

# Rendering the inedible edible: Circumvention of a millipede's chemical defense by a predaceous beetle larva (Phengodidae)

(Diplopoda: *Floridobolus*/Coleoptera: *Phengodes*/defensive glands/benzoquinones)

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**ABSTRACT** The larva of the phengodid beetle, *Phengodes laticollis*, feeds on the millipede, *Floridobolus penneri*, without risking exposure to the repellent benzoquinones ordinarily ejected by the millipede from its defensive glands when attacked. The phengodid subdues the millipede by piercing the millipede's integument with its hollow sickle-shaped mandibles and apparently injecting gastric fluid. The infusion abruptly paralyzes the millipede, which thereby is prevented from discharging its glands. As the phengodid then imbibes the liquefied systemic contents of the dead millipede, the millipede's benzoquinones remain harmlessly confined to the glands, prevented from diffusing into the millipede's body cavity by the glands' impervious cuticular lining. At the end of the meal only the millipede's skeletal armor and glandular sacs remain uneaten. Analysis of such discarded sacs showed these to contain benzoquinones in amounts commensurate with those present in replete glands of living millipedes.

Millipedes are among the best protected of arthropods. Most possess defensive glands, in the form of integumental sacs arranged segmentally along the length of the body, from which they discharge such diverse toxins as 1,4-benzoquinones, phenols, hydrogen cyanide, quinazolinones, and alkaloids (1). Anyone who has handled millipedes, particularly the large 1,4-benzoquinone-discharging members of the orders Spirobolida and Spirostetida, knows that these animals can eject their secretion in copious amounts in response to even mild provocation. Not surprisingly, predators are deterred by these fluids (1).

There is, however, one group of predators, the larvae of beetles of the family Phengodidae, that feeds on millipedes as a matter of routine. Although worm-like and seemingly innocuous, phengodid larvae are voracious hunters capable of killing millipedes larger than themselves. Their predation strategy is unique and has been described in some detail by Tiemann (2) for *Zarhipis integripennis*, a phengodid from California. When a millipede comes to within proximity of a *Z. integripennis* larva, the larva races alongside it, mounts it, and throws a body coil around the millipede's front end. It then pierces the millipede's neck membrane with its mouth parts, and effects an action that causes the millipede to become quickly immobilized. The larva then burrows underground beside the millipede, only to emerge after a time to commence feeding. It consumes the soft insides of the millipede only, predigesting the millipede's tissues with its own enteric fluids, which it apparently regurgitates while feeding. Only the millipede's skeletal parts remain uneaten after the meal.

What was most intriguing about Tiemann's account is that the millipedes seemed not to discharge their defensive glands

during the attack. Did the larvae kill the millipedes in some special way that kept them from activating their defenses? Tiemann speculates that the millipedes might have their nerve cord severed when the larvae pierced their neck membrane, but he presents no supporting evidence. Also unanswered was the question of the ultimate fate of the secretion. Are the glands not inevitably ruptured when the larvae feed on the millipedes' contents? Why are the larvae not then deterred? Do they ingest the secretion?

Observations that we have made on another phengodid larva, *Phengodid laticollis* from Florida, have enabled us to address these questions. We staged encounters between this larva and one of its natural prey, the millipede *Floridobolus penneri*, and were able to establish that (i) the larva probably kills the millipede by injection of enteric fluid, not severance of the nerve cord, (ii) the millipede does indeed fail to discharge its glands when attacked by the larva, and (iii) the larva does not perforate the millipede's glands when it feeds on the millipede. In short, we showed that the phengodid can subdue and eat its prey without ever coming in contact with the prey's defensive secretion. We here document these findings.

## MATERIALS AND METHODS

**The Phengodid.** We collected *P. laticollis* larvae (Fig. 1A) near Lake Placid, Highlands County, Florida, on the grounds of the Archbold Biological Station. The habitat at the site is typical Florida "scrub," a unique dry-land ecosystem characterized by sand ridges and shrubby vegetation (3). In the years of experience that two of us (M.D. and T.E.) had at the site, we never encountered a *P. laticollis* larva by day. We collected the larvae in conventional pit traps (no. 10 tin cans buried to the rim in the sand) into which they fell during the night, indicating that they are ambulatory on the soil surface during that time. The larvae appear to be rare. Despite extensive pit trapping during summer and fall for a number of years, we succeeded in obtaining only eight larvae for our studies. Dimensions (mean  $\pm$  SD) for three individuals were as follows: mass =  $0.71 \pm 0.42$  g (range 0.26 to 1.11 g); body length =  $4.6 \pm 1.6$  cm (range 2.8 to 6.0 cm).

**The Millipede.** We collected *F. penneri* in the fall at night, with the aid of lanterns, as they crawled on the sandy soil at the same sites where we took the phengodid larvae. *F. penneri* is an endemic of the scrub habitat and can be locally abundant. They were available to us in quantity. Dimensions (mean  $\pm$  SD) for five individuals were as follows: mass =  $2.94 \pm 1.84$  g (range 0.46 to 5.41 g); body length =  $7.8 \pm 4.4$  cm (range 3.2 to 15.1 cm).

**Chemistry.** The defensive secretion of *F. penneri* had been previously studied and found to consist exclusively of 1,4-benzoquinones. Two of these quinones, 2-methyl-1,4-

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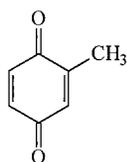
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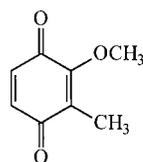
FIG. 1. (A) *P. laticollis* larva. (B) Larva in process of killing millipede (*F. penneri*). Note tiny droplets of secretion (arrows) emitted from two glands. (C) Larva commencing to feed on millipede. (D) Larva midway through its meal. (Bar = 1 cm.)

benzoquinone (compound **1**) and 2-methoxy-3-methyl-1,4-benzoquinone (compound **2**), make up 95% of the secretion (4, 5).

For our present purposes, *F. penneri* gland samples were extracted with methylene chloride, and the extracts were analyzed quantitatively for quinone content (compounds **1** and



1



2

**2**) by gas chromatography/mass spectrometry using a methylene chloride solution of 2,5-dimethyl-1,4-benzoquinone as external standard. Instrumentation was as previously described (5).

**Predation Tests.** We staged the *P. laticollis* encounters in plastic cages (24 × 30 cm, floor dimensions), filled to approximately 5 cm height with sand. Phengodid larvae were confined individually in such enclosures after capture, and for purposes of testing were offered a single millipede. The millipedes had themselves been maintained in such cages before testing. Handling of millipedes (including when initially captured) was effected with extreme care, to prevent the animals from discharging their secretion. As a rule, millipedes were not directly touched, but were transferred into or between containers by use of vials, into which they were coaxed to enter by use of brushes. In cases where individual phengodid larvae were twice tested, several weeks intervened between the two millipede offerings.

**Anatomical Studies.** Dissections were made under saline solution. Millipedes and phengodids that were dissected fresh were killed by freezing. For scanning electronmicroscopy, preparations were pretreated by critical point drying.

## RESULTS

**The Millipede's Defense.** As is typical for members of its order (Spirobolida) (1), *F. penneri* has a pair of defensive glands per each of most body segments, opening by small pores along the flanks of the body. Only the first five segments, and the last, are devoid of glands.

We noted ambulatory *F. penneri* that we encountered in the field at night to respond instantly to contact by coiling into a tight spiral, with the head at the center. If further disturbed, as by touching or mild tapping, they then typically discharged their secretion, first from the glands closest to the site stimulated, then usually from all glands. Fluid emission was abrupt, and the secretion collected as discrete golden-brown droplets over the gland openings (Fig. 2A). The millipedes at the same time came to reek characteristically of benzoquinones.

The glands, upon dissection, were revealed to conform to the structural type characteristic of spirobolids (1). Each consists of a spherical sac, devoid of intrinsic compressor muscles, fitted snugly amid the multilayered musculature of the body wall (Fig. 2B). An efferent duct, exposed by excision of this musculature, connects the sac to the gland opening (Fig. 2C).

The terminal portion of the efferent duct is ordinarily occluded, being passively maintained in this condition by the spring-like inflection of the duct wall. A special muscle that attaches to the body wall and inserts on the inflection (Fig. 2C and E, m) serves to pry open this valve. Treatment with aqueous KOH solution (to dissolve away the cellular components), showed the glands to be lined in their entirety with cuticle, attesting to their integumental origin. The cuticular lining, while membranous and flexible in the sacs, is toughened in the inflected region of the duct (Fig. 2D).

Glandular discharge presumably is effected by a contraction of the valvular muscle and simultaneous compression of the sac. Sac compression must be effected indirectly, possibly by increased hemocoel pressure or by pressure from the integumental muscles that surround the glands. One could envision both such forces being engendered by localized segmental

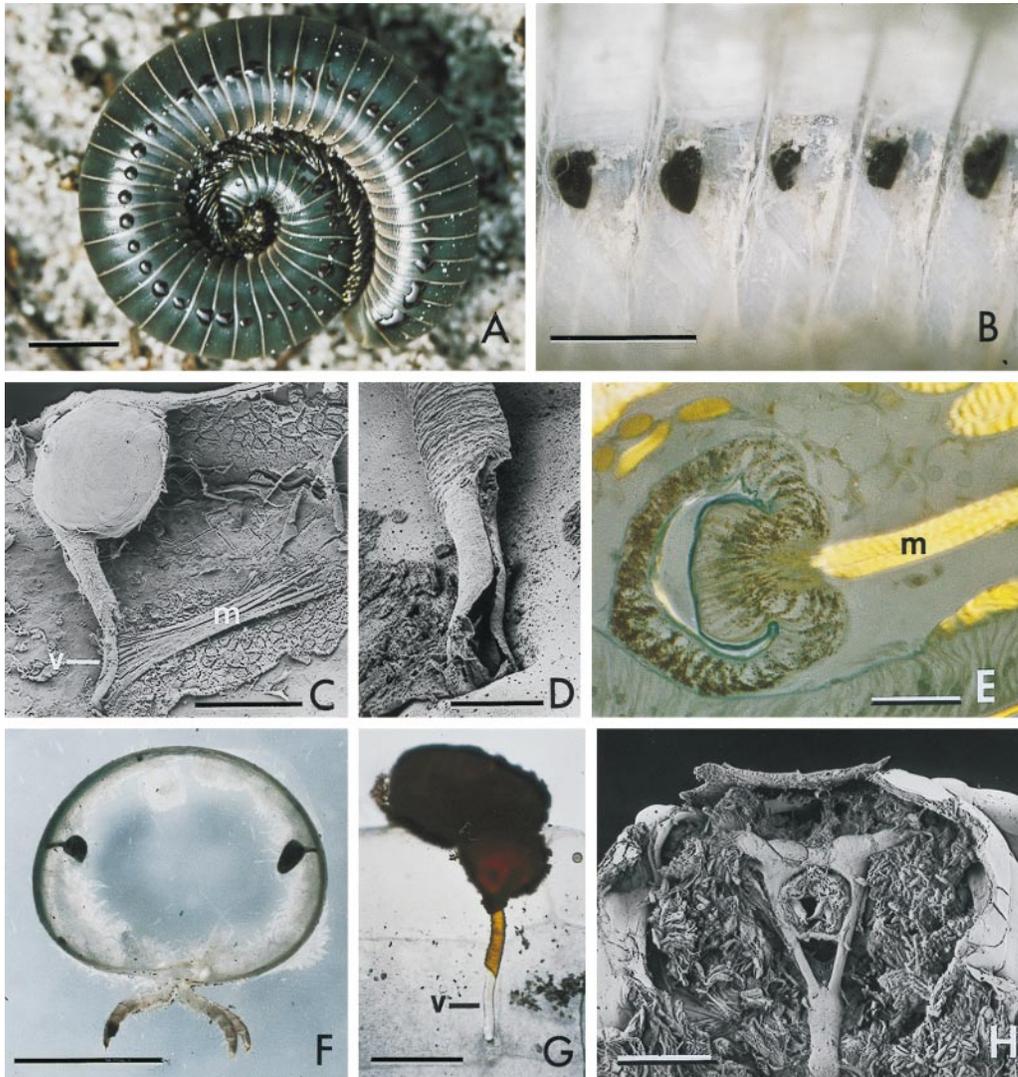


FIG. 2. Millipede (*F. penneri*). (A) Individual that has coiled in response to manual disturbance and has emitted droplets of secretion from its defensive glands. (B) Inner view of body wall, showing sacs of defensive glands, nestled amid the body wall musculature. (C) Individual defensive gland, exposed by dissecting away the body wall muscles. Note the efferent duct leading from the sac to the gland opening. The terminal portion of the duct is inflected, forming a valve (v). A muscle (m), serving to open the valve, inserts on the inflection. (D) Enlarged view of valve. The opener muscle has been dissolved away by treatment with KOH. (E) Transverse histological section through valvular portion of duct, showing relation to opener muscle (m) (partly polarized light). (F) Millipede segment, sucked out by a *P. laticollis* larva. Note the intact glands, replete with secretion. (G) Enlarged view of a gland as in F; note that the valve (v) is occluded and that no trace remains of the opener muscle. (H) Cephalic nervous system of a millipede just killed by a *P. laticollis* larva (brain, circumesophageal connectives, subesophageal commissure, and nerve cord are intact). (Bars: A = 1 cm; B and F = 5 mm; C and G = 0.5 mm; D = 0.1 mm; E = 0.05 mm; H = 1 mm.)

telescoping, triggered by contraction of the longitudinal intersegmental muscles.

**The Phengodid's Offense.** We noted the attack behavior of the *P. laticollis* larva to be essentially similar to that described for *Z. integripennis* by Tiemann (2). In three laboratory encounters that we witnessed from the outset, we noted that the larvae, which had been quiescent for days before testing, became active shortly after the millipede was introduced into their enclosure. Each larva eventually located the millipede, mounted it, and promptly threw a coil around its front end (Fig. 1B). The millipede itself coiled in response to this "embrace," and after virtually no evasive struggling, in what appeared to be a matter of seconds, suddenly "went limp." It was clear that the larva had been prodding the millipede's neck membrane with its mouth parts (the ventral region of the membrane, just in front of the millipede's first set of legs), but what precisely the larva was achieving by such action could not be determined. We judged "going limp" to signify the moment of the millipede's death. Indeed, the millipede, once flaccid,

could be readily uncoiled manually (without perturbing the larva), indicating that its intersegmental musculature had lost its tone (coiled millipedes, when live, offer considerable resistance to being uncoiled).

For minutes after going limp, the millipede continued to manifest a sporadic wiggling of its legs, but such action was feeble and uncoordinated. During the entire sequence of events that led to the millipede's death, there was virtually no secretory emission from the millipede's glands. Occasional tiny droplets of secretion made their appearance at some of the gland openings, but such effluence was minimal (Fig. 1B, arrows). The millipedes, once limp, no longer responded to being tapped by emitting secretion.

The larva maintained its coil around the millipede for minutes after the millipede went limp. Close-range observation of one larva during this period (the millipede was partly uncoiled for the purpose), showed it to have its pointed, sickle-shaped mandibles inserted through the neck membrane of the millipede.

After this period of initial engagement, the larva uncoiled from the millipede and crawled into the sand centimeters away. Within the hour it re-emerged and began the systematic task of feeding on the prey. The millipede was by now totally motionless and free of whatever traces of secretion had initially emerged from the glands.

As is true also for *Z. integrispennis* (2), the larvae consumed only the innards of the millipede. They sucked out the head capsule first, and then, proceeding posteriorly, the contents of one segment after the other of the millipede (Fig. 1 C and D). Examination of an as-yet-uneaten portion of a millipede, removed from a larva part way through the meal, showed its contents to be essentially liquefied (the prey item later was taken again by the larva). Consumption of the millipede was slow. The three larvae that we observed from the outset of attack took over 24 hr to complete their meal. Another three larvae that we monitored from when they had already commenced feeding proceeded equally slowly.

As the larvae imbibed the contents of individual segments, they reduced these to skeletal rings (Fig. 1D). At the end of the meal all that remained was a group of such rings in disorderly array.

**The Phengodid's Feeding Apparatus.** The head of the *P. laticollis* larva is prognathous (Fig. 3). Conspicuous among its features are the two sharply pointed, sickle-shaped mandibles (Fig. 3A, md), which project forward from the margins of the mouth. The mouth itself is a narrow slit (Fig. 3A, mo), subtended dorsally by the frontal plate (the fused frons, clypeus, and labrum) (Fig. 3A, fr), and ventrally by the hypopharynx. The mandibles are tubular and have orifices at each end, an oblong one near the tip, and a circular one near the base. The latter orifice, which opens into the buccal cavity, ordinarily is hidden from view, but can be exposed by excision of the frontal plate (Fig. 3 B–D).

The mandibles could clearly serve for both uptake and delivery of fluid. All the larva would need do is compress the oral slit and apply either oral suction or regurgitative enteric pressure. It thus could use the mandibles alternatively for food imbibition or gut fluid deliverance.

Dissection showed the gut of the larva (Fig. 4) to consist of a slender esophagus, a lengthy capacious midgut, and a narrow hindgut. Between esophagus and midgut there is a bulbous proventriculus. This organ is invested by a thick multilayered musculature, suggesting that it might function as a pump. The midgut itself bears an outer coating of muscle fibers. We found no evidence of special glandular sacs, such as might act as venom glands, opening into the buccal cavity.

**Fate of the Glandular Quinones.** Examination of segmental rings of *F. penneri* that had been sucked out shortly beforehand by *P. laticollis* larvae revealed these rings to be essentially free of tissue remains. Entirely missing, for instance, was the body musculature. Preserved intact, however, and replete with secretion, were the glandular sacs (Fig. 2F). These were reduced to their cuticular linings, all cellular components having apparently been dissolved away, but they were clearly imperforate [no leakage could be detected from such sacs when the segmental rings bearing them were submerged in an acidified solution of KI and starch, a mixture that discolors in the presence of benzoquinones (6)]. No trace remained of the opener muscle associated with the valve of the gland, and that valve was clearly preserved in its passive occluded state (Fig. 2G, v).

Chemical analyses of such glandular remnants showed them to contain quantities of 1,4-benzoquinones commensurate with the amounts present in live *F. penneri*. Two samples of glands ( $n = 9$  glands per sample) dissected from two millipedes killed by freezing (without having been induced to emit secretion), yielded values (for sum of compounds 1 and 2) of

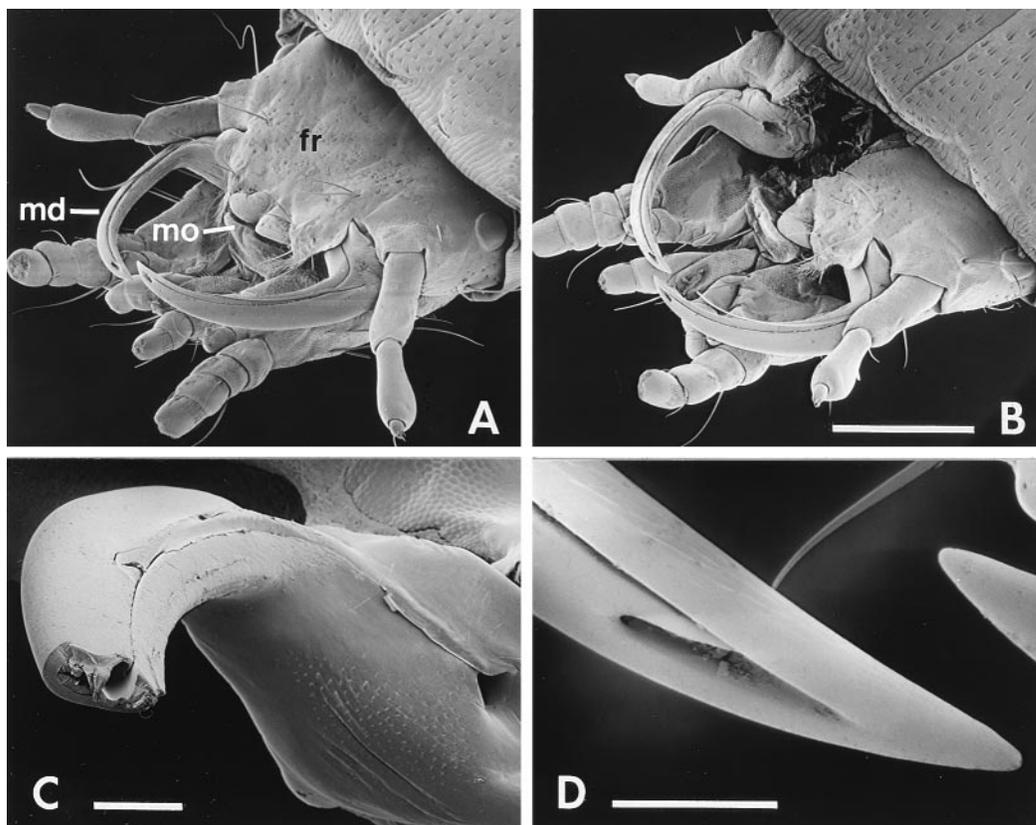


FIG. 3. Phengodid larva (*P. laticollis*). (A) Dorsolateral view of head (fr, frontal plate; md, mandible; mo, oral slit). (B) Same, with right half of frontal plate dissected away, showing orifice at base of mandible. (C) Mandible, with tip broken off, revealing the inner duct that runs the length of the mandible. (D) Orifice at tip of mandible. [Bars = 0.5 mm (B); 0.1 mm (C); and 0.05 mm (D).]

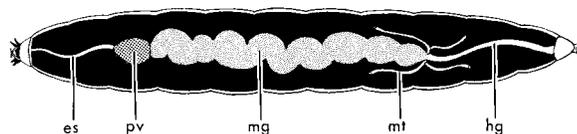


FIG. 4. Diagram of digestive system of the *P. laticollis* larva (es, esophagus; pv, proventriculus; mg, midgut; mt, malpighian tubules; hg, hindgut).

16.2 and 13.1  $\mu\text{g}$  quinone per gland, respectively. The corresponding values for two samples of glands ( $n = 9$  glands;  $n = 2$  glands) removed from segmental remnants of two millipedes killed by *P. laticollis*, were 19.9 and 22.6  $\mu\text{g}$  quinone per gland, respectively.

**Cause of the Millipede's Death.** We ruled out the possibility that *P. laticollis* kills *F. penneri* by severing the nerve cord of the millipede. A millipede that we retrieved from a phengodid larva after the larva had thrown a coil around it and the millipede had "gone limp," proved upon dissection to have a seemingly intact cerebral nervous system (Fig. 2H). We also found that severance of the nerve cord, induced by decapitation, does not cause the millipede to "go limp." A single millipede that we decapitated abruptly with scissors continued to writhe and flail its legs for minutes after the severance, while also retaining the ability to respond to tapping by emitting secretion.

On the presumption that the phengodid larva might kill its prey by injection of enteric fluid, we drained the midgut contents from two freshly dissected larvae into separate containers, and injected an undiluted (8  $\mu\text{l}$ ) and diluted (8  $\mu\text{l}$  in 25  $\mu\text{l}$  of saline solution) sample of each into four individual millipedes. The injections were effected by microsyringe, dorsolaterally, at the level of postcephalic segments 2–4. The saline solution was one widely used with insects (7). The two millipedes that received the full-strength enteric samples were immediately affected by the injections. They failed to walk when released on sand, flailed their legs only weakly, and went totally limp within 35 and 45 sec, respectively. They gave off some secretion initially when held down for injection purposes, but once limp no longer responded by discharging secretion when tapped. The two millipedes that received the dilute samples took over 3 min each to become immobilized and flaccid. Two control millipedes that were injected with 25  $\mu\text{l}$  of saline solution survived without ill effects (they were released to their native habitat 4 weeks later). Dissection, 24 hr after injection, of the two millipedes that received the full-strength enteric samples, showed the body contents of each to be in large measure liquefied.

## DISCUSSION

The *P. laticollis* larva evidently has an effective strategy for circumventing the defense of *F. penneri*. It kills the millipede without inducing it to discharge secretion and subsequently ingests it without rupturing the glands. The larva must benefit from not coming in contact with the millipede's secretion, because 1,4-benzoquinones are strongly irritating and repellent to insects (6, 8).

Although we lacked sufficient larvae to clarify many points in detail, we envision the phengodid-millipede encounter to proceed as follows: (i) the larva, while coiled around the millipede, pierces the millipede's neck membrane with its mandibles and injects enteric fluid, possibly in substantial amounts; (ii) the infusion quickly paralyzes the millipede, making it impossible for the millipede to activate the musculature (the valve-opening muscle of the glands; the body wall muscles) needed to discharge the glands; (iii) the larva temporarily retreats from the millipede, allowing the paralysis to take its full course and the infused fluid to initiate the internal

digestion of the millipede; (iv) the larva returns to the millipede and, by use of its mandibles, commences the slow process of imbibing the millipede's liquefied contents; and (v) the glands of the millipede, reduced to inoperative leak-proof sacs replete with secretion, are left behind by the larva, as part of the unimbibable remains of the meal.

That experimental injection of enteric fluid induced a paralysis in the millipede imitative of that elicited by the larva itself lends credence to the notion that the larva effects its kill by use of enteric fluid. It would be interesting to know whether the "going limp" on the part of the millipede is induced by special muscle relaxants or other neurologically active factors in the fluid. If so, one might wish to study phengodid larvae pharmacologically. Offhand, because phengodid larvae appear generally to be rare (9) that prospect might seem remote. However, there is evidence that under some circumstances phengodids may be abundant, as when they are driven from within the soil to the soil surface by flooding (10).

We obtained no evidence of how the phengodid larva effects the regurgitation of its enteric fluid, although one could imagine this being achieved by compression of the midgut (reverse peristalsis?) and simultaneous forward pumping of the proventriculus. During food imbibition the proventriculus presumably would pump in the conventional posterior direction.

Unexplained also is the remarkable fact that the phengodid does not elicit discharges from the millipede at the outset of the attack. Why does the millipede not eject secretion when the larva throws the coil around it, or when the larva probes the millipede's neck membrane preparatory to inserting the mandibles? One would think that the tactile stimuli inflicted by the larva during these procedures should in themselves suffice to trigger the millipede's defense.

*P. laticollis*, in central Florida, also might prey on two additional millipedes, *Narceus gordanus* and *Chicobolus spinigerus*, which also discharge 1,4-benzoquinones (4). But phengodids need not generally be restricted to quinone-secreting prey. At a field site near Denton, Texas, one of us (T.E.) found a phengodid larva feeding on a millipede of the order Polydesmida, the species of which characteristically produce cyanogenic secretions (1).

Strategies for circumvention of prey defense have evolved in a number of animals, including both predators and herbivores. Thus, ant lions subdue formicine ants without risking exposure to the defensive acidic secretion produced by the latter (11), and orb-weaving spiders encase bombardier beetles in silk, thereby shielding themselves against the hot quinonoid spray ejected by these beetles (12). Similarly, a number of insects that feed on leaves of latex-producing plants prepare such leaves for ingestion by first draining them of latex (13).

Phengodids are not the only predators able to circumvent the chemical defense of millipedes. In a wooded area near Ithaca, N.Y., there is a nocturnal hunter that feeds on the quinone-secreting spirobolid millipede, *Narceus annularis*. The predator is wasteful in its approach, chewing off only the glandless front end of the millipede and discarding the remainder of the body. Although presumed to be a rodent, the "decapitator" has never been caught (1).

One enemy of *F. penneri* actually may be attracted by the millipede's quinones. It is a fly, *Spirobolomyia* sp., which one of us (T.E.) noted on one occasion in the field to converge in numbers upon an *F. penneri* shortly after the millipede had been induced to emit secretion. *Spirobolomyia* are known to be parasitoids of millipedes (14), but how they find their hosts was unknown.

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