

Functional morphology of burrowing in the mayflies *Hexagenia limbata* and *Pentagenia vittigera*

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A video system, consisting of a high-resolution, infrared-sensitive, surveillance television camera, time-date generator, video recorder and high-resolution monitor, was developed for naturalistic observation of the subaquatic activities of larval mayflies. Exemplars were isolated from light, sound and mechanical disturbance, and their burrowing behaviour was recorded for subsequent slow-motion analysis. Larvae of silt-inhabiting *Hexagenia limbata* were observed to be ploughers that displaced and compacted soft substrate in the excavation of an open tunnel. The head and prothoracic legs had the principal roles in substrate displacement, while the mesothoracic and metathoracic legs drove the body forward. Five stereotyped burrowing cycles were distinguished, although one, termed the Alternate Undercut because of the motions of the prothoracic legs, predominated. Larvae of clay-inhabiting *Pentagenia vittigera* were observed to chisel and gouge out compacted substrates, also creating an open burrow. Their mandibular tusks were used to dislodge substrate, while the prothoracic legs displaced loosened substrate and widened the opening. The body was driven forward either by strong abdominal flexions or by activity of the mesothoracic and metathoracic legs. Four burrowing cycles were distinguished, two of which, the Tusk Gouge and the Chisel and Sweep, predominated. Differences in proportion and armature of the head and appendages correlated with the different substrate types excavated.

KEY WORDS:—Ephemeroptera – *Hexagenia* – *Pentagenia* – burrowing – function – morphology.

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INTRODUCTION

Larval mayflies of the families Behningiidae, Ephemeridae, Palingeniidae and Polymitarciidae burrow in the bottom or bank substrates of their aquatic environment. In addition, Gillies (1980) reported a burrowing euthyplociid from Gambia and some potamanthids may burrow as young instars (McCafferty, 1975). Burrowing thus occurs in representatives of all six families of Ephemeroidea. Individual genera and at least some species of these families appear to be sediment-type specific (McCafferty, 1975) and to demonstrate habitus associated with their particular infaunal habitat.

Burrowing may have certain benefits for organisms (Hildebrand, 1982). These include, for burrowing mayflies: (1) an increase in the predictability or stability of the microhabitat; (2) a source of food (aquatic muds are rich in algae and diatoms); and (3) predation reduction. Our observations suggest that burrowing mayfly larvae remain in their burrows until maturity. Similar conclusions were drawn by Spieth (1936) and Hunt (1953) for *Ephemera simulans* and *Hexagenia limbata*, respectively. Some larvae may leave their burrows to feed (Britt, 1962), because of high temperatures or low dissolved oxygen (Swanson, 1967), or possibly because of fluctuating water levels or overcrowding. Nevertheless, the excavation of a burrow is a predominant activity for these organisms.

General mechanical considerations are common to all burrowing animals. For one part of the body to advance, some other part must provide anchorage (Clark, 1964; Trueman & Jones, 1977). Hard substrates must be loosened, soft substrates kept from collapsing, and loosened substrates either transported or compacted. Loosening of hard substrate involves a relatively large force applied to a small area. Pointed, hardened structures are associated with this role. Dislodged material may require further breaking up before transport. Transport is facilitated by large, broad surfaces, formed from panels, webs or stiff hairs. Preventing the collapse of a burrow in soft substrates requires either a continuous outward pressure on the burrow walls, or application of a supportive burrow lining.

Habitus of burrowers is generally characterized by long, slender, cylindrical body types that minimize the volume of excavated substrate and facilitate in-burrow manoeuvring. Either the legs are reduced or absent and the head stiffened or hardened with well-developed neck musculature; or the legs are digging tools with a powerful musculature. Even in the latter, however, a tendency exists to reduce the length of proximal elements (bones or leg segments).

A number of burrowing strategies have been recognized for vertebrates (Gans, 1974; Hildebrand, 1982). These include soil crawling (snakes, lizards, amphibians), scratch digging (turtles, mammalian den-building carnivores, ground squirrels), chisel-tooth digging (rodents), and humeral-rotation digging (moles). Soft-bodied invertebrates burrow in a stepwise manner, alternately using two types of anchors (Clark, 1964; Trueman, 1975). The body is anchored while one region is advanced, then this advanced region is anchored and the remainder of the body is pulled forward by longitudinal muscles. Hard-bodied invertebrates burrow in a more continuous manner, with multiple appendages scraping out a cavity as well as providing anchorage (Trueman, 1970; Trueman & Jones, 1977). Mayflies use the latter approach.

Larvae of *Hexagenia limbata* (Serville), family Ephemeridae, and *Pentagenia vittigera* (Walsh), family Palingeniidae, are the subjects of this study. Both construct and maintain simple open burrows. There is no evidence of any tunnel system, although *H. limbata* reportedly can relocate itself by tunnelling away from its old burrow (Lyman, 1943). Although general facies of these burrowers are similar, they differ considerably in the morphological detail of structures used in burrowing (i.e. the mandibular tusks, the head processes and the explanate prothoracic legs). They also inhabit different, but intergrading, substrate types. Larvae of *P. vittigera* reside in very fine, cohesive, compacted clay or silt/clay; *H. limbata* resides in loosely compacted silt. Larvae of *H. limbata* are apparently sediment processors and filterers, whereas *P. vittigera* larvae are seston filterers.

Descriptions of burrowing behaviour and functional morphology of burrowing mayflies have been published but have generally been superficial and non-comparative. Lyman (1943), Wesenberg-Lund (1943) and Sattler (1967) reported on burrow architecture. Comments on aspects of burrowing behaviour were included in Ide (1935), Spieth (1936), Hunt (1953) and Edmunds & Traver (1959). Functional morphology notes on burrowing appeared in Morgan (1911, 1913), Clemens (1915), Morgan & Grierson (1932), Lyman (1943), Hunt (1953) and Tsui & Peters (1974). Needham (1920), Edmunds (1972), McCafferty (1975, 1979) and Gillies (1980) characterized morphology associated with burrowing.

Ephemeroid phylogeny has received considerable study (Tshernova, 1970; Edmunds, 1972; McCafferty, 1971, 1972, 1973, 1979; McCafferty & Edmunds, 1976, 1979; McCafferty & Sinitshenkova, 1983). Our future goal is to place structural-functional relationships in an evolutionary framework as suggested by Ross (1974) and Lauder (1981, 1982). Adaptive interpretations thus do not appear in the discussion of functional morphology below. Representatives of six midwestern ephemeroid genera have been examined to date as part of a study to compare the functional morphology associated with burrowing in all N American Ephemeroidea. Our study differs from previous ones in the use of video for data gathering, the degree of detail thereby resolved and its broad comparative goals. Our protocol for functional analysis was naturalistic observation followed by behavioural and morphological description and analysis. Hypotheses of functional morphology were then synthesized.

METHODS

Larvae of *H. limbata* were collected from bottom substrates along the margins of Little Pine Creek in Tippecanoe County, Indiana. This substrate was composed of organically enriched silt/clay, loosely compacted to a depth of c. 5–20 cm (md. phi >4, phi scale from Cummins, 1962) and devoid of coarse sands or gravel. For the most part, rooted vegetation was absent. Larvae of *P. vittigera* were collected from the eroded, vertical clay (md. phi 6) banks in strong current of the Wabash River in Fountain County, Indiana. Breaking off portions of the bank and bottom substrates in water up to 1.5 m deep yielded numerous larvae. All evidence to date suggests that each species has a narrow range of substrate tolerance. Collected specimens were placed in natural substrate in large laboratory aquaria.

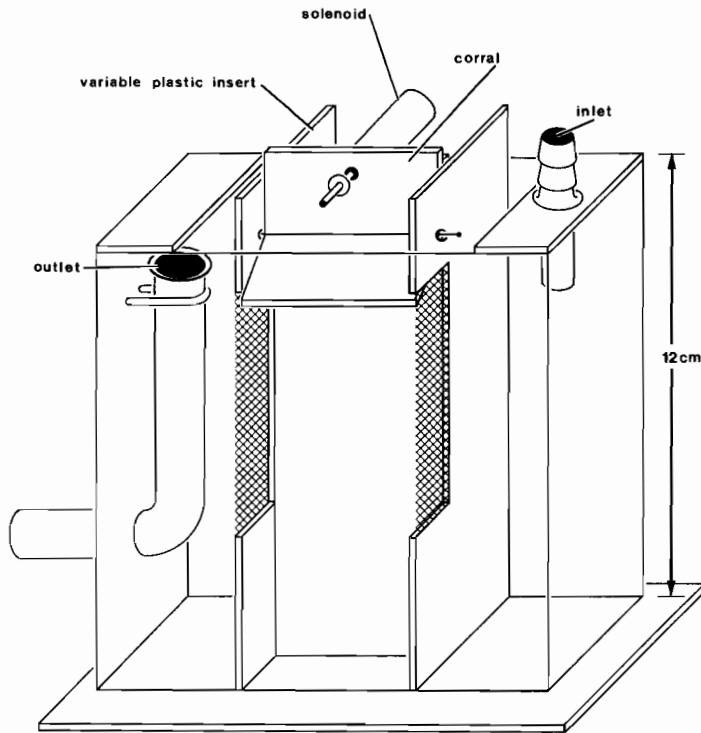


Figure 1. Viewing aquarium and corral.

An objective of the observational methodology was to maximize the fidelity of observation while minimizing disturbance to the study organisms by simulating their natural environment. The recording of observations on a permanent medium is essential for the study of complex motion. Here video was the medium of choice for several reasons: (1) the cost of video (equipment and materials) was about 10% that of film; (2) results were available immediately; (3) special features such as extreme low-light sensitivity, high resolution (black and white), expanded spectral sensitivity, slow-motion playback, and time-lapse recording were all available; (4) adding time and date information and titles to the picture was easily accomplished through in-line accessory devices; and (5) the audio tracks on the video tape were available for experimental note-taking.

For observation, specimens were removed from the holding aquaria and placed in a corral mounted on top of the viewing aquarium (Fig. 1). These were fabricated from acrylic plastic, but the viewing aquarium had a glass front plate. Acrylic inserts allowed modification of the confinement area to adjust for specimen size. Typically these inserts defined a space 11 cm high \times 3.5 cm wide \times 1 cm deep, the bottom 5 cm of which contained substrate. These inserts were screened along their upper sides, and inlet and outlet ports allowed for a continuous, controlled current of water that facilitated constant aeration and steady temperature. All initial observations were made in natural substrates taken from the habitat of the study specimens. Artificial substrates of glass beads (<2 mm diameter), filtration beads (e.g. Sephadex G-25) and gelatin were also used.

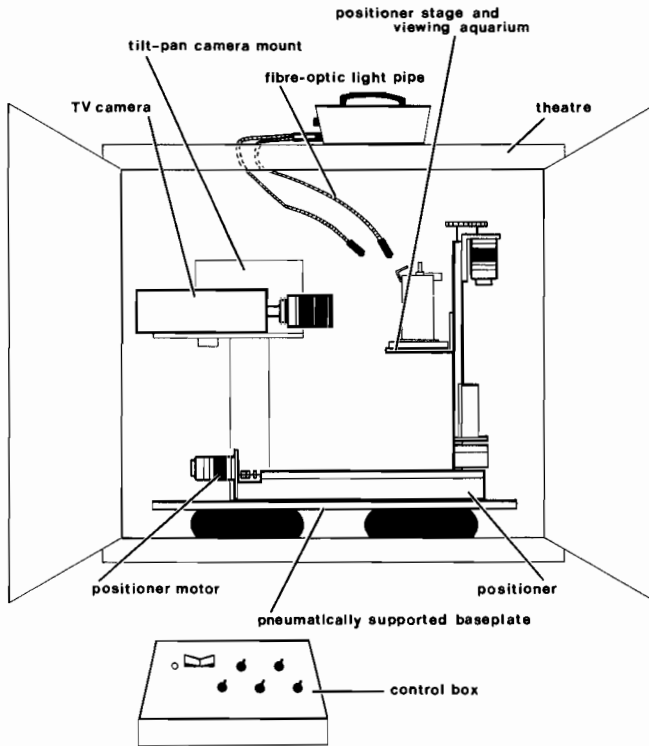


Figure 2. Remote-controlled positioner and observation theatre.

The viewing aquarium and corral were placed on the positioner stage. The positioner (Fig. 2) was an assembly of three motorized, orthogonally mounted dovetail slides. The positioner and tilt-pan camera mount were bolted to a pneumatically supported, 1.25 cm steel baseplate. This entire assembly resided in a large, light-tight, sound-proof theatre (*c.* 1 × 1 × 1 m). Subjects were thus isolated from mechanical shock and noise, and the quality of lighting on the subject was completely controlled.

A fibre-optic illuminator, mounted on the roof of the theatre, supplied a source of 'cold' light for the camera. Gelatin filters were mounted to the ends of the light pipes, allowing control over the portion of the electromagnetic spectrum to which the study specimens were exposed. A high-resolution surveillance television camera (Panasonic WV-1850) equipped with a 1" (2.5 cm) Extended Red Newvicon pickup tube (S4119) was used for viewing the specimens. This pickup tube was extremely sensitive (minimum recommended illumination is 1 lx) well into the infrared (to wavelengths of *c.* 900 nm). With 75 mm of extension on a 50 mm Macro-Switar cine lens, a magnification of 35 × was provided on the display monitor, with a subject-lens distance of 6 cm. The camera signal was routed through a time-date generator (Panasonic WJ-810) that was also capable of providing a stop-watch function with a readout in hundredths of a second. The video signal was next fed to the recorder (Panasonic NV-8950). The video signal was displayed on a high-resolution (>700 lines horizontal) 9" (23 cm) black and white video monitor (Panasonic

TR-932. A video logbook catalogued the time and date of every recording session, the subject, the experimental viewing set-up and a short description of the type of activity shown.

The objectives of the video analysis were to determine what morphological features were used in burrowing and what were the range, timing and role of each. This was plotted on a SIMO (simultaneous motion) chart. Charts were composites of behavioural observations of many individuals (*c.* 20 for *H. limbata* and 45 for *P. vittigera*). This analysis was facilitated by special features of the video recorder, including forward and reverse slow-motion playback, freeze frame and frame advance. Using frame advance, movements occurring in intervals as short as 0.03 s could be studied.

Structures identified during the behavioural analysis as having a role in burrowing were then analysed morphologically. For this analysis, the spatial relationships, shape and detail of structures were of main concern. Microscopic examination of all relevant structures on 10 individuals of each species was conducted. Drawings were made and notes were taken on articulation, mode of attachment, range of movement and orientation. The shape, including outline and surface modulation, and the extent and type of surface detail (setation and armature) were described.

Written and pictorial descriptions of the modal and variant burrowing patterns were assembled. In the written description each pattern was called a cycle and subdivisions thereof were called stages (following the terminology of Trueman, 1968). Such cycles were labelled with descriptive names in accordance with their perceived role. Stages were defined as continuous, synchronous, directional movements of a structure or a set of structures.

RESULTS

General morphology

A mature larva of *P. vittigera* is illustrated in Fig. 3. It shows typical features of burrowing mayfly larvae: a head with large compound eyes, paired lateral ocelli, long, filiform antennae, and complete mandibulate mouthparts with the mandibles bearing tusk-like projections; a thorax with a pair of legs on each segment, those on the pro- and mesothorax orientated anteriorly, those on the metathorax, posteriorly, and two pairs of wing pads; an abdomen with gills that are fully developed on segments 2–7, and three tails (a median terminal filament and two cerci) that issue from segment 10. Each leg is composed of six substructures: coxa, trochanter, femur, tibia, unsegmented tarsus and claw. The joint formed where these leg segments articulate is, in all cases, dicondylic.

The femur, tibia, and tarsus of all legs were considered to be laterally flattened and, since the flat plane is generally oriented vertically, the faces were referred to as inner (medial) and outer (lateral). The margin of the leg segment that was dorsal or sometimes anterior was always referred to as the dorsal margin. The same applied to the ventral margin, which may have been oriented ventrally or posteriorly. In the descriptions that follow, when angles are given they are always interior angles.

Movements associated with burrowing are reviewed here. These movements were similar for the two species, but the details of how these motions were

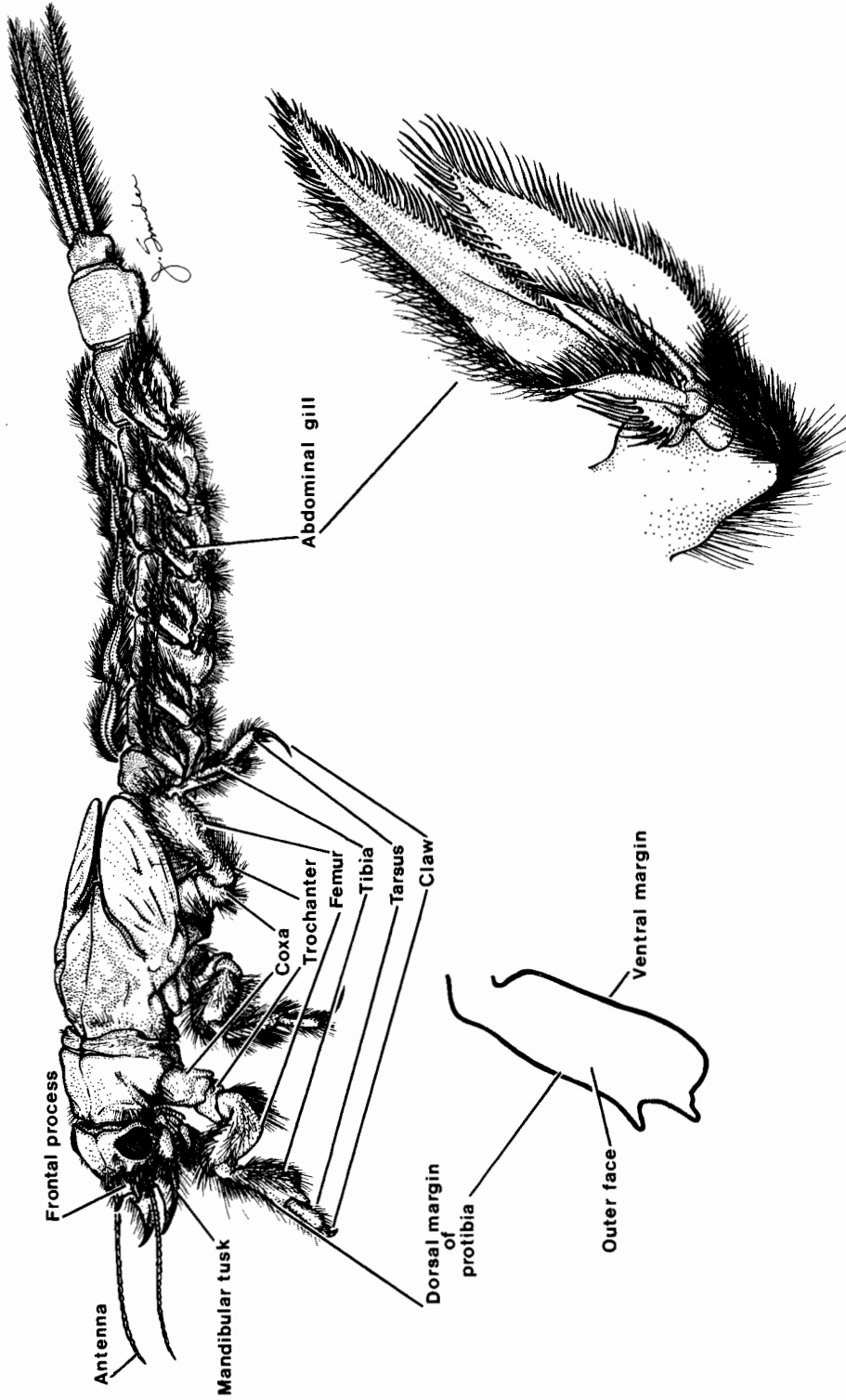


Figure 3. Typical burrowing mayfly larva (*P. vittigera*). Length from frontal process to base of tails *c.* 21 mm.

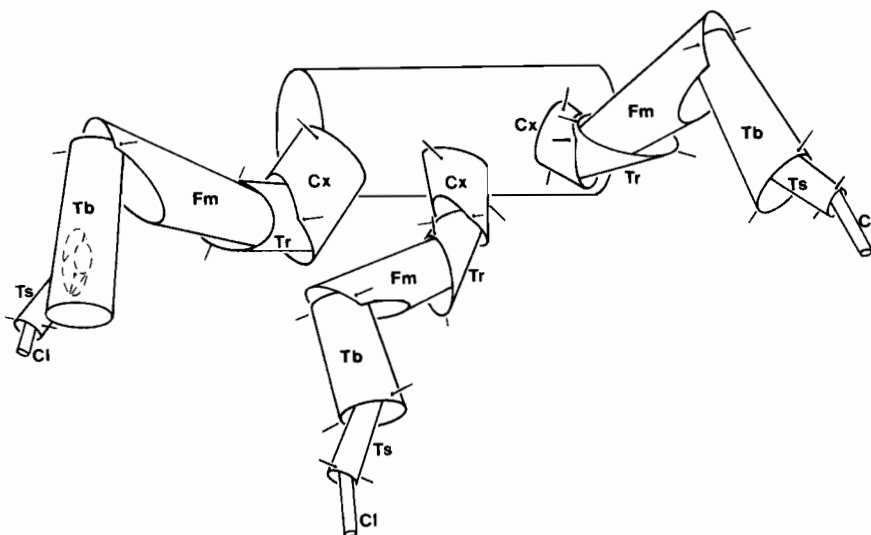


Figure 4. Schematic diagram of leg jointing, foreleg is left (based on *P. vittigera*). Lines through joints indicate axes of the dicondylous hinges. Cx = coxa, Tr = trochanter, Fm = femur, Tb = tibia, Ts = tarsus, Cl = claw.

co-ordinated with those of other structural units differed. The head and prothorax moved dorsoventrally, more-or-less as a unit. Leg movements were more complicated (see Fig. 4). Abduction/adduction was principally effected by muscle action on the coxa, about the body-coxal hinge. Elevation/depression of the leg occurred about the coxal-trochantoral hinge. Inversion/eversion occurred about the trochantoral-femoral hinge. The tibia was flexed/extended about the femoral-tibial hinge. The tarsus and claw were elevated/depressed relative to the tibia about the tibial-tarsal hinge. The abdomen moved dorsoventrally from about segment 2. Gill pairs, brought together over the abdomen synchronously and progressively from anterior to posterior, produced a wave-like gill beat or pulse.

Burrowing behaviour

Hexagenia limbata

Summary of burrowing behaviour: Larvae of *H. limbata* were ploughers that displaced and compacted the soft substrate in the creation of a tunnel. The prothoracic legs had the principal role in substrate displacement. The head was used to make initial penetration (particularly the tusks), to displace substrate vertically and to anchor the body during digging. Although the antennae were active, their role in burrowing was uncertain. The meso- and metathoracic legs, once below the substrate surface, were used to drive the body forward. The gills beat continuously, but slowly, and provided only a minor current during most of the tunnel excavation. *Hexagenia limbata* displayed five burrowing cycles one of which was modal, the others being less frequent or auxiliary.

Principal burrowing cycles of H. limbata: CYCLE I: Alternate undercut (Fig. 5). This was the modal cycle, the characteristic sequence of motions by which *H. limbata* larvae burrowed. This cycle occurred on initial contact with the

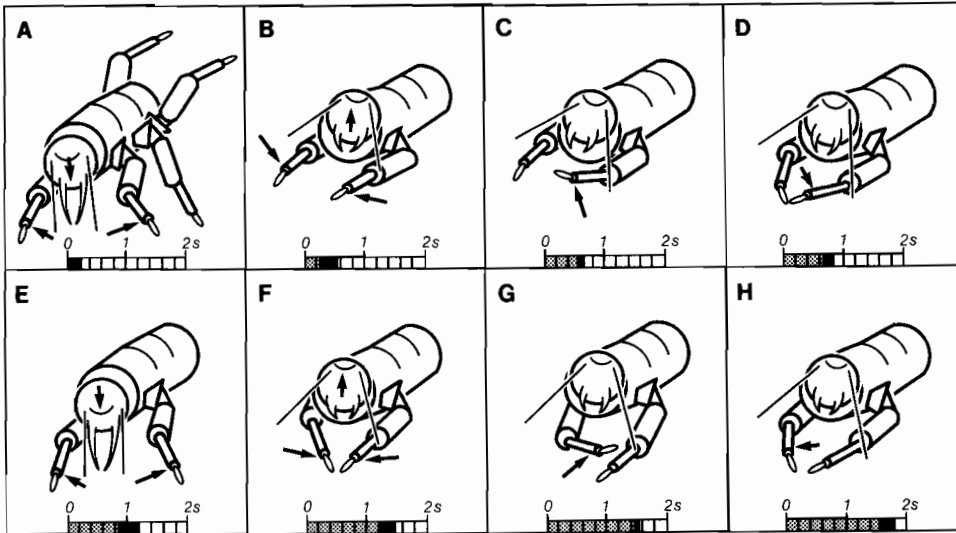


Figure 5. Frontolateral view of the modal burrowing cycle of *H. limbata*, the Alternate Undercut. Boxes delimit stages, the duration of which is indicated in black in the corresponding time bar. Arrows indicate the direction of movement that precedes successive stages.

substrate, it was often briefly preceded by cycle II, cycle III, or sometimes both in sequence; cycle IV sometimes interrupted it (these other cycles are described below). This cycle is divided into stages.

Stage 1, prothoracic leg abduction (Fig. 5A). From a starting position with the protibiae extended (*c.* 120°) beneath the tusks and the tarsi crossed, the prothoracic legs were abducted through an angle of 30–45° from the saggital body plane. This abduction, effected by movement of the coxae, was accompanied by elevation and some eversion of the legs (thus the legs were swept outward, and the outer flattened surfaces of the femora and tibiae were pulled and rolled upward). As the prothoracic legs were abducted the head and prothorax were depressed and the antennae were adducted. The mesothoracic legs were generally not abducted simultaneously with the prothoracic legs. (It appeared that the timing of the mesothoracic leg movements was determined by their position. The mesothoracic legs could be abducted through *c.* 90°. When this angle was achieved they were adducted. Thus the timing of their movement was largely independent of prothoracic leg action.) The duration of this stage was 0.2–0.3 s. The velocity of the abduction slowed as it proceeded.

Stage 2, prothoracic leg adduction (Fig. 5B). A period of up to 0.2 s elapsed between abduction and subsequent adduction. In this stage one leg was adducted before the other, and it was this leg that executed the undercut (see below); the time difference in onset of adduction was 0.05–0.15 s. During initial adduction the tibiae were flexed onto the femora as both were everted and elevated. This sequence was rapid, lasting *c.* 0.1 s. The tibiae were then extended and the femora depressed and inversion begun. Total time of adduction to return to starting position of stage 1 was less than 0.2 s. The head was elevated and the antennae were abducted simultaneously with or just preceding the initial adduction of the prothoracic legs. (The antennae moved

through an arc from anterior to 30° laterad; their motion was counter to that of the prothoracic legs. The distal half of the antenna moved passively with the basal half in a whip-like manner.)

Stage 3, single prothoracic leg undercut (Fig. 5C). The leg that initiated adduction was further adducted, depressed and inverted to a point below the head and tusks. As the undercut proceeded the tibia was flexed. The non-undercutting leg was brought to the starting position for abduction. The undercut lasted *c.* 0.1 s and its effect was to cut or shovel the substrate directly beneath the head.

Stage 4, lead prothoracic leg abduction (Fig. 5D). The undercutting leg was then abducted, tibia extended, femur elevated and everted to the starting point of stage 1. This required 0.05–0.20 s. The elapsed time from abduction to abduction was 0.9–1.0 s.

Stage 5. The legs were abducted as in stage 1 (Fig. 5E).

Stage 6. The previously non-undercutting leg began the adduction (Fig. 5F).

Stage 7. This leg executed the undercut (Fig. 5G).

Stage 8. This leg was abducted, elevated, everted and the tibia extended to the starting position (Fig. 5H).

Time for the complete cycle, including both legs alternately undercutting, was 1.8–2.0 s. Variation was present in the sequence of execution of this cycle. For example, one leg frequently made two or more successive undercuts; this sometimes led to a change in direction of burrowing.

CYCLE II: Abdominal undulation. This cycle occurred infrequently and then only during initial penetration, presumably to break the surface. It was an extension of swimming behaviour. The legs were drawn in to the body: the prothoracic legs were extended forward and were angled along and under the tusks (with the protarsi and claws directed anteriorly), the mesothoracic legs were flexed to the thorax, and the metathoracic legs were flexed over the abdomen. A forward propulsion was provided by dorsoventral flexions of the abdomen, beginning at about segment 2. The gills were abducted and held horizontally. This drove the head and prothoracic legs into the substrate. This cycle lasted 1–2 s before digging was initiated, with 8–10 abdominal undulations per second.

CYCLE III: Unison prothoracic leg sweep. Like cycle II, this cycle was observed infrequently, and only during initial penetration. When it occurred, the movements were repeated 1–5 times after which either cycle I or cycle II would follow. This cycle differed from the Alternate Undercut in that the prothoracic legs were simultaneously brought together under the tusks and swept outward, the other legs were inactive. Also, the prothoracic leg abductions were less extensive (never exceeding 30°), the frequency of the cycle was higher (*c.* 2–3/s), and the head and antennae were stationary.

CYCLE IV: Abdominal undulation during digging. This cycle occurred only during the early period of burrowing, while the abdomen was above the level of the substrate. The undulations were rapid dorsoventral flexions with gills spread. They differed from cycle II in being of lower frequency (*c.* 5/s) and of shorter duration (2–6 undulations, lasting 0.5–1.0 s). The effect was similar, thrusting the body into and perhaps loosening the substrate.

CYCLE V: Digging with metathoracic leg support. Once burial reached the level of the thorax, the metathoracic legs came into play. Normally they were

directed posterolaterally at *c.* 60°, with the flattened surfaces of the femora and tibiae lying horizontally in the dorsal plane of the thorax. The metathoracic legs were flexed, rotated anteriorly and extended into the substrate, providing anchorage and propulsion for the organism. Their timing, like that of the mesothoracic legs, depended mostly on their position.

Further notes on H. limbata: The abduction/adduction of the mesothoracic legs was similar to that of the prothoracic legs although there was no undercut. Adduction began with flexion of the tibiae on the femora, followed rapidly by elevation and inversion of the femora. The femora were then depressed and the tibiae extended as adduction brought the legs to a position below the side of the body. Time for adduction was 0.1–0.2 s. Abduction was accompanied by extension of the tibiae and elevation and eversion of the femora. The tusks were never actively manipulated during burrowing. They were held with one tip overlapping the other. During abduction the sickle-shaped protarsus/claw was directed anteroventrally and acted somewhat like a plough to break up the substrate. The gill beat during initial penetration was fairly constant and slow, typically 1–2 beats/s; however, completion of the tunnel was facilitated by a gill-generated current (as described by Lyman, 1943). The gills were depressed *c.* 45° and then elevated to vertical. Only the gills on segments 3–4 made much contact with each other over the abdomen; those of other pairs at most touched tips. The cerci, which were spread at 120°, were drawn together as they entered the substrate.

Pentagenia vittigera

Summary of burrowing behaviour: Larvae of *P. vittigera* were chisellers and gougers that dislodged compacted substrates in the formation of a burrow. The tusks had a dominant role in opening a burrow. The antennae were inactive. The prothoracic legs moved loosened materials and widened the tunnel. Once penetration was sufficiently advanced, the meso- and then the metathoracic legs were used to drive the body forward into the substrate. During burrow excavation the gills beat continuously but slowly, producing a minor current which was insufficient for loosening substrate but possibly was useful for suspending soil particles. Abdominal flexions, to drive the body forward, were common prior to full burial. Larvae of *P. vittigera* displayed four burrowing cycles; the two modal cycles predominated but had numerous variations.

Principal burrowing cycles of P. vittigera: CYCLE I: Chisel and sweep (Fig. 6). This was a modal cycle used especially during initial penetration, although it also occurred after considerable penetration beyond the level of the metathoracic legs. It predominated in hard substrates and included seven stages. Chiselling occurred by abdominal undulation (stage 2) and sweeping was effected by movements of the prothoracic legs (stages 5 and 7). Other stages provided positioning or additional loosening of the substrate.

Stage 1, leg adduction (Fig. 6A). In the starting position the head was depressed and the tusk tips pointed anteriorly. The antennae were held dorsolaterally at 45° to the sagittal body plane. The pro- and mesothoracic legs were drawn in to the sides of the body. The profemora were angled dorsally, the protibiae were extended parallel to the tusks and inverted somewhat so that the flat plane sloped mediad. The protarsi and claws were directed anteriorly. The mesofemora were horizontal; the mesotibiae extended at about 120°

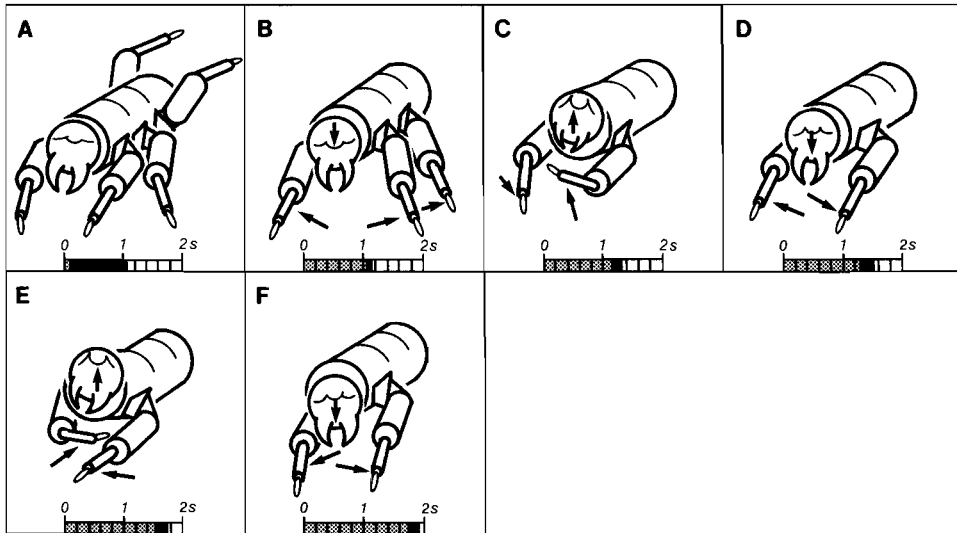


Figure 6. Frontolateral view of a modal burrowing cycle of *P. vittigera*, the Chisel and Sweep. Boxes delimit stages, the duration of which is indicated in black in the corresponding time bar. Arrows indicate the direction of movement that precedes successive stages.

anteroventrally, the mesotarsi and claws angling ventroposteriorly. The metathoracic legs were extended posterolaterally and were not active during this cycle. A powerful dorsoventral flexion of the abdomen forced the body forward. Duration was <0.1 s.

Stage 2, abdominal undulation. This undulation was an application of the swimming motion. The legs were held as in stage 1, and the abdomen was repeatedly flexed dorsoventrally from about segment 2. The tips of the tusks, the proclaws, the processes of the head, and the mesoclaw were repeatedly thrust against the substrate, chiselling and loosening it. Duration was $c. 1.0$ s, with 10 undulations/s.

Stage 3, head depression (Fig. 6B). The head was rapidly depressed and the thoracic legs were abducted slightly. The mesothoracic legs were abducted, pushing the body forward. Duration was $c. 0.1$ s.

Stage 4, head elevation and prothoracic leg undercut (Fig. 6C). The head made a gouging cut against the substrate (in hard substrates this effected a push-off). As the head was raised, the prothoracic legs were adducted beneath the head (coxal adduction, trochantal depression, femoral inversion, tibial flexion). As in *H. limbata*, one leg adducted prior to the other and it executed the more posterior undercut (both legs were adducted to a position beneath the head). The legs overlapped under the head at least to the apices of the tibiae. The mesothoracic legs continued their abduction (as in *H. limbata*, the timing of their abduction/adduction appeared to be related to effectiveness for pushing since they would usually be adducted and reset once abducted beyond 90°). Duration was $c. 0.1-0.2$ s.

Stage 5, prothoracic leg abduction (Fig. 6D). A lateral sweep was made with the prothoracic legs (coxae abducted, trochanters elevated, femora everted, tibiae extended). As the legs passed the lateral margin of the head, the head was

depressed. The prothoracic legs abducted to an angle of about 15° lateral to the sagittal plane. Thus the sweep was a relatively short stroke. Duration was *c.* 0.2 s.

Stage 6, head elevation and prothoracic leg undercut (Fig. 6E). The head was elevated as the body was pushed forward by the mesothoracic leg abduction. The prothoracic legs were elevated, then adducted and inverted, and the tibiae flexed into the cavity beneath the head. This was a repeat of stage 4. Duration was 0.1–0.2 s.

Stage 7, prothoracic leg abduction (Fig. 6F). This consisted of a short lateral sweep that was a repetition of stage 5. Duration was 0.1–0.2 s.

Total time for the complete cycle was 2 s. Variations of cycle I occurred. The number of undercut/sweeps was usually 2–3/cycle, but this varied depending on substrate hardness. On occasion this prothoracic leg activity would go on almost long enough to be considered a distinct cycle. This cycle graded into cycle II (see below) as the substrate softened or loosened and as the metathoracic legs were able to contribute to forward propulsion. Occasionally the head would remain elevated and stages 5 and 6 would be repeated several times. Another variation involved a modification of stage 6. The prothoracic legs did not undercut the head but were adducted merely to the lateral margin of the head before subsequent abduction.

CYCLE II: Tusk gouge (Fig. 7). This modal cycle predominated once burrowing passed the point of initial penetration, and during initial penetration in softer substrates. The head and tusks were used to excavate the burrow and the legs provided propulsion.

Stage 1, body advance (Fig. 7A). In the starting position the head and prothorax were depressed *c.* 60° to the frontal body plane, with the tusk tips pointing anteriorly. The prothoracic legs were abducted beyond the side of the head, but not widely. The mesothoracic legs were adducted to the body. The metathoracic legs (given sufficient penetration) were abducted, with their tibiae flexed. The head and body were then pushed forward by abduction of the mesothoracic legs (and extension and adduction of the metathoracic legs) and by further abduction and eversion of the prothoracic legs. Duration was 0.1 s.

Stage 2, head elevation (Fig. 7B). The head and prothorax were elevated while the legs continued their forward push. The prothoracic legs pushed down into the substrate aiding head elevation. Duration was 0.2 s.

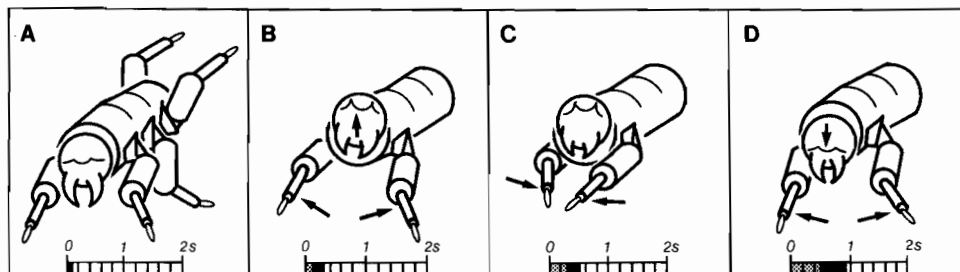


Figure 7. Frontolateral view of a modal burrowing cycle of *P. vittiger*, the Tusk Gouge. Boxes delimit stages, the duration of which is indicated in black in the corresponding time bar. Arrows indicate the direction of movement that precedes successive stages.

Stage 3, prothoracic leg adduction (Fig. 7C). With the head fully elevated, the prothoracic legs were simultaneously adducted to a point just below the lateral margins of the head; the legs did not overlap. Duration was *c.* 0.2 s.

Stage 4, head depression (Fig. 7D). The head was depressed as the prothoracic legs were being adducted. The prothoracic legs were abducted as head depression continued. The mesothoracic legs were adducted and reset towards the end of this stage (also the metathoracic legs), depending on their current position. Duration was 0.4 s.

Total time for the complete cycle was less than 1 s. Variations of cycle II occurred. The advance might be followed by a brief elevation and depression of the head without any intervening prothoracic leg adduction. The duration of the cycle seemed to vary with substrate hardness. The cycle was sometimes executed with full elevation and depression of the head without prothoracic leg adduction. Several of these movements might be strung together into a series of long and short gouges without prothoracic leg activity. In such cases the cycles were of shorter duration. Rather than being adducted, the protibiae were sometimes flexed and extended (while being held abducted), evidently providing a rasping effect on the tunnel sides. Again this motion coincided with head depression, and several tibial flexions might occur in the interval.

CYCLE III: Unison prothoracic leg sweep. This cycle was observed only on initial contact with the substrate (and was different from the unison sweep that was a variation of cycle II). It was not possible to determine whether or not this cycle was invariably performed on first contact with the substrate, so its use in substrate testing is dubious. Movement consisted of the simultaneous and rapid inversion and eversion of the profemora. The protarsi were also actively moved through the substrate.

CYCLE IV: Alternate sweep. This cycle was observed during initial penetration in soft substrates (silts and silt/clays). The pro- and mesothoracic legs on one side of the body were adducted abducted in opposite phase to the legs on the other side.

Further notes on P. vittigera: Initial penetration was the most difficult phase of burrowing for these organisms, certainly more difficult than for *H. limbata* which burrowed rapidly out of sight in their natural substrate of loose silt. Once *P. vittigera* was able to use the head and legs to brace and anchor itself, burrowing proceeded quite rapidly. The tusks were held stationary, narrowly separated during burrowing and moving only as projections of the head. The claws on the protarsi were 'hooked' outwardly during leg sweeps and thus acted to cut the substrate. During undercuts they were ineffectual as femoral inversion effectively put the point on its side. The tails were pulled together as they entered the substrate. A gill current was not used in the opening of a burrow; however, the gills did provide a current to flush out debris. This was witnessed most clearly once the burrow was completed. The gills of *P. vittigera* were observed on occasion to make several consecutive reverse beats (posterior to anterior pulse or wave) thereby moving water forward over the body. Observations of in-burrow activity revealed how larvae positioned themselves in the burrow. The tails were spread, the abdomen touched the substrate on sterna 2-7, the metathoracic legs were outstretched, the mesonotum touched the substrate, the mesothoracic legs were extended ventrally touching the substrate, the prothoracic legs were flexed and not in contact with the substrate (their

setae formed seston catchnets), the head and prothorax were somewhat depressed and the antennae were directed anteriorly with the tips downcurved. Turning within the burrow was accomplished by depression of the head beneath the thorax and then pushing with the legs while wriggling the body. Once the thorax was turned, the abdomen was pulled over, completing the manoeuvre.

DISCUSSION

Size and proportion

Larvae of *P. vittigera* are smaller than equivalently mature larvae of *H. limbata* (c. 3/4 the length of *H. limbata*). They appear more robust because of their relatively larger head and thorax (c. 4/5 that of *H. limbata*), and relatively smaller legs (c. 2/3) and tails. This correlates with the predominant burrowing roles of the head and tusks in *P. vittigera*, and the legs (particularly the prothoracic ones) in *H. limbata*. Size, however, may be related to factors other than the exigencies of burrowing.

Head

(Figs 8 & 9)

The heads of both species are large and have an extensive dorsal surface, the area of which is increased by the presence of a frontal process. This extensive surface has a principal role in anchoring the body while the legs dig or sweep the substrate. In soft substrates a secondary result is the opening of a cavity by the elevation of the head and perhaps also the compacting of the substrate above the head. The most striking difference between the heads involves the armature. The much more heavily armed *P. vittigera* has the sclerotized, upcurved points (often with additional medial crenulations) of the frontal and supra-antennal processes (Fig. 8B), and the much more massive tusks (Fig. 9B) with their heavily sclerotized tips and spuriferous setation. In contrast, armature is absent from the head of *H. limbata* (Fig. 8A) and the tusks are slender, with a smooth outline, and though upturned they are not heavily armed or sclerotized (Fig. 9A). As noted above, behavioural observations of *P. vittigera* clearly demonstrated the use of these tusks in gouging out a tunnel in compacted substrates. The tips of the tusks appeared most important in actually cutting or breaking loose the substrate. The dorsal processes may, then, act as cutting, disintegrating and/or anchoring structures. It appears that the spurs along the tusks, though they may have a rasping effect, serve mainly to break up the larger chunks of loosened substrate. Further breakdown was accomplished by the prothoracic legs and these also swept suspended particles rearward. The tusks of *H. limbata* seem to act as a prow, cleaving the substrate, although they may also provide anchorage while the legs dig.

The antennae of *H. limbata* (Fig. 8A) have dense whorls of long setae proximally and make rhythmic lateral sweeps in opposition to the movements of the prothoracic legs. Their net effect may be to help in dislodging and dispersing substrate, to create a minor current that pushes suspended soil particles rearward, or to keep the head and the eyes free of silt. The bare antennae of *P. vittigera* (Fig. 8B) are not active during burrowing.



Figure 8. Comparison of heads. A, *H. limbata*; B, *P. vittigera*.

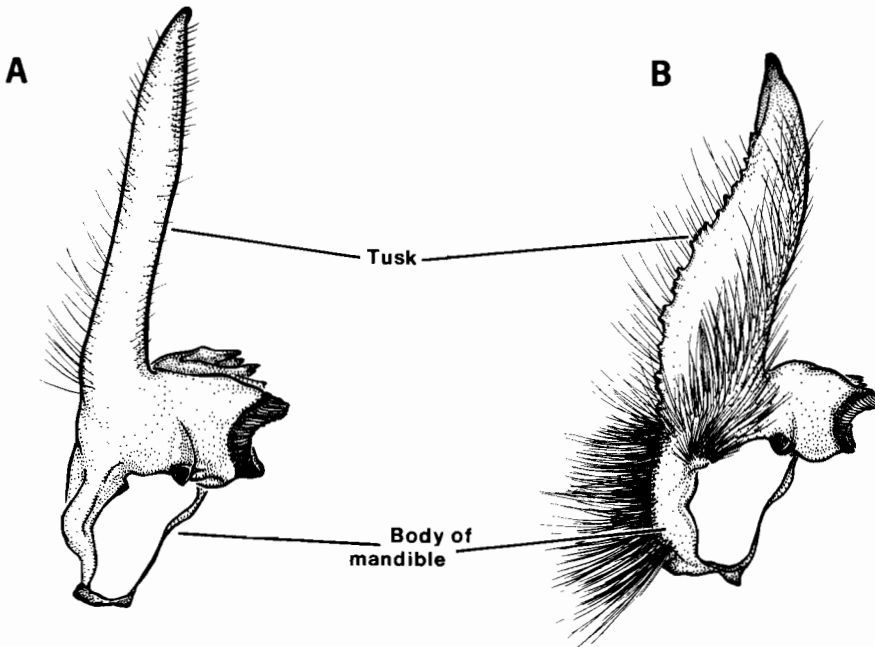


Figure 9. Comparison of tusks (not drawn to scale). A, *H. limbata*; B, *P. vittigera*; left mandible, dorsal view.

Prothoracic legs (Fig. 10)

Of all the legs, the prothoracic legs have the greatest number of structural differences between the observed species. The extensive pattern of setation, except for that associated with the seston catchnet of *P. vittigera*, is similar. Shape, particularly of the tibiae, orientation and the hinging patterns of the coxa–trochanter–femur differ markedly.

As argued above for tusks, one would expect the more heavily armoured, spur-bearing form to be associated with the cutting and breaking up of compacted substrates. The mechanical problem of penetrating such soils is the application of a relatively large force to a small area. This is effected with regard to the prothoracic legs of *P. vittigera* (Fig. 10B) by the point of the claws, the tibial processes, the spurs along the dorsal margin of the tibiae and the stout setae of the tibiae and tarsi. Whether the role is more to loosen the substrate initially or to break up chunks dislodged by the tusks is unclear. The outswEEPing tibiae, due to the nature of femoral eversion (as well as shape), make contact with the compacted substrate on their dorsolateral edge (the spur-bearing edge with the outcurved distal processes). The outer face contacts loosened substrate both on the outer sweep and on the undercut.

The different hinging arrangements of the procoxa–trochanter–femur are striking in light of the similarity of such arrangements in the meso- and metathoracic legs. The profemora of *P. vittigera* (Fig. 10B) are laterally displaced relative to those of *H. limbata* (Fig. 10A) since the body–coxal hinge is at a much

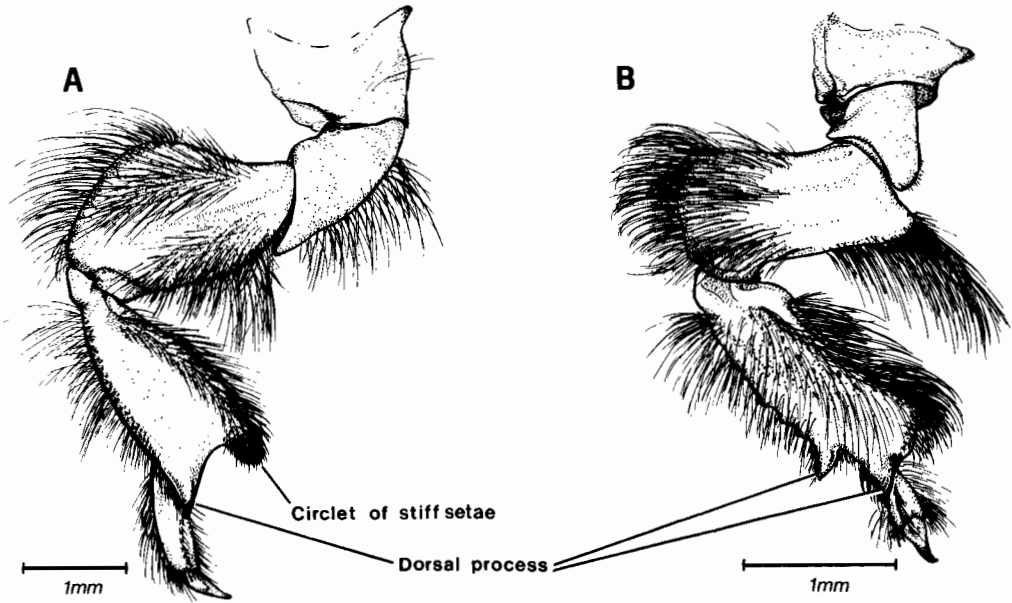


Figure 10. Comparison of prothoracic legs. A, *H. limbata*; B, *P. vittigera*; left leg, lateral view.

greater angle to the sagittal body plane (at *c.* 60° vs. 30°) and the trochanters are inserted on the inner face of the femora. This would seem to allow greater abduction of the leg and perhaps restrict adduction (as is the case for the mesothoracic legs, which are set at a greater angle than the prothoracic legs in both species). But observation revealed that the prothoracic legs of *P. vittigera* were generally abducted less widely than those of *H. limbata* and that the undercut was as deep if not deeper. The outer faces of the protibiae lie in nearly the same plane when the procoxae of *H. limbata* and *P. vittigera* are similarly positioned. The difference in femoral placement is therefore made up in part by the shape of the protibiae of *P. vittigera*, which are bowed out and are also thicker ventrally, and in part by the shape of profemora of *P. vittigera*, which are more bowed.

The different hinging arrangements of the procoxa-trochanter-femur may reflect the demands (using evolutionary shorthand) of burrowing in markedly different (resistant) substrates. The trochantal articulation in *P. vittigera* is well onto the inner face of the femur, and this might provide greater power during leg abduction for femoral eversion. Placement of the hinge distad would improve leverage for basally inserted muscles. Another possible explanation for these hinging differences involves seston filtering behaviour rather than burrowing. To feed, the larva of *P. vittigera* faces into the current circulating through the burrow (generated either actively through gill movements or possibly passively by the architecture of the burrow or its placement with respect to the stream current). This apparatus consists of the long setae on the ventrobasal angle of the femora (see Figs 10B & 11), the long setae on the ventral margin of the tibiae and the rows of long setae present on the inner face of the tibiae. When the prothoracic legs are held elevated with the tibiae partly flexed, a functional basket-like catchnet is formed. The legs are periodically

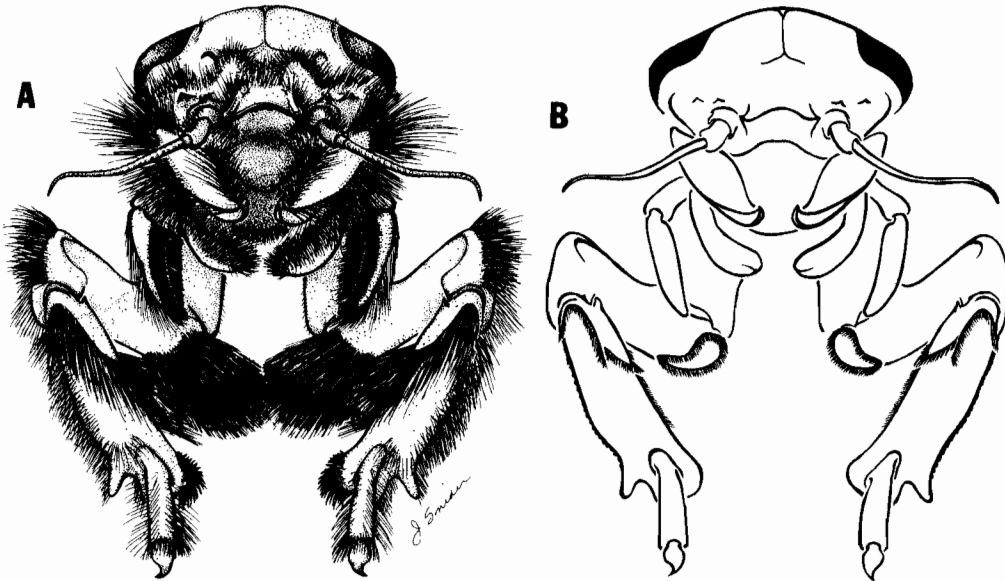


Figure 11. Seston catchnet of *P. vittigera*, frontal view. A, Detailed illustration; B, setae comprising catchnet indicated by short hatching.

raised and the net is swept clean by the maxillary palpi. The long setae on the head and prothoracic legs perhaps form a seal with the burrow walls and increase the efficiency of the apparatus by channelling the current. This argument suggests that coxal placement is a means of achieving a large, wide catchment area.

Mesothoracic legs (Fig. 12)

The large mesotarsi of *P. vittigera* (Fig. 12B) are effective in the pushing role of the mesothoracic legs. Observations showed that this occurred mainly through leg abduction. The angulation of the long, ventrally pointed claw, however, is such that it would not hook into the substrate. It is possible that the claw is employed effectively during in-burrow manoeuvring or to anchor the body in the tunnel (in conjunction with the metathoracic legs).

The mesotarsi of *H. limbata* (Fig. 12A) are not so large or expanded and they angle dorsally from the tibiae. The tibial-tarsal hinge runs almost lateromedially and the tarsi thus move in an arc mostly in and below the flat plane of the tibiae. The point of the claw is not very long and it points laterally. These legs push the body forward as in *P. vittigera*, but they are also somewhat effective in sweeping and compacting substrate.

Metathoracic legs (Fig. 13)

These legs are mounted laterally on the body and move laterally and posteriorly in the dorsal body plane. During burrowing they aid in pushing the

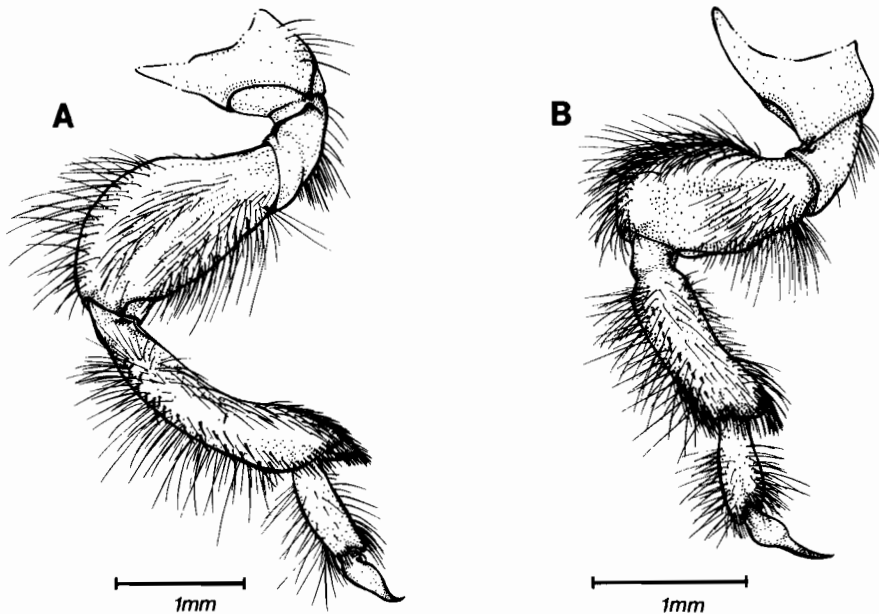


Figure 12. Comparison of mesothoracic legs. A, *H. limbata*; B, *P. vittigera*; left leg, lateral view.

body forward once penetration has reached a sufficient depth. The push is accomplished through the extension of the tibiae, the depression of the trochanters and somewhat by the adduction of the coxae. The trochanters are relatively very long and attach broadly to the femora, increasing the length of the leg and probably facilitating the transfer of power during depression of the trochanter.

The tibial process of the metathoracic legs of *H. limbata* (Fig. 13A) is long and dorsally and laterally curved, making a hook almost opposable to that of the claw (both actually point laterally). In combination they may aid in securing the leg during burrowing and help to anchor the organism in the burrow.

The tibial process of the metathoracic legs of *P. vittigera* (Fig. 13B) is not so elongated (the apex is relatively blunt) and the securing function may instead be assumed by the series of stout setae on the outer face. The tarsi and claws are angled dorsally and curve medially. The long, sharply pointed claws appear to function (with the mesoclaws) for anchorage within the burrow.

Gills (Fig. 3)

The gills have a number of roles. Besides being sites of oxygen uptake and possibly osmoregulation, their co-ordinated movement creates a current flow over the body that pulls food particles and oxygenated water into the burrow and clears the burrow of excavated or loose substrate. In the case of *H. limbata*, gill-generated water flow aids in the completion of the burrow and functions to maintain the integrity of the burrow in the silt. (In general, tunnelling in soft soils requires a constant outward pressure on the walls of the tunnel to prevent collapse.) The gills maintained a steady but relatively slow beat during

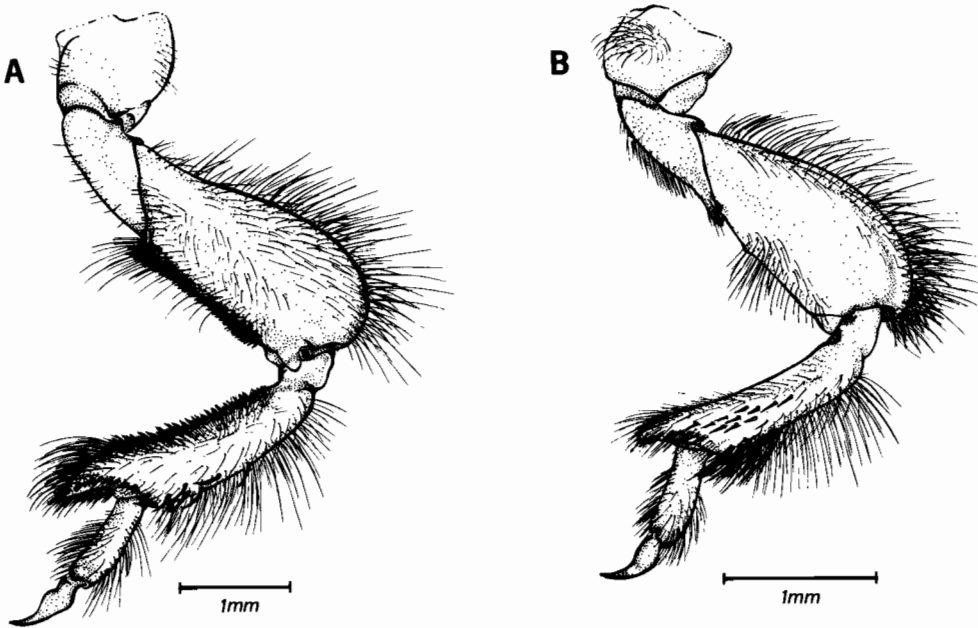


Figure 13. Comparison of metathoracic legs. A, *H. limbata*; B, *P. vittigera*; left leg, lateral view.

burrowing for both species (rates varied, 1.5–3.0 beats/s were typical), and thus they were not considered to play a major role in excavation. They were, however, important for flushing debris from the newly created tunnel, where gill beat rates reached 5–7/s (*P. vittigera*).

Setation

The setae, which are of elaborate types (Fig. 14) and arrangement on these organisms, are likely to play a number of roles. Some sensory function is suggested at least by the stiff setae (Fig. 14A & G) on the ventral apices of the protibiae and mesotibiae. These are arranged as apical circlets in *H. limbata* (Fig. 10A) and as flattened mats on the inner face in *P. vittigera*. Given the observations that suggest some behavioural patterns for substrate testing (e.g. the unison prothoracic leg sweep), the architecture and placement of these setae make them likely sensory candidates.

Another role of setae is the protection of delicate structures, or the reduction of wear to them, or to any structure exposed to abrasion by the substrate. The long setae (Fig. 14E & H) around the eyes, on the thoracic margins and around the bases of the gills are notable examples. Some setae on the legs, particularly on the outer faces, also may be primarily protective.

Densely spaced, long setae may have various functions. In this regard the profemoral and protibial setae (Fig. 14I) involved in seston filtering have been mentioned already. The long setae on the margins of the tibiae sweep substrate material that is in suspension and they may be somewhat effective in excavating soft substrates. Additionally, some lateral setae on the abdomen and tails may be swimming hairs.

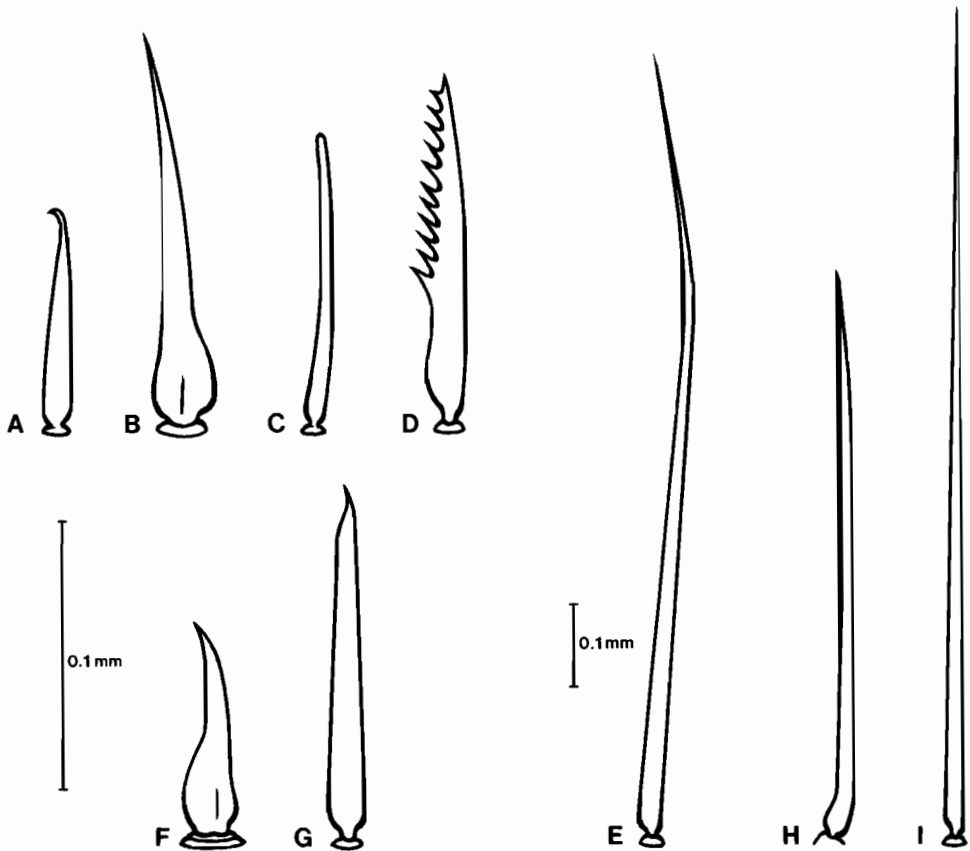


Figure 14. Comparison of setal types. A-E, *H. limbata*. A, Stiff protibia, apical circling; B, curved, stout metatibia, outer face; C, short, fine metatibia; D, stout metatibia, gill cleaner; E, long metatibia, outer face. F-I, *P. vittigera*. F, Curved, stout protibia, outer face; G, stiff protibia, apical circling; H, long protibia, ventral margin; I, long protibia, inner face.

The bands of curved stout setae that are present on the ventral margins of the metafemora and tibiae of *H. limbata* (Fig. 14D) are gill cleaners. The abdomen is flexed laterally while the tibia on that side is extended. The tibia is then flexed onto the femur and the gills are pulled through the comb-like rows of setae.

Roles in burrowing for the stout setae on the legs (Fig. 14B & F) and the spurs on the legs and tusks (Fig. 9B) of *P. vittigera* have been described already in connection with breaking up large chunks of loosened substrate. Such armature prevents or reduces slippage of the legs during power application, acting in this case like cleats. Stout setae are present on the outer faces of all tibiae.

A few general concluding remarks about this vein of research appear to be appropriate. The application of new technology for discovering and describing some of the most fundamental of organismal phenomena is most exciting. Through advances in electronics, a wealth of observational data is becoming accessible to functional and behavioural researchers. Yearly, cameras and recorders become more rugged, compact and versatile, simpler to use and affordable on modest research budgets. Digital television, which promises film-

like resolution and an extraordinary ability to manipulate the video information, is the next advance.

The possible analytical ramifications of the newly accessible observations are considerable but, of course, dependent on the extent to which comparative data become available. An evolutionary context for our burrowing study awaits further data collection on a wider range of taxa. Still, the subtlety and sophistication in morphology and behaviour shown by these animals, the number of burrowing cycles and their variations, suggesting a specific response to the nature of the material in which they burrow and to the progress of their work, have been a considerable encouragement in itself.

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