AMERICAN MUSEUM
Novitates

PUBLISHED BY
THE AMERICAN MUSEUM
OF NATURAL HISTORY
CENTRAL PARK WEST AT 79TH STREET
NEW YORK, N.Y. 10024 U.S.A.

NUMBER 2640 JANUARY 3, 1978

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Number 2640, pp. 1-24, figs. 1-36, tables 1-3 January 3, 1978

The Bionomics and Immature Stages of the Cleptoparasitic Bee Genus *Protepeolus* (Anthophoridae, Nomadinae)

JEROME G. ROZEN, JR.,[1] KATHLEEN R. EICKWORT,[2] AND GEORGE C. EICKWORT[3]

ABSTRACT

Protepeolus singularis was found attacking cells in nests of *Diadasia olivacea* in southeastern Arizona. The following biological information is presented: behavior of adult females while searching for host nests; intraspecific interactions of females at the host nesting site; interactions with host adults; oviposition; and such larval activities as crawling, killing the host, feeding, defecation, and cocoon spinning. In general, adult female behavior corresponds to that of other Nomadinae. Females perch for extended periods near nest entrances and avoid host females, which attack parasites when encountered. Females apparently learn the locations of host nests and return to them frequently. This may account for the high rate of cell parasitism (47%) in five nests excavated by the authors. Females oviposit in open cells and hide their eggs in the cell walls as do all Nomadinae. As this is considered to be an autapomorphic feature of the Nomadinae, *Protepeolus* and the other Nomadinae are believed to have had a common parasitic ancestor in spite of numerous biological dissimilarities. The first instar *Protepeolus* attacks and kills the pharate last larval instar of the host before consuming the provisions, a unique feature for nomadine bees.

First and last larval instars and the pupa are described taxonomically and illustrated. Brief comparative descriptions of the other larval instars are also given. Larval features attest to the common origin of *Protepeolus* and the other Nomadinae. Cladistic analysis using 27 characters of mature larvae of the Nomadinae demonstrates that *Isepeolus* is a sister group to all the other Nomadinae known from larvae, including *Protepeolus*, and that *Protepeolus* is a sister group to the Nomadinae excluding *Isepeolus*. Because of this and because larval *Isepeolus* and *Protepeolus* differ in numerous autapomorphic features, *Isepeolus* is placed in its own tribe, the Isepeolini, new tribe.

Appended is a brief account distinguishing the four larval instars of the host, *Diadasia olivacea*.

INTRODUCTION

The Anthophoridae contain more cleptoparasitic bees than any other family of the Apoidea, in terms of number of species, genera, and tribes. Systematists have had difficulty in determining how many times cleptoparasitism evolved among these taxa and what their interrelationships are because of numerous convergences of adult features that attended the development of clep-

[1]Deputy Director for Research and Curator of Hymenoptera, the American Museum of Natural History.
[2]Research Associate, Department of Entomology, Cornell University, Ithaca, New York.
[3]Associate Professor, Department of Entomology, Cornell University, Ithaca, New York.

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toparasitism. It has generally been concluded that cleptoparasitism in Melectini, Ctenioschelini (=Ericrosini), and Rathymini had a separate origin from its development in the other major group of parasitic anthophorids, the Nomadinae. Recently Rozen (1966, 1969) corroborated the hypothesis of the separate origins of and lack of close relationships between these two groups on the basis of the mature larvae. He further indicated that the Melectini as a parasitic group arose independently from the Ctenioschelini and Rathymini. These two tribes presumably had a common ancestor and, as Bohart (1970) suggested, might well be grouped in the same tribe.

Rozen (1966), studying all available mature larvae of Nomadinae, concluded that they were probably a monophyletic group although larvae of a number of tribes were then, and for that matter still are, unknown. However, he recognized that South American *Isepeolus* was a remarkably divergent element differing in numerous apomorphic ways from all other Nomadinae. A similar observation was made by Lucas de Oliveira (1966). Bohart (1970) subsequently questioned the inclusion of the tribe Protepeolini, which includes *Isepeolus*, in the Nomadinae. Clearly, before the onset of the present investigation, relationships of the Protepeolini (comprised of three genera, *Protepeolus*, *Isepeolus*, and *Leiopodus*) with the other Nomadinae were uncertain and demanded further investigation.

Hence, when the second author (K.R.E.) discovered adults of the rare *Protepeolus singularis* Linsley and Michener literally at her doorstep at the Southwestern Research Station, 5 miles west of Portal, Cochise County, Arizona in mid-August of 1973, she and the other two authors investigated its biology in depth until early September. The present paper is the result of these field investigations and of a detailed study of the immatures of *Protepeolus singularis* in the laboratory. The taxonomic status of species of *Protepeolus* has been investigated by Eickwort and Linsley (in prep.).

This parasite was flying in association with *Diadasia olivacea* (Cresson) and *D. diminuta* (Cresson) and attacked the nests of the former. The nesting activities of adults of both species of *Diadasia* at the Southwestern Research Station were described by Eickwort, Eickwort, and Linsley (1977).

Although each author has reviewed all parts of this paper, the Eickworts were responsible for the section on Behavior of Adult Females, and Rozen for the sections on Ethology of the Immature Stages, Morphology of Immature Stages, and Discussion and Conclusions.

We extend our appreciation to Mr. Vincent Roth, Resident Director of the Southwestern Research Station of the American Museum of Natural History, for his hospitality and his assistance during the course of the field studies. Mrs. Marjorie Favreau, Mr. Ron McGinley, and Mr. Kenneth Rozen assisted with the field excavations. Dr. E. Gorton Linsley of the University of California at Berkeley read the section on behavior of adults. Mr. Robert J. Koestler, Interdepartmental Laboratory, the American Museum of Natural History, was responsible for the scanning electron microscope examination of the first instar of *Protepeolus*.

This work was supported in part by NSF grants GB-32193 and GB-35954.

Eggs, larvae, pupae, and adults of *Protepeolus singularis* obtained during the investigation are in the collection of the American Museum of Natural History.

BEHAVIOR OF ADULT FEMALES

Observations were made of *Protepeolus* behavior from August 17 to 29, 1973 in the nest aggregation of *Diadasia olivacea* and *D. diminuta* described in a companion paper (Eickwort, Eickwort, and Linsley, 1977). The nests of *D. diminuta* were more numerous than those of *D. olivacea*, and most of the *D. diminuta* nests were with conspicuous turrets in bare ground. In contrast the *D. olivacea* nest entrances were usually concealed under mat plants, *Brayulinea densa* (Willd.) Small (Amaranthaceae). More than 200 *D. diminuta* nests were found in a small area, about 9 $m.^2$, from August 12 to 29. However, each nest of this species did not remain active for that entire period. On August 29 there were 53 active *D. diminuta* nests and 31 *D. olivacea* nests in this 9 $m.^2$ area. The mean distance from a *D. diminuta* nest to its nearest neighboring conspecific nest was only 8 cm., whereas the same

measure for *D. olivacea* nests was 22 cm. Despite the greater numbers of *D. diminuta* nests present, *P. singularis* females were only observed to visit nests of *D. olivacea*.

The *Protepeolus* females were not marked, since the marking procedure carries a risk of killing or damaging the bee, and we believed that there were few bees in the local population, probably fewer than 10. In addition, *P. singularis* is not a large bee nor is it brightly colored, and it was easy to lose sight of it in flight. Hence our observations were sometimes fragmentary and required cautious interpretation.

Protepeolus singularis females were active at the nesting site from 9 a.m. until 3 to 4 p.m. Many parasitic bees (e.g., *Sphecodes*, *Nomada*) are most often seen flying in a search pattern near the ground, investigating holes or turrets. *Protepeolus singularis* were occasionally observed to behave in this fashion, but they spent a large portion of their time sitting on "perches" (miscellaneous small objects such as small rocks, erect plants 5 to 15 cm. aboveground, and dead leaves). Frequently a perch was near a *D. olivacea* nest entrance, and the parasite sat oriented toward the nest entrance, in a dorsoventrally flattened position with antennae forward, giving an overall impression of "furtive alertness," like a predator waiting to ambush its prey. If the parasite left this perch it often flew to another nearby. When they flew, they flew close to the ground and sometimes interspersed brief flights with short rapid walks on plants.

On 46 occasions *P. singularis* adult females were seen to enter *D. olivacea* nests and their stays within the nests were timed:

(1) On 37 of these occasions the parasite remained within the nest for 20 seconds or less; in four of these cases it was known that another bee was in the nest, either the host or another parasite, which might account for the precipitous exit. In the other cases the parasite possibly did not find a cell ready to parasitize.

(2) In seven instances the parasites remained in the nests for periods varying from 110 to 122 seconds. It is probable that in these cases oviposition occurred. Just before leaving a perch to enter a nest, the *P. singularis* female usually pumped her abdomen for a few seconds. After a two-minute visit to a nest most individuals first made an orientation flight, then perched on a nearby plant and preened. After preening for about 30 seconds, the bee flew away.

(3) Only two cases were noted of bees remaining in the nest more than 20 but less than 110 seconds; one bee remained only 44 seconds before exiting, presumably after encountering the host bee, which was known to be in the nest. The second exited after 80 seconds; previous to entering this individual had engaged in an aerial combat with another *Protepeolus* near the nest entrance.

One *Protepeolus* appeared to have pollen smeared about her mouth when she exited from a nest 12 seconds after entering. She then proceeded to preen her antennae and mouthparts. This single observation needs confirmation but suggests that the hosts' provisions might provide a protein source for the adults as well as the food for the immature stages of the parasitic bee. The one female that was preserved for dissection did have large amounts of malvaceous pollen in its midgut.

As described in the companion paper, the nest entrances of *D. olivacea* were well hidden. Although there were often short vertical turrets, the turrets (and consequently the nest entrances) were in most cases concealed under mat plants or other objects. The parasitic bees behaved as if they had learned the positions of the nest entrances and returned to oviposit in the same nest several times. Since individuals were not marked, this was not based on direct observations, but is inferred from the following: (1) The parasites often entered well hidden nests without the slightest hesitation, even if this required, as in one case, going through a hole in a dried leaf that covered the nest entrance. (2) Upon leaving the nests the parasites often made extensive "orientation flights" (circling about the entrance several times). Such flights upon exiting are typical of ground-nesting bees and wasps and are thought to be required so that the insect can memorize landmarks surrounding the nest location (Graenicher, 1906). In other cases the exiting orientation flights were less extensive, as is typical of bees and wasps leaving a nest on subsequent flights after the initial orientation flight. (3) Individuals were seen to fly on numerous occasions from a perch near one nest entrance to a

perch near another nest, then to return to the original perch or another one near the first nest it had been watching. (4) A few nests were repeatedly visited by parasitic bees, whereas others were apparently never disturbed. The former nests were no less well concealed than the undisturbed nests. Some of the nests where frequent *Protepeolus* visits had been recorded were later chosen for excavation, in order to obtain immature stages of the parasites. These five nests had high rates of parasitism, as listed below:

Parasitized Cells	Unparasitized Cells	Percentage of Parasitism
5	7	42%
3	7	30%
1	4	20%
3	4	43%
9	2	82%

The overall parasitism rate was 47 percent in the 45 cells.

The parasitic bees seemed to have two ways by which they originally found *D. olivacea* nests. First, they may have found them simply by searching over the area investigating holes. The bees were not often seen engaging in this sort of search. Secondly, they may have located nests by observing the host bees or other *P. singularis* entering and leaving nests, especially if these nest entrances were near others that had been previously located. On several occasions a *Protepeolus* sat on a perch in an alert posture with antennae pointed toward a nest until after the host bee left; then she entered the nest.

Intraspecific Interactions. Our attention was first drawn to the presence of *Protepeolus* at the nest aggregation when K.R.E. saw two parasitic bees in aerial combat (chasing each other in flight) near a *D. olivacea* nest entrance. This same intraspecific antagonistic behavior was noted at least three more times, twice on August 17 and once on August 25. During the first such interaction, seen at 9:07 a.m., the two parasitic females hovered facing each other several times in flight near the nest entrance; then first one, then the other entered the nest. Both came out after only a few seconds, presumably after having met each other in the burrow. The observer had placed a jar over the entrance while the bees were in the nest. When they re-emerged they grappled briefly in the jar. One of the specimens was collected. About 9:30 a.m. a second contest was seen near the entrance to another nest. After the two females had faced each other in flight several times, one flew away. The other sat on a plant 5 to 8 cm. from the nest entrance, in a dorsoventrally flattened position with antennae pointed toward the nest. While this bee continued to perch there, the host bee returned with pollen and a few minutes later it left the nest without pollen. Then the parasite entered the nest, left two minutes later, made an orientation flight, settled on a nearby plant, preened, and finally departed, as described above.

Interactions with Diadasia olivacea Adults. The adult females of *D. olivacea* are much larger than those of *P. singularis*, and the latter usually avoided contact with the host bees. As noted above, a parasite sometimes seemed to wait till she saw the host leave the nest before entering. Sometimes the parasite left the nest vicinity when the owner returned, without any behavior on the part of the latter to indicate that the parasite had been seen. As noted above, when parasites did enter nests occupied by the host bees, they left in a matter of seconds. In one case, the host followed the *Protepeolus* part of the way out of her nest. However, the *Diadasia* did not confine their attacks on *Protepeolus* to those individuals that were actually caught intruding within nests. The females of the host species also were observed repeatedly chasing away these parasites from the vicinity of their nests. In some cases the *Protepeolus* had been perching several centimeters from the nest entrances on rocks or plants; in others the parasites were flying nearby. In one case an intraspecific combat between two *Protepeolus* near a nest entrance was ended when the host bee returned and chased them both away. Whenever they were pursued by *Diadasia*, the *Protepeolus* females took flight. Nevertheless the pursuing *Diadasia* female sometimes managed to hit the slower flying parasite several times in flight before the latter made its escape. The *Diadasia* females were not seen chasing any other insects away in this fashion, although they did react in a similar fashion to other females of their own species that approached the wrong nest entrances.

Discussion. In general, the behavior of *P. singularis* females above the ground conforms well to the pattern described by Linsley and MacSwain (1955) for *Nomada opacella* Timberlake, a parasite of *Andrena* spp. One detail in which it differs is that putative oviposition visits in *N. opacella* took from four to six minutes, rather than two as in *P. singularis*; but the *Nomada* lays two eggs per cell. *Nomada opacella* was also reported by these authors to spend more time in nest-searching behavior (flying low over the ground in a search pattern) than we observed in *P. singularis*. This may, however, have been due to the fact that when *P. singularis* is flying it is hard to follow with the eye, and most individuals had already found host nests to which they were returning by the date on which observations began.

The behavior of *P. singularis* females in learning the location of the host's nest entrances is obviously an important adaptation. The nests are well concealed and may not be ready to be parasitized when they are first found. Hence it is to the reproductive advantage of the parasite to be able to find the same nest again. Of course they also can return to parasitize more cells as they are prepared by the host. Similar nest-location learning behavior has been reported in *Nomada opacella* (Linsley and MacSwain, 1955), *Coelioxys rufitarsis* Smith and *Epeolus minimus* (Robertson) (Graenicher, 1906), and *Melecta separata callura* (Cockerell) (Thorp, 1969) and may be widespread among parasitic bees.

A *P. singularis* female perching outside a nest entrance sometimes waited until the host bee first returned, entered, and then left her nest again. This behavior both insures the host bee's absence during the parasite's visit to, and may help the parasite relocate the exact position of, the nest entrance. *Nomada opacella* also acts in the same fashion (Linsley and MacSwain, 1955).

The avoidance of the host bees by *P. singularis* may have evolved to serve two adaptive purposes: first, to avoid the direct attacks by the host females that were sometimes observed; second, to avoid disturbing the host to the extent that she abandons her nest.

One interesting detail of the parasite's behavior is its habit of preening the legs, antennae, and abdomen after a two-minute visit to a nest. This may simply be necessary to clean delicate sensory organs but it may also be adaptive to prevent the spread of nest pathogens such as fungi. This same behavior occurs in *Epeolus minimus* (Graenicher, 1906) and *Nomada opacella* (Linsley and MacSwain, 1955).

Bohart (1970) stated that solitary bees that are hosts of parasitic bees usually ignore the inquilines when the latter approach or enter their nests. This is not true in the present case nor, as Bohart mentioned, is it true of *Anthophora* when parasitized by *Melecta* (Thorp, 1969). Custer (1928), on the other hand, reported that *Svastra obliqua* (Say) paid no attention to *Triepeolus* females that entered their nests. This is also reported by Smith (1844) to be the case with *Eucera* parasitized by *Nomada*.

One of the most intriguing questions raised by the present study is: Why did the *P. singularis* females confine their visits exclusively to the nests of *D. olivacea* when *D. diminuta* nests were more common and more conspicuous within the same area? Is it possible that some unknown aspect of the life cycle of *D. diminuta* makes it an unsuitable host? We observed several behavioral tactics on the part of *D. diminuta* that may provide a partial answer. First, each individual nest remains open for only a few days; then it is closed and the female starts another nest. Secondly, the entire aggregation of this species apparently moves to a new site in subsequent years (Eickwort, Eickwort, and Linsley, 1977). Both of these behavioral patterns would foil a parasitic bee that searches for nests in the area from which it emerges and then learns the location of individual nests, to which it returns repeatedly. Also, as described in the companion paper, *D. diminuta* females just before they initiate digging nest burrows (whether their first or subsequent ones) spend a good deal of time entering other burrows of the same species. Such behavior would create a considerably increased probability of disturbance to a *P. singularis* female attempting to parasitize a nest. Since the *D. diminuta* engaging in this behavior never evicted the nests' original owners, what appears to be attempted nest usurpation may have an adaptive value as a mutualistic behavioral defense against parasites. This could also explain the selective advantage to *D. diminuta* of having its nests clustered so closely together, with an average distance of only 8 cm. to each nest's nearest neighbor.

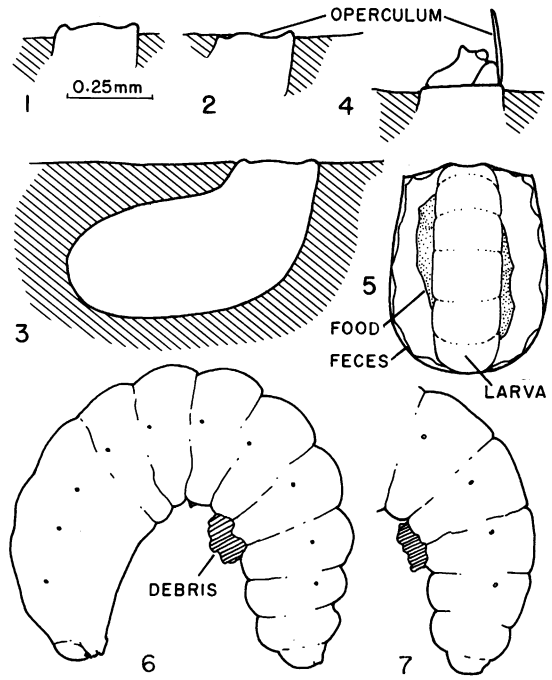
ETHOLOGY OF IMMATURE STAGES

Oviposition. Females of *Protepeolus*, like all other Nomadinae, apparently enter and oviposit in open, newly constructed host cells while host females are away gathering provisions. This is indicated by the discovery of an egg of *Protepeolus* in a cell still being provisioned.

Eggs were uniform in size, approximately 0.75 mm. long and 0.4 mm. in maximum diameter. They exhibited the unusual shape shown in figure 3 and were short relative to their width, compared with eggs of other bees. The chorion, except for the operculum, was translucent, whitish, smooth, faintly shiny. Some eggs displayed a broad white diffused band along the ventral length. The chorion was sufficiently thick so that it did not collapse after eclosion. The operculum, 0.25 mm. in diameter, was semitransparent. Before eclosion it was light grayish, not unlike the color of the cell wall, and possessed a narrow, raised rim, and slightly bulging center. After eclosion it was disclike, white, with the inner surface slightly concave and the outer surface correspondingly convex.

Most eggs were embedded so that all but the opercula were covered with soil, although with some, part of the operculum was obscured by soil (fig. 2) and with others, the anterior end of the egg projected slightly (fig. 1). As can be seen in figure 3 the main part of the egg, although hidden from view, was only a short distance from the cell lumen. In most cases the cell wall contiguous to the egg was undifferentiated from the rest of the cell surface and no space separated the wall from the egg. In several cases, however, a somewhat irregular area of the cell surface to one side of the operculum was slightly raised and perhaps less smooth than elsewhere. Two such areas measured 1.0 by 0.6 mm. and 1.5 by 0.75 mm. They suggest that the parasite female made a large hole or perhaps an oblique slice into the cell wall, inserted an egg and then either packed soil into the hole or cemented the flap from the slice over all of the egg except the operculum. The raised area of the wall presumably represented the filled hole or cemented flap.

Of 17 eggs whose positions in the cells could be determined, one was in the top one-fifth of the cell, two in the next lowest one-fifth, four in the middle one-fifth, eight in the next to the lowest one-fifth, and two in the bottom one-fifth.



FIGS. 1-7. *Protepeolus singularis.* 1-2. Opercula of eggs, showing variation in their position in cell wall, side view. 3. Entire egg. 4. Anterior part of egg with operculum open and head of first instar partly extended, side view. 5. Fourth larval instar feeding on provisions of *Diadasia olivacea.* 6-7. Postdefecating larvae with debris on venter, side views. Scale refers to figures 1-4.

Nineteen cells containing *Protepeolus* eggs were found. Of these, 15 had only one egg. Eggs were difficult to discover because of their small size, mold growing profusely over the cell surface, and the need to partly destroy a cell to examine it. However, the number of eggs per cell could often be confirmed by cast head capsules of first and other instars, at least in cells that still contained young larve. Four of the 19 cells had two eggs each.

The incubation period was unusually long compared with other Nomadinae. Of 20 dead host larvae, all were killed in their third or fourth stadium by the first instar of *Protepeolus*. In contrast, other Nomadinae destroy the eggs or first

instars of their hosts. Even *Diadasia* larvae that were too decomposed to be analyzed were obviously large, i.e., in their third or fourth stadium. Hence the incubation period of the *Protepeolus* egg equaled the host's incubation period plus the duration of at least the first two larval stadia and, as discussed below, usually the length of the third stadium as well. We do not believe that the parasite larva hatches early and waits for the development of the third or fourth instar *Diadasia*. If this were true we would have encountered first instar *Protepeolus* in the numerous cells containing live young immatures of the host.

Three eggs collected from cells in the field on August 27, 1973, hatched in the laboratory on August 30 and 31, 1973; we do not know when they were deposited.

The operculum appeared darker shortly before hatching, probably because the head capsule of the embryo became pigmented. In each case the operculum split around most of the periphery but remained weakly attached to the chorion at one point which served as a hinge. As the operculum was slightly oval rather than completely circular, this point was on one of the longer sides of the oval. The hinged operculum opened and the head of the larva was partly extruded as shown in figure 4. Each larva remained in this position for an extended period of time, in one case more than 105 minutes. This individual contracted its labrum about once every five seconds as if it were ingesting fluid, and at irregular intervals flexed its mandibles. Occasionally it would extend its head slightly farther into the cell causing the operculum to swing open more and then would withdraw again allowing the operculum to close somewhat. The purpose of the waiting period is not known. Each of two larvae, while sitting in the chorion with operculum partly open, was oriented so that the labiomaxillary region and the mandibular apices were touching the operculum. The maxillary pad-like structures, presumably the palpi (see description of first instar), may be the main point of contact of the head with the operculum.

The emergence of a first instar was observed once. The larva began by extending its head farther from the chorion, thereby forcing the operculum to open more widely. It flexed its body very actively, primarily dorsally and ventrally, and extruded itself, segment by segment, from the opening. The emergence may have been assisted by the somewhat posteriorly directed, dorsal, setalike spicules and the erect bristles on the anterior lateral regions of the body in that once these structures had sprung from the opercular rim, they served as a lock mechanism, preventing the larva from accidentally wriggling back into the chorion. Although it strongly bent its body, it emerged perpendicular to the cell wall and did not touch the wall until all but the last abdominal segments were freed. Emergence spanned about five minutes. No integument such as an embryonic cuticle was left behind; the lumen of the chorion was vacant.

The opercula remained attached on most vacated choria. In some cells, however, the opercula were missing, presumably rubbed away by the passage of older larvae. In a few cases the opercula were found a short distance from the emergence hole.

Activities of Larvae. The newly emerged *Protepeolus* larva (fig. 9) is tiny (approximately 1 mm. long) by comparison with the host; it is only slightly longer than the diameter of the egg of *Diadasia olivacea*. Immediately after emergence it was able to crawl rapidly although like larvae of all higher Hymenoptera it possesses no legs. It crawled by elevating the apex of its abdomen, bringing it forward and then pressing against the substrate with its bilobed terminal abdominal segment. It then extended each of the preceding segments successively. The segment in front of the extended segment became longitudinally compressed and enlarged in diameter. Consequently, there was a rhythmical wave from the apex of the abdomen anteriorly to the head. As the wave reached the head, the head was raised, thrust forward and then anchored by being pressed against the substrate. Immediately afterward, the apex of the abdomen was again elevated, brought forward, and then pressed against the substrate to start the next wave. The setalike spicules on the body may have aided the larva in crawling between the provision mass and cell wall as well as helping with eclosion.

The most conspicuous adaptation of the first instar to its cleptoparasitic role is its highly modified mandibles (figs. 10, 11, 13, 14, 22, 23). The

mandibles of the first instars of many other groups of parasitic bees are elongate, sickle-shaped and used to kill the host. Almost certainly the mandibles of *Protepeolus* are used in a similar fashion, although their shape is atypical of other nomadines in that they have a broad, spiculate base and a narrow, smooth, fanglike apex (described in detail below).

How the first instar finds and attacks the larva of *Diadasia* may be suggested by the fact that two first instars from the same cell moved to the bottom of the plastic rearing-dish and there elevated themselves on the apices of their abdomens. Perhaps thus standing erect, the *Protepeolus* larva awaited passage of the ambulatory *Diadasia* larva, climbed onto it, and killed it after it became quiescent. However, the *Protepeolus* first instar could crawl actively and could crawl around the pollen mass until it encountered the already quiescent *Diadasia*. We discovered no *Protepeolus* larvae in cells with live *Diadasia* larvae, even though we examined many cells. This suggests that the first instar rapidly eliminated the host larva and that the emergence of the first instar was well synchronized with the time when the host larva was quiescent and vulnerable, as discussed below.

We have little information concerning interactions between two first instars of *Protepeolus* in a single cell. The two larvae mentioned above opened their opercula within 215 minutes of each other. Both emerged and crawled to the bottom of the dish during the night, but did not attack each other. In another cell with two hatched parasite eggs, two first instar head capsules were found stuck to the provision mass, one apparently the cast skin of the survivor, the other the remains of the assassinated rival.

An analysis of 18 host larvae killed by *Protepeolus* shows two unusual phenomena. First, as indicated above, only third or fourth instars were attacked, a situation we believe unique among nomadine cuckoo bees. Second, the ratio of age groups of the dead host larvae was: one third instar, three fourth instars, 16 third instars nearly ready to molt. With the last group, the fourth instar's pigmented mandibles could be seen clearly within the mandibles of the third instar in each case. Hence the pharate *Diadasia* larva about to undergo ecdysis is the preferred host, presumably because such larvae are quiescent. A quiescent host would almost seem to be necessary because the length of the *Protepeolus* is approximately 1 mm., whereas the host is 10 or 12 times longer.

After killing the host, the first instar of *Protepeolus* increased greatly in diameter and more than doubled its length (fig. 8) before molting to the second instar. There is no indication that it fed on the host, which slowly decomposed on the pollen mass. On the other hand, large first instars when dissected revealed no pollen grains in their intestines which were filled with amorphous finely granular material; pollen grains were readily observable in later instars. We suspect that the curved mandibles, the apices of which fit into the sclerotized buccal cavity (fig. 22), may be adaptations whereby an individual pollen grain (approximately 0.05 mm. in diameter) is held either at the entrance of the cavity or perhaps within it and the sharp tips of the closing mandibles split it apart. Its contents are then ingested, perhaps with the aid of salivary secretions as suggested by the unusually close proximity of the salivary opening to the buccal cavity; presumably the pollen wall is discarded.

Accurate figures on duration of larval stadia are lacking. Casual observations indicate that the first stadium, at least from the time the host is killed to molting, was short, perhaps two to three days. The second stadium was of about the same duration. One larva molted to the third instar at 11:00 p.m., August 25, and then molted to the fourth at 9:30 a.m., August 28, a period of two days, 10 hours, and 30 minutes. The first three instars consumed only a very small part of the available food. The fourth instar had the longest stadium and consumed most of the food. A larva that molted to the fourth stage on August 26, almost finished the provisions by the end of September 3, a period of more than eight days.

Like the first instar, the remaining three instars were capable of moving about the provision mass while they ate. The general mode of locomotion was similar to but less energetic than that of the first instar. Movement was assisted by the spiculated dorsum with its somewhat elevated segments and by the ventral protrusion on abdominal sternum IX, a feature absent in the first instar. Although abdominal segment X was somewhat bilobed in the second instar, the bilobed

condition was completely obliterated in the third and fourth. By being pushed against the cell surface, the elevated anal area may aid the larva to crawl. However, the anal area more probably is an adaptation for applying feces to the cell wall.

Protepeolus commenced to defecate early in the fourth stadium and plastered feces over the entire inner surface of the cell including the closure as it ambulated. At least with larger larvae, the provision mass was circled by the venter while the dorsum of the larva was pressed against the cell surface (fig. 5). The food mass was thus held away from the feces. As the larva fed, the provisions were at first reduced to a nearly spherical form, but later became more elongate. The rather symmetrical reduction of the food mass indicated that the fourth instar moves in relation to the food mass as well as in relation to the cell wall. At no time were the provisions deeply furrowed as they were by the intermediate instars of *Diadasia*. Hence, when first opening cells we usually could identify those parasitized by older larvae of *Protepeolus* even if we saw no larva or dead host, because of the much smoother surface of the food. After the provisions became somewhat elongate they appeared faintly sculptured rather than completely smooth (fig. 5). When the provision mass was greatly reduced it apparently was held stationary by the last instar which circled its body forward to eat.

The movement of the larval head that accompanies feeding was quite different from that of *Diadasia*. Whereas *Diadasia* moved its head from side to side while taking bites from the moist surface of the pollen mass, the second, third, and fourth instars of *Protepeolus* moved their heads forward and back as they scraped the surface layer from the pollen with their mandibles which were maintained in a nearly closed position. The head completed about one back and forth cycle per second. The mandibles may have been moved somewhat more in the fourth instar, but the movements were not the bold mandibular extensions and retractions of *Diadasia olivacea*. As the larva fed, the top of the labrum, mandibles, maxillae, and labium, but never the antennae, came in contact with the food. The larva ambulated slowly forward while continuing the shaving motion with its head. It also moved the anterior part of its body somewhat from one side to the other.

In the second and third instars several conspicuous, bright yellow, tubular organs, probably Malpighian tubules, meandering nearly the full length of the body, were visible through the transparent integument of live *Protepeolus* larvae. Present as very thin structures in the early second instar, these yellow glands gradually enlarged and became pronounced in the third. Such yellow glands are characteristic of live, predefecating larvae of other Nomadinae and can often be used as a field identification of these parasitic larvae when they are excavated. Only larvae of the Melitomini and Exomalopsini are known by us to also possess similarly appearing glands. When starting to defecate, larvae of *Protepeolus* first discharged a greenish semitransparent liquid that contained no pollen grains. Concomitantly the yellow glands reduced in diameter, suggesting that the voided material was the content of the glands. The feces became opaque yellowish and filled with pollen less than a day later when the glands were no longer visible. For the rest of its feeding period the larva continued to defecate while ingesting food.

All the food in the cell was consumed, the only remains being debris composed of the mandibles and other integumental parts of the host, perhaps part of the cast skins of *Protepeolus*, and also small stones. Presumably too large to be ingested, the debris accumulated on the venter of the abdomen where they hardened into a single mass (figs. 6, 7, 26) in some cases. In other cases, perhaps as a result of laboratory rearing, the debris seemed less consolidated, being widely spread over the venter of the parasite.

Feces were extruded and applied as moist pellets to all surfaces of the cell with the modified anal area (fig. 27). By the time a larva had finished defecating, the feces were built up so that the larva was surrounded by a nearly dry coating ranging from about 0.25 to 0.5 mm. in thickness. The feces seemed thicker on the cell closure than on the wall itself. The inner surface of the plastered feces was moderately smooth and exhibited patches of white mold.

Some individuals of *Protepeolus* spun cocoons but others did not, apparently depending upon whether they became diapausing, hibernating lar-

vae or whether they pupated immediately. Two larvae pupated and one was about to pupate when preserved, all in the 1973 season, and none had spun a cocoon. On the other hand, seven postdefecating larvae produced cocoons and then became quiescent. In summary, no quiescent postdefecating larvae were found without cocoons, and no pupae were discovered with cocoons in the late summer or fall of 1973.

Larvae that pupated did so shortly after defecating and without becoming quiescent beforehand. Larvae that spun cocoons started spinning either near the end of the defecating period or more probably immediately after it, and in two cases took less than four days to spin cocoons and become quiescent.

All cocoons examined were spun in the laboratory in cells that had been reoriented from their normal position in the ground. These cocoons corresponded in general to the shape of the cell wall and did not exhibit a nipple or other distinctive features. Each consisted of a single thin layer, less than 0.25 mm. thick, of partly fibrous and partly sheetlike silk. The cocoon did not incorporate fecal material, all of which was between the cell wall and the cocoon. The inner surface of the cocoon, reddish tan to pale tan, was moderately rough in texture because of abundant fibrous silk; sheetlike silk glistened between the fibers. The cocoon fabric was minutely fenestrated but was sufficiently tough to be removed by forceps from the cell without tearing. When it was so removed, fecal material adhered to much of the outside. Where there were no feces, the cocoon was semitransparent.

Diapausing larvae were slightly flaccid, entirely inactive, curved but not curled in contrast to the host in which the abdominal apex comes in contact with the anterior part of the dorsum. Larvae did not adhere to the inner surfaces of their cocoons.

Discussion. Although the ethological characteristics of *Protepeolus singularis* differ in many ways from those of other known Nomadinae, two features are characteristic of the subfamily in general: (1) oviposition in still-open cells that are being provisioned, and (2) insertion of the egg in the cell surface. Only the Nomadinae among the parasitic bees exhibit these two features, and it seems unlikely that they would have arisen once in *Protepeolus* and again in the other Nomadinae. For that reason they are considered synapomorphies, attesting to the common origin of *Protepeolus* and the other Nomadinae about which we have such information. (These two features are probably functionally interrelated, for a parasite egg must be hidden if a returning host female is capable of detecting and destroying an exposed parasite egg.) *Protepeolus* and the other Nomadinae (including *Isepeolus*) also have active larvae that can crawl around the cells, find the host immatures and possess sclerotized head capsules with specialized mandibles for killing the hosts. However, these features have arisen independently among various, clearly unrelated parasitic bees (e.g., *Coelioxys*, *Dioxys*, Melectini, Ctenioschelini) so they are less reliable as indicators of relationships.

Unfortunately, we do not know many of the ethological features of *Isepeolus* and none of *Leiopodus*, the other two genera in the Protepeolini. Michener (1957) and Lucas de Oliveira (1966) reported that the first instar larva of *Isepeolus viperinus* has a sclerotized head capsule and elongate sickle-shaped mandibles for killing the host. We do not know where the *Isepeolus* egg is laid nor whether it is inserted in the cell before cell closure. Among the Nomadinae only *Isepeolus* and *Protepeolus* are known to be capable of spinning cocoons (not known for *Leiopodus*), but this feature is plesiomorphic for bees and therefore does not imply close relationship.

Protepeolus has a number of ethological features by which it differs from all other Nomadinae (unfortunately these features are unknown for *Isepeolus*): (1) extended incubation period; (2) unique shape of egg (egg shape highly specialized in other ways among some other Nomadinae); (3) ability and propensity to destroy a large host immature that is already a pharate last larval instar (Michener, personal commun., stated that he found only small larvae of *Colletes* killed by *Isepeolus viperinus*). The interpretation of these features will be treated at the conclusion of this paper.

IMMATURE STAGES

Descriptions of the first and last larval instars and pupa are presented below and are followed

by an account of the major anatomical changes that take place from one instar to the next.

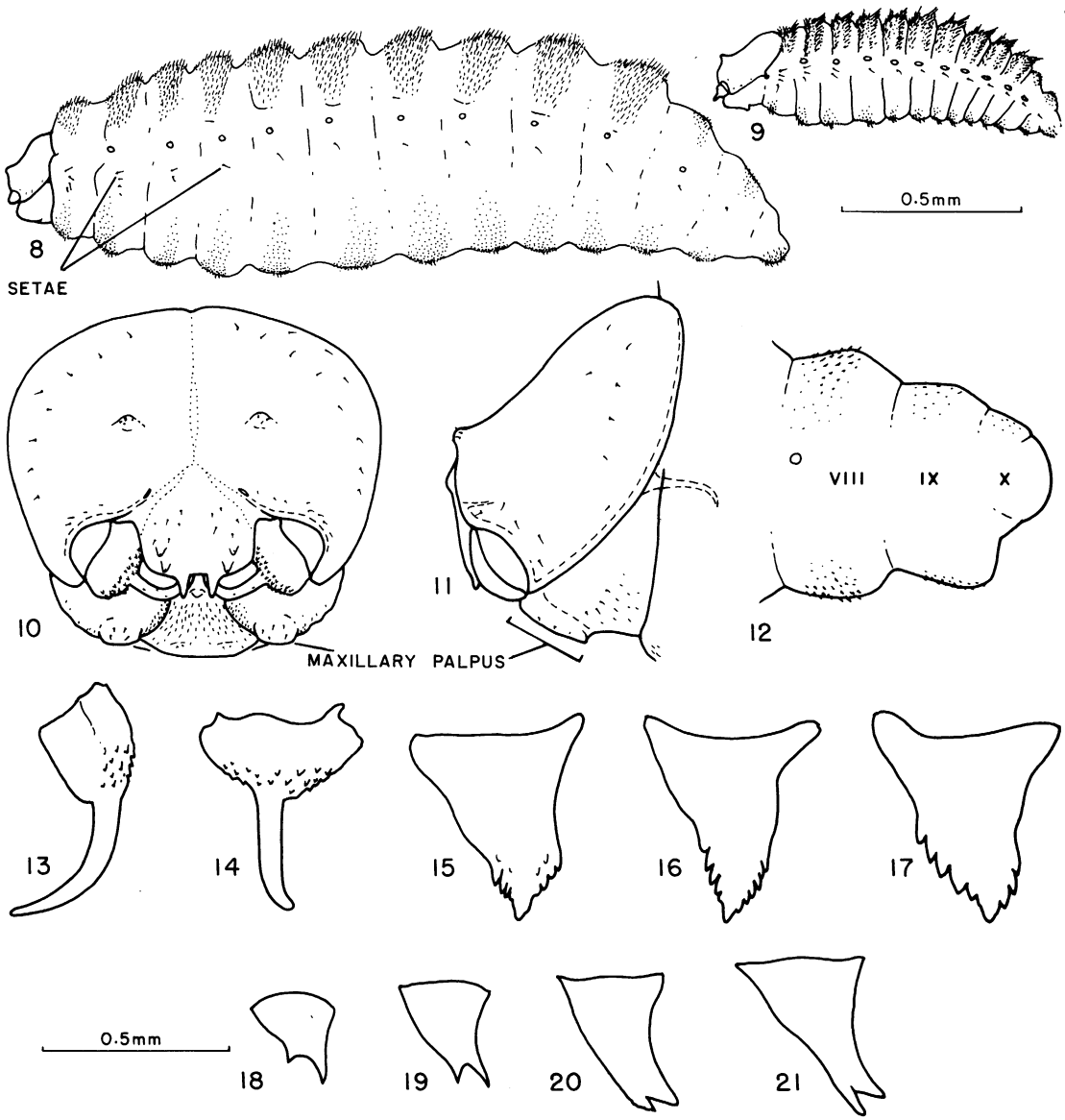
First Larval Instar
Figures 8-11, 13, 14

The following description is comparative insofar as possible with that of the first larval instar of *Isepeolus viperinus* (Holmberg) (Michener, 1957; Lucas de Oliveira, 1966). The two forms are so radically different that a formal diagnosis in addition to the description is scarcely needed. This study is based upon SEM, as well as optical microscopic examination. For SEM study, previously preserved specimens were taken from 100 percent ethyl alcohol, dried in a critical point drying apparatus, and then coated with gold.

Total length 1.0 mm. (newly hatched) to 2.1 mm.

Head (figs. 10, 11). Hypognathous, unusually short and not depressed, unlike head of *Isepeolus viperinus*, which is prognathous, strongly depressed, and unusually elongate. Head capsule pigmented except for coronal ecdysial lines; mandibles distinctly pigmented; labrum more faintly pigmented; rest of head, including all of ventral surface, unpigmented; head capsule with scattered moderately long setae, apparently less dense than those of *I. viperinus*. Tentorium possibly complete, but anterior arms becoming very thin although anterior pits quite distinct; posterior pits on hypostomal-posterior thickening and posterior arms pronounced, being pigmented and extending posteromedially; these arms tapering and becoming unpigmented and very fine as they bend and run medially; posterior thickening of head capsule well developed and unlike that of *I. viperinus*, pigmented sclerotization of capsule not spreading backward beyond posterior margin; hypostomal ridge well developed, appearing as continuation of posterior thickening; pleurostomal ridge moderately well developed at least near anterior mandibular articulation; epistomal ridge laterad of anterior tentorial pits moderately well developed but apparently absent mesiad of pits; anterior tentorial pits well developed; coronal cleavage line extending from posterior margin of head nearly to labroclypeal sulcus; parietal bands not evident; head not strongly constricted behind; genal area not produced anteroventrally as in *Coelioxys*. Antennal papilla evident as swelling, less well defined than in *I. viperinus*; papilla arising gradually from moderately conspicuous prominence and bearing four to six cone-shaped sensilla. Labrum large, rounded anteriorly, with three pairs of tubercles, each bearing a single sensillum; in contrast labrum of *I. viperinus* small and tapering anteriorly to single median tubercle; apical tubercles longest, subapical tubercles next longest; remainder of labrum with scattered short setiform sensilla, none on tubercles. Mandibles (figs. 13, 14) moderately broad at base, tapering rapidly to elongate, narrow, fanglike apical part; basal area beset with numerous apically directed spicules as well as scattered peg-shaped sensilla on adoral surface (figs. 22, 23); apical fanglike part very gradually tapering to simple apex, circular in cross section, and bearing four or five scattered cone-shaped sensilla on outer surface; apex without pore, indicating that mandible not connected to poison gland; apical part strongly curved and moderately short, compared with mandible of *I. viperinus*; tip of each mandible when in repose curving into pigmented and sclerotized buccal cavity (figs. 10, 22, 23). Maxillae, labrum, and hypopharynx distinct; not formed into a single sclerotized ventral plate as in *Isepeolus viperinus*. Maxilla with ventroapical part possessing large, flat, elongate oval padlike structure bearing sensilla (figs. 10, 11, 22, 23), presumably palpus; apex of maxilla bending gradually adorally and bearing numerous moderately short setiform spicules; base of maxilla laterally with moderately long setiform spicules intermixed with some setae of similar length; galea not evident. Labium recessed, apex not extending anteriorly or ventrally as far as maxillae; most of the surface covered with posteriorly directed moderately short setiform spicules; palpus apparently a flattened, rather large area bearing approximately two cone-shaped sensilla and not covered with spicules. Hypopharyngeal area not produced and reduced; salivary opening circular, close to mouth.

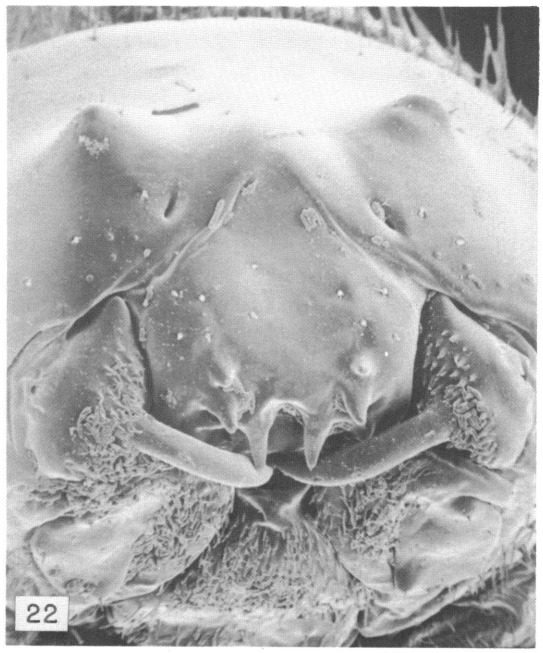
Body. Form (figs. 8, 9) moderately robust, straight, not depressed; body segments without evident intrasegmental lines; middorsal tubercles absent; lateral swellings not conspicuous as in *Isepeolus viperinus*; abdominal segment X di-

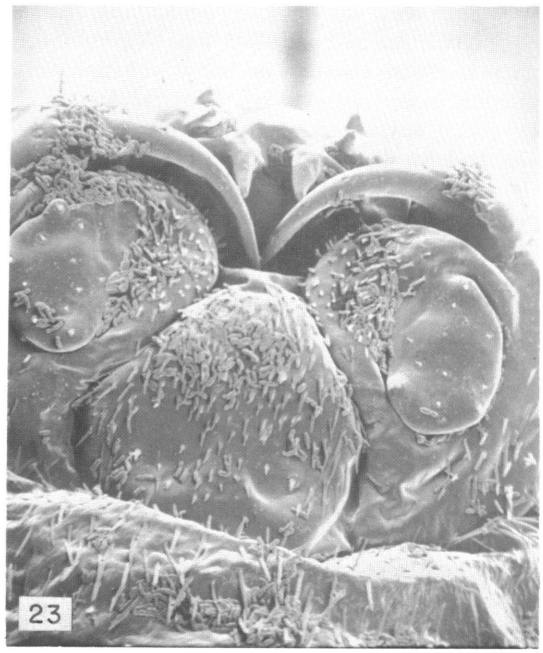


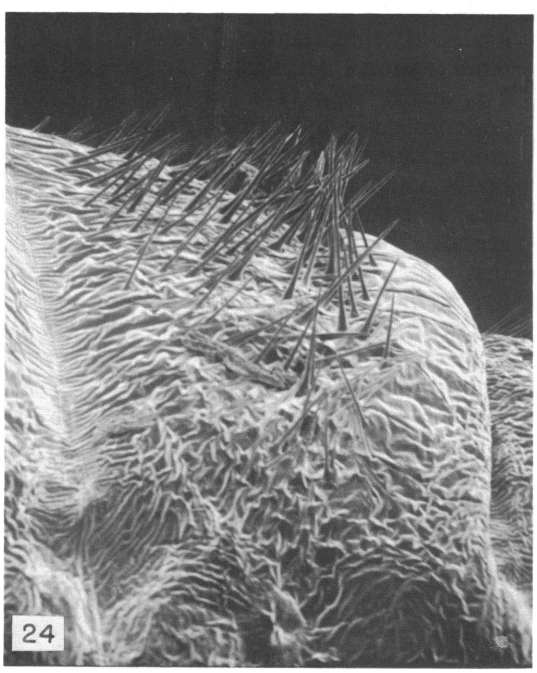
FIGS. 8-17. *Protepeolus singularis.* 8. First instar shortly before molting, lateral view. 9. Same, immediately after hatching, lateral view. 10. First instar head, frontal view. 11. Same, lateral view. 12. Tip of abdomen of second instar, lateral view. 13. Right mandible of first instar, lateral view. 14. Same, outer view. 15. Right mandible of second instar, outer view. 16. Right mandible of third instar, outer view. 17. Right mandible of early fourth instar, outer view. Mandibles drawn at various magnifications.

FIGS. 18-21. *Diadasia olivacea*, mandibles, outer view. 18. First instar. 19. Second instar. 20. Third instar. 21. Fourth instar. Mandibles drawn to same scale.

Scales refer to figures 8 and 9 and 18 through 21, respectively.







FIGS. 22-25. Scanning electron micrographs of *Protepeolus singularis*. 22. First instar head, frontal view. 475×. 23. Same, ventral view, 500×. 24. Same, dorsum of body segment, showing setiform spicules, lateral view, 475×. 25. Fourth instar, dorsum of anterior body segment showing transverse spicules and scattered short setae, anterior view, 500×.

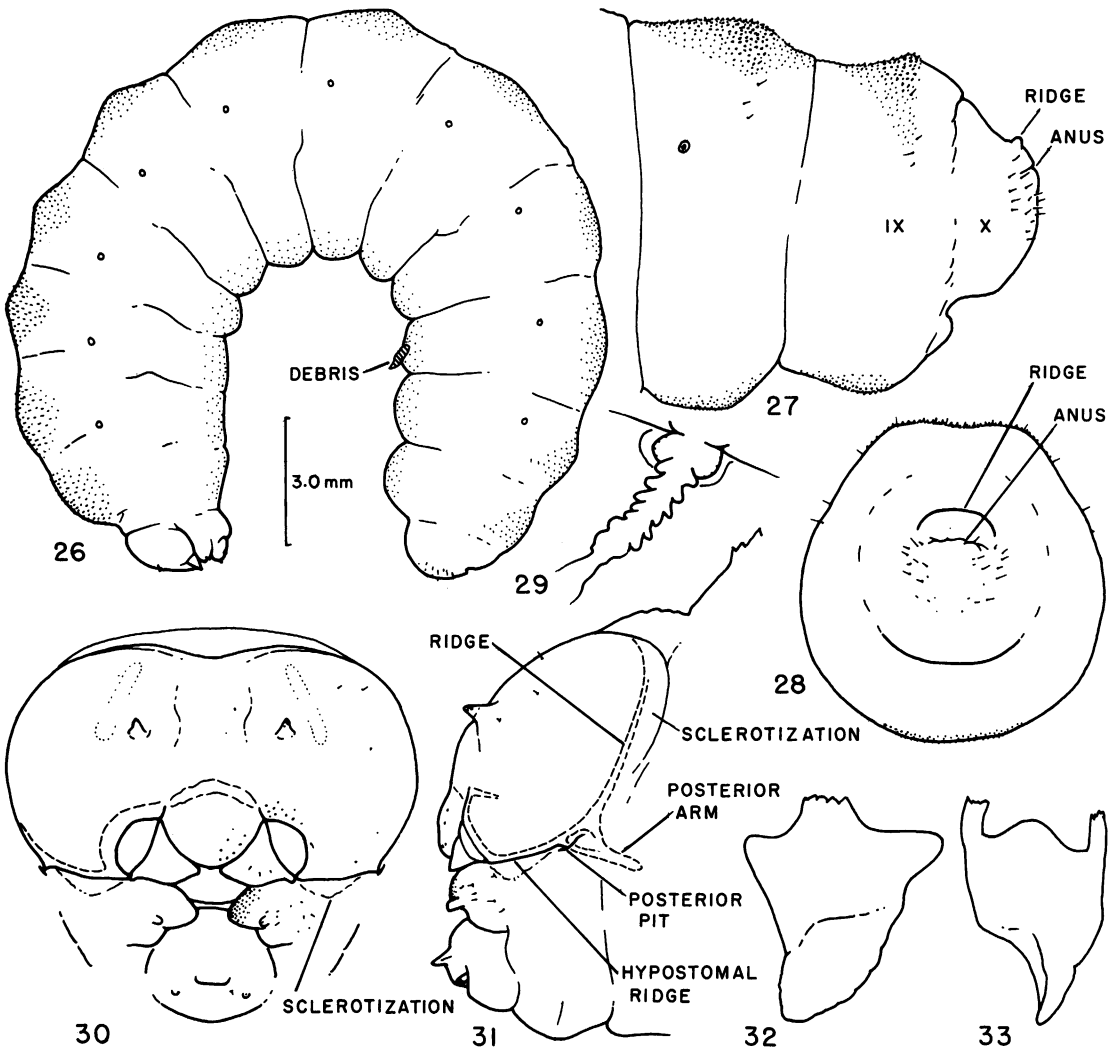
vided into two lateral lobes which serve as a pygopod during locomotion (see Activities of Larvae); stout spicules probably assisting this function. Anus not evident. Thoracic dorsa and abdominal dorsa I to VII with dense, extremely long setiform spicules (fig. 24); dorsa of segments VIII and IX with scattered much shorter setiform spicules; setiform spicules of all dorsa intermixed with widely scattered setae; lateral area of first thoracic segment with approximately four very long erect setae as well as with very fine setiform spicules which may be decumbent (because of their obscure appearance not shown in figures 8, 9); lateral areas of mesothorax and metathorax each with approximately two or three erect elongate setae in addition to very fine setiform spicules on each side; abdominal segments I through VIII each with single erect elongate seta in addition to finer setiform spicules on each side; lateral fine spicules becoming less evident toward posterior end of body; lateral area of segment IX apparently with seta on each side; all lateral setae on thoracic segments and abdominal segments I to IX situated short distance below level of spiracles; venter of all body segments with numerous, elongate, setiform spicules as well as with few widely scattered setae; most of tenth abdominal segment with numerous spicules which tend to be stouter and perhaps shorter than other body spicules; presence of setae on abdominal segment X uncertain. Spiracles uniform in size; atrium not projecting above body wall, large, shallow (about twice as wide as deep) and apparently with peritreme and with very finely spiculate wall; subatrium undifferentiated from trachea.

Material Studied. Seven specimens and seven head capsules of first instars (either cast skins or killed siblings), Southwestern Research Station, 5 mi. west of Portal, Cochise Co., Arizona, August 24 to September 4, 1973 (G. Eickwort, K. Eickwort, M. Favreau, J. G. Rozen).

Fourth Larval Instar
Figures 17, 26-33

Diagnosis. Because last larval instars of this species possess a produced labiomaxillary region adapted for cocoon spinning, they can be distinguished from last instars of all other Nomadinae except *Isepeolus*. The pronounced body tubercles of *Isepeolus viperinus* (Rozen, 1966, fig. 2; Lucas de Oliveira, 1966) contrast with the lack of such tubercles in *Protepeolus singularis*. Numerous other differences between these two taxa are present in the following description.

Head (figs. 30, 31). Head capsule faintly pigmented except for internal ridges and mandibles which are moderately dark; scattered sensilla present, some in form of short setae; apices of maxillae with distinct sharp-pointed spicules; other areas of the head nonspiculate. In frontal view, head approximately as wide as long; in lateral view (fig. 31), capsule evenly rounded in front and, in contrast with that of *I. viperinus*, labroclypeal area not strongly projecting. Tentorium complete; anterior arms moderately thin; pits well developed; dorsal arms thin but present; posterior arms (fig. 31) extremely thick, much thicker than those of *I. viperinus*; instead of being directed mesiad or perhaps somewhat anteriorly as those of *I. viperinus*, posterior arms directed posteriomedially for a considerable distance, narrowing abruptly and connecting to moderately thin, transverse tentorial bridge; posterior tentorial pit slightly behind and below hypostomal ridge as a result of thickness of posterior arms; hypostomal ridge and posterior arm apparently functioning as single structural brace; position of posterior margin of head capsule uncertain because sclerotization of head capsule extending posteriad of pronounced ridge that represents upward curving continuation of hypostomal ridge; hence, posterior margin of head capsule is either boundary of sclerotization of head capsule or ridge extending dorsally as continuation of hypostomal ridge; hypostomal ridge well developed, pigmented; pleurostomal ridge moderately well developed, pigmented; epistomal ridge moderately developed, short, and darkly pigmented laterad of each anterior tentorial pit; mesiad of pit, ridge absent but area between pits occupied by broad unpigmented area which arcs slightly upward medially; head capsule without median frontal ridge but with somewhat sinuate, pigmented ridge on each side approximately halfway between antenna and median line; these ridges approximately parallel; parietal bands faint. Antennal papillae moderately pronounced; each approximately as high as basal diameter and bearing approximately six sensilla; antennal protuberances slightly devel-



FIGS. 26-33. *Protepeolus singularis*, mature larva. 26. Entire larva, lateral view. 27. Tip of abdomen, magnified, lateral view. 28. Same, anal view. 29. Spiracle, side view. 30. Head, frontal view. 31. Same, lateral view. 32-33. Mandible, inner and ventral views.

oped just beneath antennae. In contrast to that of *Isepeolus*, labrum scarcely projecting, approximately in same plane as frontoclypeal area; labrum without tubercles, with apical edge rounded and with sensilla scattered along anterior apical margin; epipharynx broadly swollen basally. Mandible (figs. 17, 32, 33) short and broad at base; apex broadly rounded on postdefecating form but on specimen still feeding moderately pointed; upper and lower apical margins indistinctly serrate on postdefecating form but distinctly serrate on specimen still feeding; cusp moderately produced, nondentate; apical concavity moderately poorly developed. Maxillae moderately broadly fused to labium, moderately produced; cardo nonsclerotized; stipes faintly sclerotized; maxilla just below hypostomal ridge sclerotized as continuation of head capsule; unlike that of *Isepeolus viperinus* apex of maxilla bent strongly mesiad and abruptly narrowed

mesiad of palpus as seen in frontal view (fig. 30); galea absent; palpi moderately elongate, somewhat longer than basal diameter and not extremely long as in *I. viperinus*. Labium moderately projecting, divided into prementum and postmentum; palpi somewhat smaller than maxillary palpi but distinct and well developed; hence palpi not extremely elongate as in *I. viperinus*. Hypopharyngeal area not produced and not differentiated from labium; hypopharyngeal groove absent. Salivary opening on moderately narrow projecting lips; these lips not so broad as those of *I. viperinus*.

Body. Integument of postdefecating form wrinkled; dorsum of thoracic segments beset with numerous transverse spicules (figs. 25, 26); dorsum of abdominal segments also with spicules but spicules becoming less transverse and somewhat smaller toward caudal end of body; venter of thorax and abdominal segments I through IX densely but finely spiculate; a few short, fine setae present on dorsum of posterior abdominal segments; setae on abdominal segment X somewhat longer, more numerous than those of preceding segments and grouped primarily below anal region in position similar to that of the setae of *Diadasia*. Form moderately robust, with head being rather small in relation to rest of body, unlike situation in *I. viperinus*; in lateral view body generally curved; body segments not divided dorsally into caudal and cephalic annulets; dorsum of pronotum perhaps somewhat swollen; dorsolateral areas of most segments slightly swollen though not raised into distinct tubercles; lateral tubercles absent; abdominal segment IX not produced ventrally but seeming large by comparison with small segment X; abdominal segment X dorsal in attachment to IX; anus transverse, somewhat dorsal in position rather than apical as in *Isepeolus*; perianal area defined dorsally by a distinct transverse ridge (fig. 28); in general, abdominal segment X surprisingly similar to that of certain species of *Diadasia*. Spiracles (fig. 29) uniform in size, not on elevations; atrium globular, projecting slightly above body wall with distinct rim; atrial wall with faint spicules arranged in lines; peritreme present; primary tracheal opening with collar; subatrium annulate, of moderate length. Imaginal discs of male genitalia situated mesially on ventral surface approximately on intersegmental lines separating abdominal segments IX and X; cuticular scar transverse, linear, short, somewhat protuberant and unpigmented on nonpostdefecating forms; discs of male not discernible on postdefecating forms; characteristics of female unknown.

Material Studied. Twelve specimens including eight postdefecating larvae, Southwestern Research Station, 5 mi. west of Portal, Cochise Co., Arizona, August 25 to September 4, 1973 (G. Eickwort, K. Eickwort, M. Favreau, J. G. Rozen), most larvae preserved on day of collection but some preserved during following months.

Pupa

Figure 34

Diagnosis. This pupa can be distinguished from the known pupae of other Nomadinae on the basis of the key in Rozen and McGinley (1974).

Head. Integument nonspiculate; setae absent. Scape and frons without tubercles; vertex without tubercles; genal tubercle absent; clypeus modified and without tubercles; mandible with moderate swelling on ventral surface; dorsal surface without tubercles or swelling.

Mesosoma. Integument nonspiculate; setae absent. Lateral angles of pronotum not produced; posterior lobe of pronotum indistinctly produced; mesepisternum without tubercles; mesoscutum with pair of large, dorsally projecting tubercles; axilla and metanotum without tubercles. Tegula without tubercles; wing without tubercle or swelling. All coxae without tubercles; foretrochanter, but not others, with distinct rounded ventral tubercle; femora without tubercles, fore- and mid-tibia each with small apical tubercle.

Metasoma. Integument nonspiculate except as indicated below; setae absent. Tergum I with several apical tubercles; terga II-V each with apical irregular row of sharp-pointed tubercles; sterna without tubercles; terminal spine absent.

Material Studied. One female, Southwestern Research Station, 5 mi. west of Portal, Cochise Co., Arizona, collected as larva, August 25, 1973 (Eickworts and J. G. Rozen), preserved September 11, 1973.

FIG. 34. *Protepeolus singularis*, pupa, lateral view.

Developmental Changes

Protepeolus larvae undergo considerable change from one instar to the next. Table 1 gives the maximum width of the head capsule of the four larval stages. Significant features of the second, third, and fourth instars whereby they differ from the previous instar are presented in the following synopsis.

Second instar: Head capsule less pigmented; hypostomal ridge somewhat more angled in relation to posterior thickening of head capsule; area immediately behind posterior thickening of head capsule sclerotized as continuation of capsule; antennal papilla smaller in relation to head capsule but projecting more than that of first instar; labral tubercles greatly reduced but their sensilla present; mandible (fig. 15) broad at base but with apical part short and dentate, not fanglike and curved and not fitting into buccal cavity; maxillary palpus moderate-sized, vague swelling, relatively smaller than palpus of first instar; labial palpus represented only by sensilla, not produced; salivary opening farther away from nonsclerotized, unpigmented buccal cavity. Body form straight or nearly so; venter of abdominal segment IX protruding strongly (fig. 12); abdominal segment X only indistinctly bilobed; dorsal spicules no longer setiform, now shorter and mostly transverse; lateral setae on thorax greatly reduced, those on abdomen apparently absent; widely scattered dorsal setae present on most segments; setae in anal region absent or inconspicuous.

Third instar: Head capsule pigmented about as much as that of second instar; hypostomal ridge somewhat more angled in relation to posterior thickening of head capsule as compared with the second instar; sclerotization behind thickening somewhat more extensive; antennal papilla projecting even more than that of second instar; labral tubercles absent but sensilla present; mandible (fig. 16) approximately same as described in second instar; maxillary palpus with basal diameter smaller in relationship to maxilla but more pronounced, more strongly projecting; labial palpus now slightly produced; salivary opening still not on projecting lips. Body form curved; venter of abdominal segment IX protruding strongly just as in second instar; abdominal segment X not bilobed; dorsal spicules

TABLE 1
Maximum Head Width of Larval Instars of *Protepeolus singularis* (in Millimeters)

Instar	Mean	Range	Number of Measurements
First	0.26	0.25-0.30	5
Second	0.41	0.38-0.43	7
Third	0.61	0.60-0.63	2
Fourth	0.95	0.85-1.00	11

even more transverse than those of second; lateral setae on body either inconspicuous or absent; widely scattered dorsal setae present on most segments; setae in anal region now conspicuous.

Fourth instar: Head capsule pigmented as in second and third instars; hypostomal ridge in relation to posterior thickening of head capsule slightly more angulate; sclerotization behind posterior thickening perhaps somewhat wider than in previous instars; antennal papilla somewhat smaller and projecting less than that of third instar though still conspicuous; tubercles absent; mandible (fig. 17) at least of specimen that just molted to the fourth instar with dentate condition of upper and lower apical edges similar to that of second and third instar; these teeth apparently abrading as the larva feeds, becoming inconspicuous in postdefecating forms (fig. 32); maxillary palpus moderately elongate, narrow in diameter; labial palpus not extremely elongate but more pronounced than those of third instar; salivary opening now with projecting lips. Body form curved, venter of abdominal segment IX protruding strongly on early fourth instar but protrusion becoming less evident as larva increases in size; abdominal segment X not bilobed; dorsal spicules broadly transverse (fig. 25); widely scattered dorsal setae present on most segments; setae in anal region conspicuous (fig. 27).

Certain structures change little, if at all, during the larval stadia. Most of these are evident and need not be pointed out. However, two such features are thought to be unique for bee larvae and therefore warrant emphasis. The anterior-posterior compression of the head capsule is consistent in all instars. Also the posteriomedial direction of the posterior arms of the tentorium is clearly evident in all stages. The adaptive significance of these two features is not understood but perhaps their functions are in some way interrelated.

DISCUSSION AND CONCLUSIONS

The numerous distinctive features of the life history and immature stages of *Protepeolus singularis* raise questions concerning the affinities of the genus (1) to the subfamily; (2) to *Isepeolus* and *Leiopodus*, the other genera of the Protepeolini; and (3) to the other genera and tribes within the subfamily. These questions are discussed below.

Affinities to the Subfamily. In spite of the various unique features of *Protepeolus*, it shares a number of synapomorphies with the other Nomadinae with respect to biology and anatomy of first and last larval instars. These characteristics are as follows:

1. Cleptoparasitic way of life
2. Eggs deposited in cell walls
3. Females ovipositing in open host cells
4. First instars ambulatory with the aid of bilobed abdominal segment X
5. Ability of first instars to kill host immatures with modified mandibles
6. Mandibles of mature larva with simple apex and reduced cusp
7. Modified position of posterior tentorial pits and/or posterior arms

These are judged apomorphic because such features are not generally found in other subfamilies of the Anthophoridae or in some cases in other bees. Although we recognize that some (e.g., 1, 5, and 6) have appeared *de novo* elsewhere in the Apoidea and even in the anthophorid Melectini-Ctenioschelini-Rathymini complex, others (e.g., 2 and 3) clearly have not. Hence, in our judgment the Nomadinae, as now constituted, had a single evolutionary origin and *Protepeolus* should be retained in it.

Affinities to Other Protepeolines. The mature larvae of *Protepeolus* and *Isepeolus* share numerous similarities. However, analysis of the features (table 2, fig. 35) shows that the similarities are plesiomorphic; *Isepeolus* appears to be the sister group of all other Nomadinae. On the basis of larval morphology it is unreasonable to include *Isepeolus* in the Protepeolini on either phenetic or cladistic grounds. Michener (1944), while retaining *Isepeolus* in the Protepeolini, suggested that it might belong to a separate tribe on the basis of adults. Therefore, we here propose a separate tribe for *Isepeolus*, the Isepeolini, new tribe. The Protepeolini now contains *Protepeolus* and probably *Leiopodus*.

Affinities to Other Nomadinae. The cladistic analysis based on mature larvae is the result of a series of studies (Rozen, 1966; Rozen, 1977;

TABLE 2

Characters of Mature Larvae Used in Cladistic Analysis of Nomadinae

Determination: 1 = out-group comparison with Anthophorinae; 2 = out-group comparisons with other Apoidea; 3 = character correlation

Character	Plesiomorphic State	Apomorphic State	Determination
1 Head capsule anteriorly-posteriorly compressed	no	yes	1
2 Posterior thickening of head capsule	single ridge	double ridge	1
3 Area behind posterior thickening of head	membranous	sclerotized	1
4 Tentorium	well developed, complete	reduced or incomplete	1
5 Posterior tentorial arms	present	absent	1
6 Direction of posterior tentorial arms	medially	posteroventrally	1
7 Hypostomal ridge	well developed	weak	1
8 Vertex	recessed	produced	3
9 Labrum exceeding frontoclypeal region	yes	no	1
10 Labrum bearing	at most weakly developed paired swellings	a) single median tubercle b) paired sharp-pointed tubercles	1, 3
11 Labrum	small to moderate size	enlarged	1
12 Mandible	elongate	short	1

TABLE 2 – (*Continued*)

Character	Plesiomorphic State	Apomorphic State	Determination
13 Maxillary palpus	elongate	a) very elongate b) moderately short c) short d) virtually absent	1
14 Apex of maxilla	bent mesiad	straight	2
15 Labium divided into prementum and postmentum	yes	no	1
16 Labium exceeding hypopharynx	yes	no	1
17 Labial palpus	elongate	a) very elongate b) moderately short c) virtually absent	1
18 Salivary lips	well developed	a) reduced b) absent	1
19 Development of hypopharynx	normal	greatly reduced, undifferentiated	1
20 Hypopharynx	spiculate	nonspiculate	3
21 Body setae	present	absent	2
22 Body spicules	normal	transverse	1
23 Dorsal body tubercles	present	absent	3
24 Lateral body tubercles	absent	present	1
25 Length of abdominal segment X	normal	a) short b) elongate	1
26 Venter of abdominal segment X	normal	elongate	1
27 Atrium of spiracle	without elongate spines	with spines	3

TABLE 3
Character States in the Mature Larvae of Nomadinae that Have Evolved *de novo* Two or Three Times

Apomorphic State	Appearance
14 Apex of maxilla not bent mesiad	*Isepeolus*–sister group to *Protepeolus*
20 Hypopharynx nonspiculate	*Protepeolus*–Ammobatini–sister group to *Holcopasites*
23 Dorsal body tubercles lost	*Protepeolus*–sister group to *Paranomada, Melanomada* and *Triopasites*
25a Abdominal segment X short	*Isepeolus–Neopasites*

Ehrenfeld and Rozen, 1977; Rozen and McGinley, 1974) as well as the present one. Some of the characters presented in table 2 have been explained more fully in the previous papers; other characters, particularly those pertaining to the Protepeolini, are introduced here for the first time. A few used in previous analyses have been dropped because they now seem to be of little importance.

The polarity of the character states (i.e., the determination of the plesiomorphic-apomorphic sequence) presented in table 2 in most cases was based on which state was found in tribes of the related subfamily Anthophorinae, other bee families, and even related wasps. In other cases, the polarity of otherwise indeterminate sequences was deduced on the basis of correlation of other characters after the initial cladogram was established. These two methods of determining polarity are termed out-group comparison and character correlation, respectively, in table 2.

In most cases, out-group comparisons were made with Anthophorinae. In a few cases, namely characters 14 and 21, the out-group comparisons were broader and were based on the fact that the plesiomorphic state occurs in the Sphecidae and elsewhere among the Apoidea.

Figure 35 is a cladogram of the Nomadinae[1] based on the characters in table 2. As indicated in table 3, the cladogram postulates a number of multiple origins of character states. In each case the assumption of an opposite polarity would have dictated far more parallelisms among the other characters.

The apomorphic states of characters 2 and 3 (i.e., posterior thickening of head with two transverse ridges and head capsule sclerotized behind posterior thickening) are regarded as separately evolved states since the biomechanics of the shallow head capsules of *Protepeolus* and of the projecting labroclypeal area of *Nomada* and the Ammobatini are so different.

Only one character state, i.e., 16, labium exceeding hypopharynx, seems to have undergone a reversal. To assume otherwise would require postulating independent origin of the apomorphic condition in four lineages. A plesiomorphic feature in *Isepeolus* and *Protepeolus*, character 16 was lost in the sister group to *Protepeolus* but reappeared in *Neopasites* and *Neolarra*.

Whereas figure 35 shows only the sequence of branching, figure 36 presents the same sequence with the length of each line being equal to the number of synapomorphic and/or autapomorphic features used in the analysis. As such, it not only shows the sequence of branching but also gives an impression of the amount of evolutionary change that has occurred in the mature larvae. It corresponds in a general way to our subjective evaluation of the overall differences and similarities we see in these larvae compared with their hypothetical ancestors. For example, there is no question that *Protepeolus* and *Isepeolus* are very different from each other in spite of their shared plesiomorphic features; they are also

[1]Michener (1974) combined the Epeolini with the Nomadini. However, larval epeolines and larval *Nomada* have separate synapomorphies and furthermore the Nomadini appear to be diphyletic (figs. 35, 36), with *Nomada* having a separate origin from *Paranomada*, *Melanomada*, and *Triopasites*. Therefore the Epeolini retain their tribal status in figures 35 and 36.

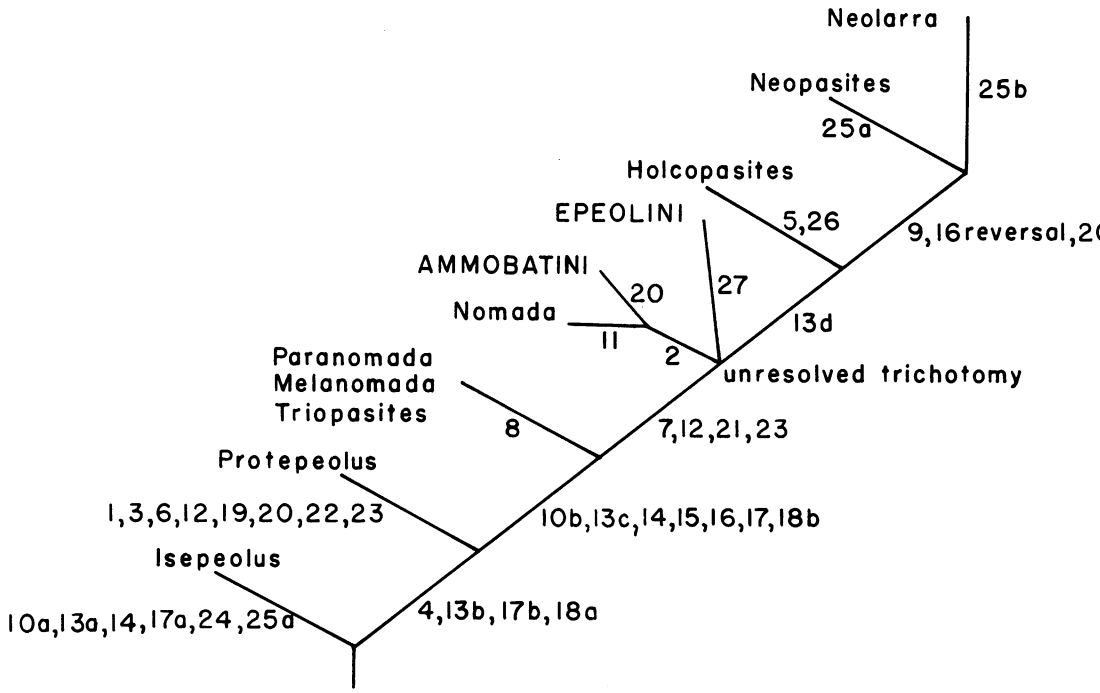


FIG. 35. Cladogram of Nomadinae based on mature larvae. For explanation see text.

very different from the better known Nomadinae like *Nomada* and *Epeolus*. A cladistic analysis of adult features of the Nomadinae should present the same sequence of branching as presented in figure 35. However, it would be surprising if the length of the lines presented in figure 36 would be the same; it is quite obvious that among certain groups of bees the larvae have undergone considerable change whereas the adults have not, while among others the larvae have been the more conservative.

APPENDIX
Larval Instars of *Diadasia olivacea*

During the course of the study of *Protepeolus* we needed to recognize the various instars of the host, *Diadasia olivacea*, to determine which instars were attacked. As the numbers of larval instars seem to vary in bees and as little has been written as to how to identify them, we include this information here for *D. olivacea*, which has four such instars. A young larva of this species was figured by Linsley, MacSwain, and Smith (1956). The first instar is apparently restricted to the same position as the egg, that is, to the underside of the pollen mass, and presumably does not crawl. One was found ingesting liquid and some pollen with only its head exposed from the chorion. Apparently the chorion and first instar exuviae are shed at the same time as the second instar emerges. All instars except for the first are active, crawling energetically around the pollen mass while feeding. All four instars can be recognized by their distinctive mandibles (figs. 18-21) as well as head capsule size. The following are the main diagnostic features of each instar.

First instar: Mandible as figured (fig. 18); no dorsal tubercles on most body segments; venter of abdominal segment IX not conspicuously produced; setae on abdominal segment X absent; abdominal segment X rounded, without ridges.

Second instar: Mandible as figured (fig. 19); middorsal body tubercles low and transverse; venter of abdominal segment IX strongly produced; setae on abdominal segment X absent; abdominal segment X without ridges.

Third instar: Mandible as figured (fig. 20);

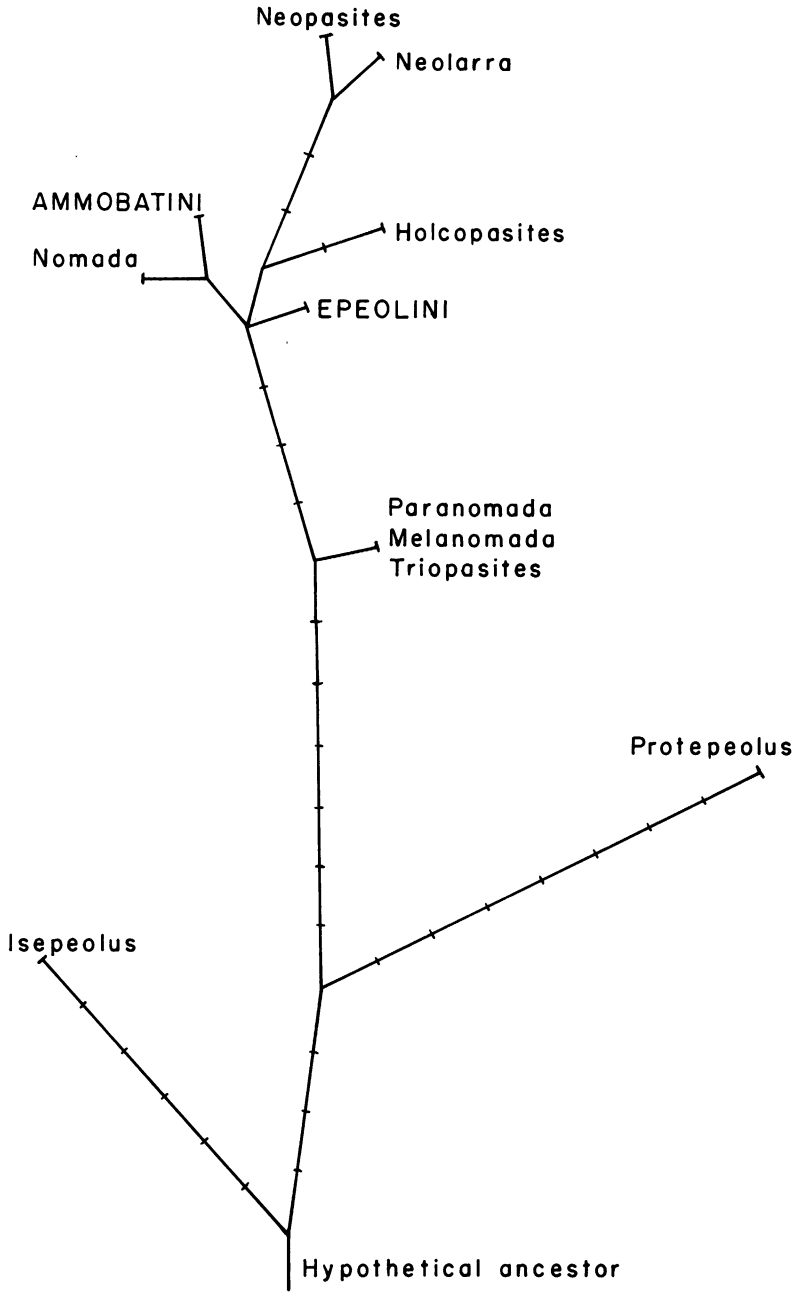


FIG. 36. Branching diagram of the Nomadinae based on mature larvae. Sequence of branching derived through cladistic analysis; length of line approximates amount of evolutionary change as expressed by synapomorphies and autapomorphies.

middorsal body tubercles elevated, restricted to median line, more or less conical; venter of abdominal segment IX strongly produced; setae on abdominal segment X present but short and inconspicuous; abdominal segment X without ridge above anus.

Fourth instar: Mandible as figured (fig. 21); middorsal body tubercles elevated as those of third instar; venter of abdominal segment IX produced; setae on abdominal segment X conspicuous; abdominal segment X with somewhat pigmented, rugose ridge above anus.

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