complex, than those of the pellets feeders in both lineage 2 (Fig. 5B) and 3 (Fig. 5F). The apex of each hook can be drawn backwards by an inward ‘nodding’ of the basal sclerite effected by a strong flexor muscle in the stipes, perhaps combined with an inward movement of the stipes. It seems doubtful, however, whether the maxilla can be stretched forward as may be the case in *Scarabaeus* and in several other scarabaeids (e.g. Bürgis, 1981a & b). The maxilla is much more bulky than in *Scarabaeus* (compare Fig. 3A with 5A or 6D), probably because the stipes must accommodate the large flexor muscle.

We suggest that the galeal hooks and the epipharyngeal tooth may work synergistically, particularly in the detritus feeders. The two ends of a stiff, large plant fragment lying across the sharp edge of the tooth could be forced downwards by a

‘nodding’ movement of the hooks, which might cut or break the item into two smaller pieces. The complicated structures protruding medio-basally on the hooks might hold on to the ends of the plant fragment during the process.

CONCLUDING REMARKS

The present study has highlighted the cutting and grinding structures in the mouthparts of *Pachysoma*. The clypeal scraper, the epipharyngeal tooth and the unique galeal hooks must in some way (cf. the above suggestion) cut/break large food items into smaller pieces, an indispensable process in detritus feeders. And grinding must be performed by the strongly developed dorso-anterior part of the molae.

Are the differences between mouthpart morphology in the examined *Pachysoma* species primarily related to phylogeny or to feeding habits? With our limited sample of species, lineages and food preferences, few reasonably well-substantiated conclusions are possible. But the extreme reduction of the conjunctive within lineage 3 may be related to taxon rather than to food because it occurs in species with all possible food choices. By contrast, the morphology of the molae and of the epipharyngeal tooth/spine may be related chiefly to food choice. These structures are rather similar in pellet feeders of both examined lineages, and markedly different from those of detritus feeders in lineage 3.

The capacity for macerating the food, in all probability lost by the wet-dung feeding ancestors, cf. Introduction, has clearly been re-established in *Pachysoma*, as shown by the finely comminuted gut contents. On the other hand, there is no indication of “evolution in reverse” (e.g. Teotónio & Rose, 2001; Porter & Crandall, 2003) with re-acquisition of the normal biting mouthparts presumably present in a remote ancestor of wet-dung

feeders. On the contrary, the apparently well-functioning tools evolved for cutting and breaking up large food items in *Pachysoma* are novel and unique, quite different from normal mandibular incisors. Moreover, mouthpart evolution appears to have proceeded in the usual way, by reduction of some existing structures (e.g. the conjunctive) and enhancement (e.g. the dorso-anterior part of the mola) or other modifications (e.g. median brush → epipharyngeal tooth) of others. The results of these processes are essential adaptations for the arid habitats colonised by *Pachysoma*.

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