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Hospicidal Behavior of the Cleptoparasitic Bee *Coelioxys* (*Allocoelioxys*) *coturnix*, Including Descriptions of Its Larval Instars (Hymenoptera: Megachilidae)

JEROME G. ROZEN, JR.[1] AND SOLIMAN M. KAMEL[2]

ABSTRACT

In an attempt to determine whether *Coelioxys* and *Radoszkowskiana*, both cleptoparasitic members of the Megachilini, had a common cleptoparasitic ancestor, an investigation of the nesting biology and immature stages of *C.* (*Allocoelioxys*) *coturnix* Pérez was undertaken in Egypt. The purpose was to compare these aspects of this species with the results of a recent study of *R. rufiventris* (Spinola) and certain other species of *Coelioxys* (Rozen and Kamel, 2007). The egg of *C. coturnix* is deposited on the egg of *Megachile minutissima* Radoszkowski after the host female departs to collect cell-closure material. On hatching, the first instar, still surrounded by egg chorion, bites the developing host egg and consumes the entire egg content before feeding upon the host provisions. This behavior contrasts with certain other species of *Coelioxys*, whose eggs are hidden in the host cell while it is being provisioned and third instars normally kill the young host larvae. Because the behavior of *C. coturnix* closely mirrors that of *R. rufiventris*, the authors conclude that two modes of cleptoparasitism have developed in *Coelioxys* and that *Coelioxys* and *Radoszkowskiana* possibly had a common cleptoparasitic ancestor. The five larval instars of *C. coturnix* are described and compared with those of other *Coelioxys* species, and its first instar is compared with that of *R. rufiventris*.

[1] American Museum of Natural History (rozen@amnh.org).
[2] Department of Plant Protection, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt (soliman_90@hotmail.com).

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INTRODUCTION

Well over a century ago, Ferton (1896) reported two instances where he observed that females of *Coelioxys* (*Allocoelioxys*) *afra* Lepeletier oviposited so that the anterior ends of their eggs rested on the host eggs while the posterior ends were in the provisions. This occurred while the host females were presumably gathering leaf snippets to close the cells in the same nests. In one instance, he noted that two days later the cleptoparasitic egg and host egg were in the same position. In the evening of the second day the parasite's head had become defined, and he concluded that the egg had hatched. The resulting young larva then sucked the contents of the host egg, which subsided into the food, and within two days the egg was entirely empty. Afterward, the parasite larva fed on the provisions. His report thus indicates that the *Coelioxys* egg is deposited after the host female has deposited her egg and implies that the first instar (and perhaps subsequent ones) of the *Coelioxys* kills the host egg and feeds on the egg contents before feeding on the provisions.

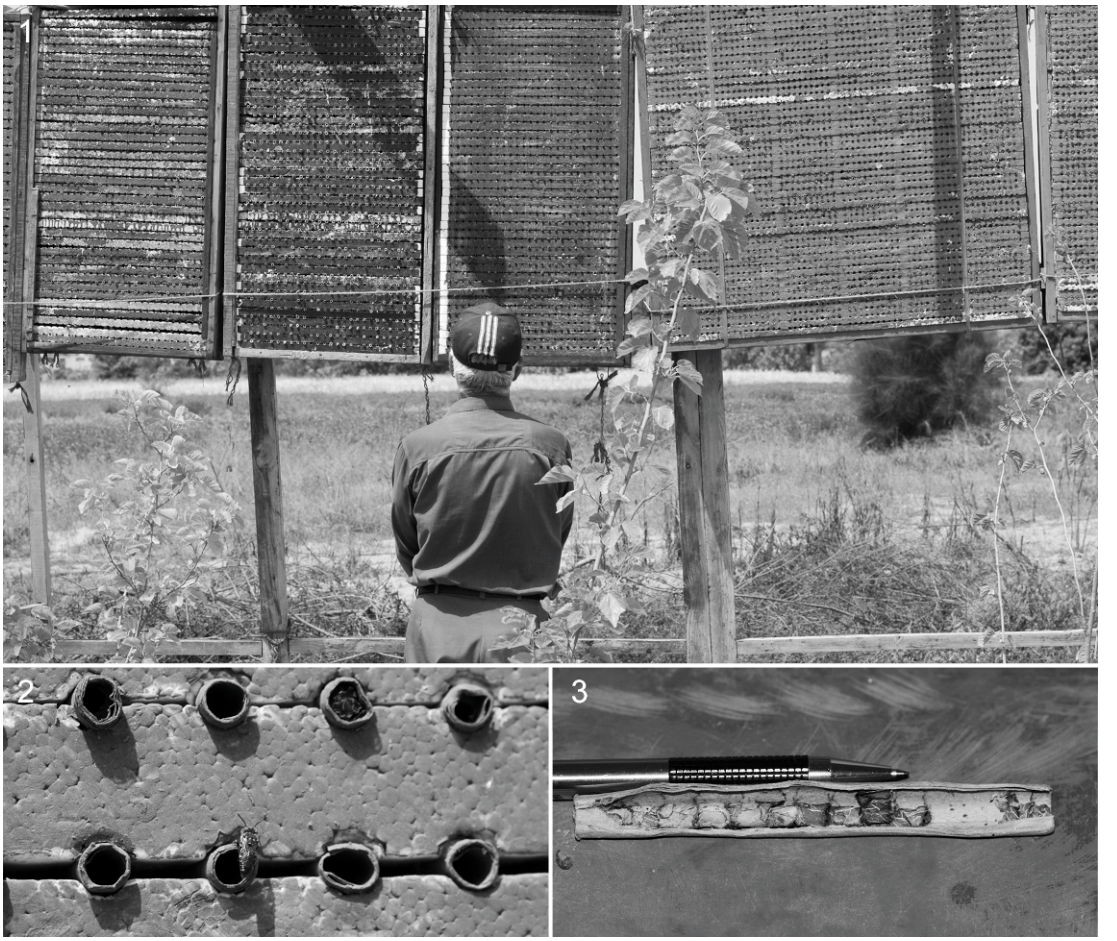
As noted by Rozen and Kamel (2007) these observations do not match the detailed account by Baker (1971), who found that the third instars of *Coelioxys* (*Boreocoelioxys*) *octodentata* Say and *C.* (*B.*) *sayi* Robertson, with highly modified head capsules bearing elongate mandibles, are primarily responsible for eliminating the host immatures (by that time larvae); furthermore, their eggs are introduced into the host cells before the hosts have deposited their eggs. Baker's finding corresponded exactly with those of Carré and Py (1981) on a species they called *C. rufocaudata* Smith, questionably a synonym of *C.* (*Allocoelioxys*) *echinata* Förster. Baker's discovery was also fully supported by our investigation of *Coelioxys* (*Liothyrapis*) *decipiens* Spinola (Rozen and Kamel, 2006, 2007).

The apparent discrepancies between Ferton's report and the subsequent ones came into focus while we were studying the biology and immature stages of *Radoszkowskiana rufiventris* (Spinola) (Rozen and Kamel, 2007). The latter genus, like *Coelioxys*, is cleptoparasitic and also a member of Megachilini. One of the questions we hoped to resolve in that study was whether these two genera had a common cleptoparasitic ancestor or whether each evolved their cleptoparasitic lifestyle independently. Sharply different modes of parasitism would suggest the latter; similar modes would argue for a common cleptoparastic ancestor.

To summarize our finding, we concluded that the

> female *Radoszkowskiana rufiventris* enters the host nest when the host is away presumably gathering closure material, deposits her egg on top of the host egg, which is resting on the surface of the provisions, and then departs. Its embryo develops rapidly so that it hatches before the host does. Still surrounded by most of its chorion, it kills the embryonic host by biting it with strongly curved but short, fanglike mandibles that bear a tiny spined second tooth basally. Without moving its head, it then proceeds to ingest the entire contents of the chorion over a period of more than a day while remaining motionless on top of the host egg. Its body slowly swells as the host egg is depleted. Except for the fanglike mandibles and short incubation period, there are no other obvious adaptations of the first instar for its parasitic role. The second instar starts feeding on the provisions by moving the anterior part of its body to one side or the other of the deflated host egg. Its mandibles are apically bifid as are those of all subsequent instars, presumably adapted for feeding on the provisions.

These results seemed highly suggestive of those of Ferton. Our next step was to verify what Ferton had reported. In the absence of *Coelioxys afra*, we had available a close relative, *C.* (*Allocoelioxys*) *coturnix* Pérez. This species is abundant in the vicinity of trap nests on the campus of Suez Canal University, where it attacks the nests of *Megachile* (*Eutricharaea*) *minutissima* Radoszkowski. This paper intends primarily (1) to report on our investigation into the behavior and anatomical adaptations involved with cleptoparasitism in this species and, on the basis of these findings, (2) to consider further the



Figs. 1–3. Trap-nest panels, Suez Canal University, Ismailia, Egypt. **1**. Five panels deployed on campus. **2**. Close-up of part of one panel showing nesting straws projecting from holes in painted foam plastic and a female of *Coelioxys coturnix* at one entrance. **3**. Nest straw removed from panel and opened to expose leaf-lined cells of *Megachile minutissima*.

origin(s) of cleptoparasitism in the Megachilini.

MATERIAL AND METHODS

Fieldwork for this paper took place from April 24 to May 10, 2007, on the campus of Suez Canal University, Ismailia, Egypt (N30°37′10″ E32°15′58″). There one of us (S.M.K.) has been pursuing a solitary-bee rearing program to develop pollination services for alfalfa and other crops because of the dwindling nesting opportunities for solitary bees in old, adobe structures (see Rozen and Kamel, 2007) due to the increasing availability of more permanent construction materials. The current study was greatly assisted by the availability of abundant immatures obtained from the array of large trap-nest panels (sometimes referred to as ''polystyrene nest blocks'') used in this program (figs. 1, 2). These panels consist of large blocks of painted foam plastic (described elsewhere in greater detail: http://www.pollinatorparadise.com/Egypt.htm) in which are inserted rows of hollow paper tubes (''straws'') about 12 cm long and 5 mm in diameter. The blocks are placed in the field close to the crop plantings, and bees such as *Megachile minutissima* that nest in preformed cavities find and use these

straws for their nests. When removed and taken to the lab, straws containing nests are slit longitudinal so that nest contents can be examined and appropriate immatures of host and cleptoparasite collected for study (fig. 3). We also availed ourselves of specimens that S.M.K. is rearing at the Ismailia Experimental Station, Agricultural Research Center, Ismailia. Because this locality is only a few kilometers from the university, observations from there are not differentiated from those recorded at the university.

In our investigations dealing with development, we relied heavily on retrieving the cast exoskeletons (hereafter termed *skins*) to distinguish instars on an anatomical basis. The skins are stacked one on top of the preceding one under the feeding larva and can be gently removed, separated from one another, and examined. Preserved instars were cleared in an aqueous solution of potassium hydroxide after the head was partly severed from the body. Heads were washed, transferred to ethanol, lightly stained with Chlorasol Black E, and studied in glycerin on well slides. Cast skins were simply retrieved, washed, and placed in glycerin on the same well slides with the preserved instar.

Because first instars remained cloaked by the egg chorions, details of their anatomy are hidden from view. We removed parts of the chorion from specimens after they had been critical-point dried and placed on a scanning electron microscope (SEM) stub by gently patting the chorion with the sticky surface of a sharply pointed sliver of cellophane tape. Patches of the chorion were thus removed.

Preserved specimens were examined with a Hitachi S-5700 SEM after being critical-point dried and coated with gold/palladium. Others were studied with a Zeiss EVO 60 SEM.

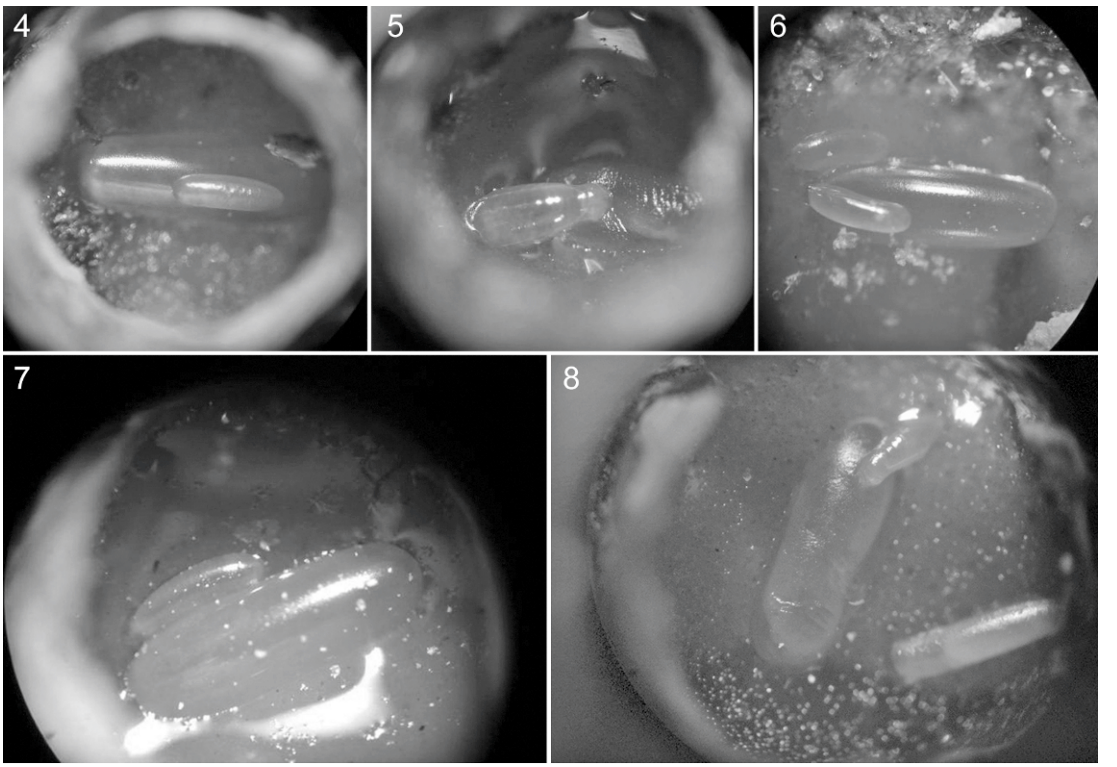
HOSPICIDAL BEHAVIOR

Adult Activity and Abundance: During our observations in April/May, females of *Coelioxys coturnix* flew in considerable numbers over the vertical trapnest panels (fig. 1), on which they occasionally alighted. They were accompanied by host females, which were arriving, departing, and searching for suitable nest straws. No males of the cleptoparasite and only a few of the hosts were observed there, but they were swept from blooming alfalfa plants adjacent to the nest panels. It seems likely that mating of *C. coturnix* does not occur at nest entrances but takes place in the vicinity of the host plants. Although we occasionally observed female *C. coturnix* enter nest straws, their visits were brief, suggesting that they did not result in ovipositing.

A number of other hymenopterans in addition to *Coelioxys coturnix* were seen around the nest panels, including *Osmia submicans* Morawitz (Megachilidae) and *Sapyga luteomaculata* Pic (Sapygidae). The latter species is a cleptoparasite of *Megachile minutissima* and *O. submicans* (Rozen and Kamel, 2007). A sampling of specimens by net sweeping over the surface of the panels on May 28 for a period of about 5 min yielded the following proportions: *M. minutissima*: 3 males, 38 females; *C. coturnix*: 0 males, 18 females; *O. submicans*: 2 males, 0 females; *S. luteomaculata*: 0 males, 2 females.

Eggs and Egg Deposition: The mature oocytes of *Coelioxys coturnix* were previously described (Rozen and Kamel, 2007). We found that four deposited eggs had an average length of 1.1 mm compared with 1.47 mm, the average length of four oocytes in the previous study (Rozen and Kamel, 2007). The average length of four eggs of *Megachile minutissima* was 2.56 mm. Thus the length of the cleptoparasite egg is considerably less than half that of the host.

We found numerous (50+) eggs of *Coelioxys coturnix*. Most were either parallel to and on the top surface of the host eggs (fig. 4), which floated on the surface of the sticky provisions, draped somewhat diagonally across the host eggs on the provisions (fig. 6), or lying on their side, firmly attached to the chorions of the hosts while both were floating on the provisions (fig. 7). Most were affixed to the rear half of the host egg, but a few were farther forward. Many of them were so far back on the host egg that their posterior ends were in the provisions (fig. 6), although others did not contact the provisions at all. In almost all cases the anterior end of the cleptoparasitic egg pointed in the same direction as that of the host.



Figs. 4–8. Macrophotographs of live eggs and early instars of *Coelioxys coturnix* and eggs of its host, *Megachile minutissima*. **4**. Egg of *C. coturnix* on host egg. **5**. Shrouded first instar of *C. coturnix* feeding on partly depleted egg of host. **6**. Live egg of *C. coturnix* with its posterior end slightly submerged in provisions and positioned slightly diagonally on host egg; note second egg of *C. coturnix* removed from host egg and resting in provisions. **7**. Egg of *C. coturnix* attached to host egg, both resting on their sides. **8**. Live first instar of *C. coturnix* feeding on host egg with large egg of *Sapyga luteomaculata*, ready to eclose, nearby on surface of provisions.

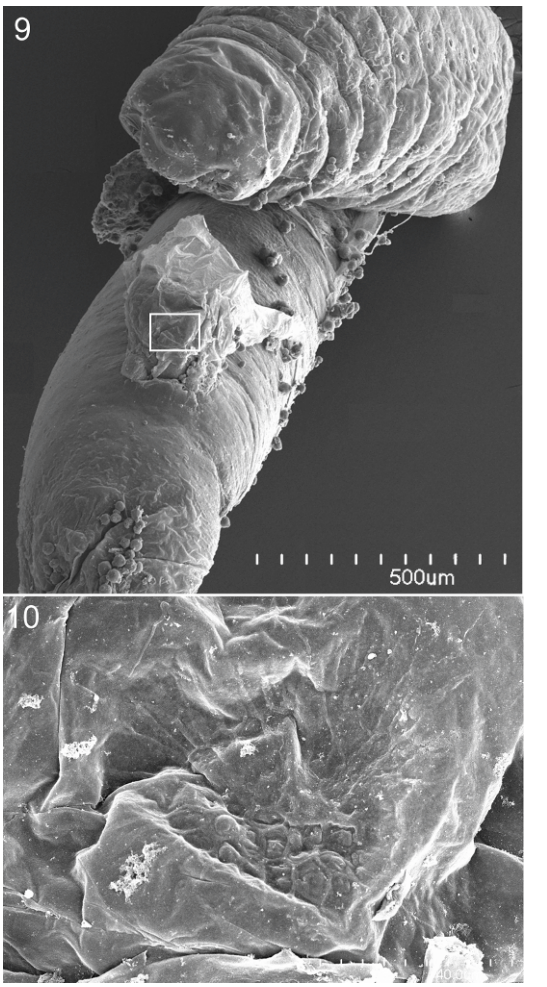
Obviously, these cleptoparasite eggs were deposited after the host female had laid her egg, probably at the time that the host female was gathering leaf snippets to close the cell. Cells containing these eggs gave no suggestion that they had been opened by the cleptoparasite after the host female had sealed them, and all cleptoparasitic eggs were deposited on freshly deposited host eggs that had no time to develop. Had they been found with older host immatures, this would imply that the cleptoparasitic female had oviposited by somehow inserting her egg into the cell after the host female had closed it.

Although most of the eggs were deposited one to a brood cell, a number of cells contained two (fig. 6) or even three eggs of *Coelioxys coturnix*. These are assumed to be depositions by different females rather than cases of double or triple oviposition by a single cleptoparasite because of the infrequency of their occurrence. We suspect that if double oviposition by females were normal as proposed for certain *Nomada* (Linsley and MacSwain, 1955), we would have encountered it more frequently. In many of these cases one of the parasitic eggs seemed to have been removed from the host egg, perhaps suggesting that the second cleptoparasite female to arrive had dislocated the earlier egg. In the few cases in which more than one egg survived for a time, we observed no cases in which both eggs survived longer than beyond the second instar. Unfortunately, we were unable to gather data as to whether a cleptoparasitic egg could survive without first feeding on the host egg.

As reported elsewhere (Rozen and Kamel, in press) eggs or young larvae of *Sapyga luteomaculata* were occasionally encountered in cells also occupied by immatures of *Coelioxys coturnix* (fig. 8), indicating that these two cleptoparasitic species are in competition for the same host (although *S. luteomaculata* also attacks *Osmia submicans*, which is not attacked by *C. coturnix*). Because the first instar of *S. luteomaculata* is highly active and substantially larger than the first instar of *Coelioxys coturnix*, one might assume *S. luteomaculata* would destroy the competitor. We did encounter this situation, but we also found a cell containing a mangled egg of *S. luteomaculata* and an active third instar of *C. coturnix*. We surmise that in this case the *C. coturnix* egg might have been deposited earlier than that of *S. luteomaculata*.

Duration of the egg stage of *Coelioxys coturnix* is estimated to be brief and is certainly shorter than that of the host, since all cleptoparasitic eggs on host eggs hatched before the host embryo started to absorb the surrounding amniotic fluid prior to hatching. Although we were unable to identify newly deposited eggs as such, the absence of older immatures of either cleptoparasite or host and the presence of numerous fresh eggs indicated that the nesting season had just begun. In a sample of 17 cells containing eggs of *C. coturnix* monitored in the laboratory, all hatched within three days of being collected. We identified hatching by the appearance of gas-filled tracheae, accompanied by the visible constriction between head and the rest of the body as well as by more or less visible body segmentation (figs. 5, 8).

DEVELOPMENT: The first instar is shrouded by its chorion as it starts to feed on the host egg. It remains motionless on the host for the next day or two (exact timing not determined, but no more than two days) during which time the host egg first becomes flaccid (figs. 5, 8). Some evidence suggests that shedding of the chorion and first instar exuviae occurs while the host egg is only partly ingested (figs. 9, 10). The host egg gradually flattens with a finely wrinkled chorion and then becomes concave, after which it flattens and floats as a dull film on the provisions. After depleting the host egg, the second instar starts feeding on the provisions.



Figs. 9, 10. SEM micrographs of second instar (identified by its mandible, as in fig. 20) of *Coelioxys coturnix*. **9**. The somewhat flaccid egg of *Megachile minutissima* to which is attached the chorion and presumably first instar skin of *C. coturnix*. **10**. Close-up of micropyle (identified by rectangle in fig. 9) matched with micropyle of mature oocyte (Rozen and Kamel, 2007: fig. 33).

During this time the cleptoparasitic larva gradually swells due to the ingestion of the host yolk and subsequently the provisions. As the larva feeds, the opaque, yellowish content of its digestive tract indicates ingestion of pollen, and the larva continues to increase in size. While still a second and third instar, it reacts to teasing with a probe by rearing its head from under the surface of the provisions and opening and closing its mandibles.

This species has five larval instars. Defecation commences at the beginning of the last larval stadium or shortly thereafter while considerable food remains to be eaten. As mentioned above, cast larval skins of previous instars presumably normally accumulate under the posterior end of the feeding instar. In one case we collected a fourth instar shedding to a fifth instar and all three previous skins were sequentially flattened onto its venter. Such a finding facilitates determination of instar numbers.

DICUSSION AND CONCLUSIONS BASED ON HOSPICIDAL BEHAVIOR

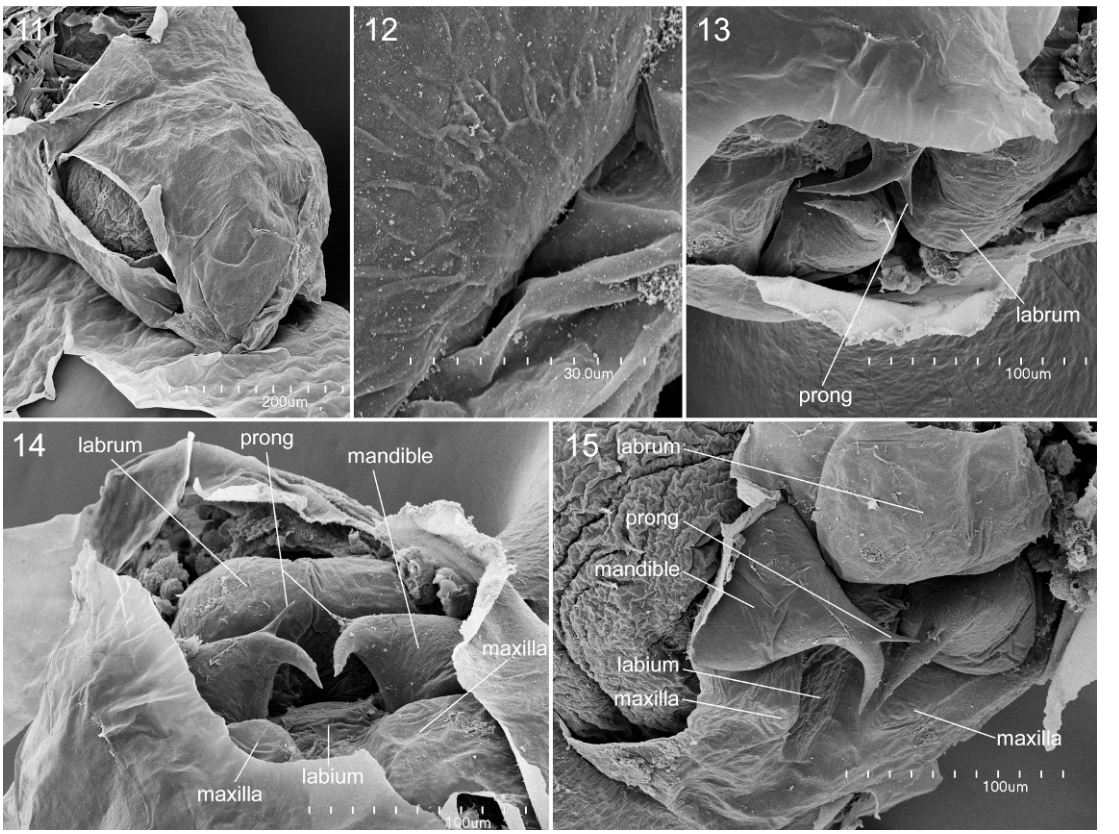
Clearly the eggs of *Coelioxys coturnix* are deposited as has been described by Ferton (1896) for the related *C. afra*, although the variation of positions of the cleptoparasite egg relative to the host egg was greater than encompassed by his account based on only two sightings. Identical in the accounts of both species is the fact that parasite eggs are deposited after those of the hosts, presumably when the host female is gathering material to close the cell. In addition, his description of the hatched larva of *C. afra* feeding on the host egg, slowly reducing it to an empty chorion, is essentially identical to our observations. In both cases, after the host egg is eliminated, the cleptoparasitic larva commences feeding on the provisions.

Thus, these two species are nearly identical in their hospicidal behavior, which is also shared with *Radoszkowskiana rufiventris* (Rozen and Kamel, 2007). This behavior was possibly derived from a common cleptoparasitic ancestor of *Coelioxys* and *Radoszkowskiana*, and, therefore, supports the hypothesis that there was a single origin of cleptoparasitism in the Megachilini. However, unexplained is the study by Carré and Py (1981) on *C. rufocaudata* in which they identified the third instar as the principle hospicidal form and stated the cleptoparasitic egg is inserted in the cell that was still being provisioned. This species is presumably a close relative of *C. coturnix* and *C. afra*, and we would have expected a close agreement in their behavior. We also note that their larvae are illustrated as having hypostomal tubercles (= "pleurostomal thickenings"), which are lacking in *C. coturnix*.

While the egg-deposition and host-killing behavior of this species of *Coelioxys* and *Radoszkowskiana rufiventris* appear identical, there is an anatomical difference between the first instar of *C. coturnix*, as detailed in the "Descriptions of Larval Instars," below, and that of *R. rufiventris* (Rozen and Kamel, 2007). Although the mandibles of both species are short and strongly curved apically, the apices of those of *C. coturnix* bend caudally whereas those of *R. rufiventris* questionably curve orally. More significantly, both species exhibit a secondary mandibular projection that in both cases we tentatively homologize with the dorsal mandibular tooth of the typical, bidentate, larval megachilid mandible. This tooth, however, is structurally very different in these two species: that of *C. coturnix* is long, tapering, apically simple, and strongly diverging from the curved mandibular apex (figs. 13–15) and that of *R. rufiventris* is short, inconspicuous, and apically bears a cluster of long (relative to the tooth) fixed spines (Rozen and Kamel, 2007: figs. 20, 21). It is difficult to postulate a scenario whereby these very different structures could have a common functional origin. Unfortunately the mandibles are tiny structures on very small larvae that are hidden beneath their egg chorions, so that in vivo observations are impossible. Perhaps studies of related species will illuminate the anatomy and function of these structures.

If one accepts the hypothesis that *Coelioxys* and *Radoszkowskiana* had a common cleptoparasitic origin, one then has to explain the origin of the second mode of parasitism within the cleptoparasitic clade, that is, what evolutionary steps could account for a shift from a situation in which (1) a parasitic egg is placed on a deposited host egg and the first instar of the cleptoparasite kills the host egg to one in which (2) the parasitic egg is hidden in the host cell as it is still being provisioned before the host egg is deposited and the third-instar cleptoparasite has the capability of killing the host larva?[3]

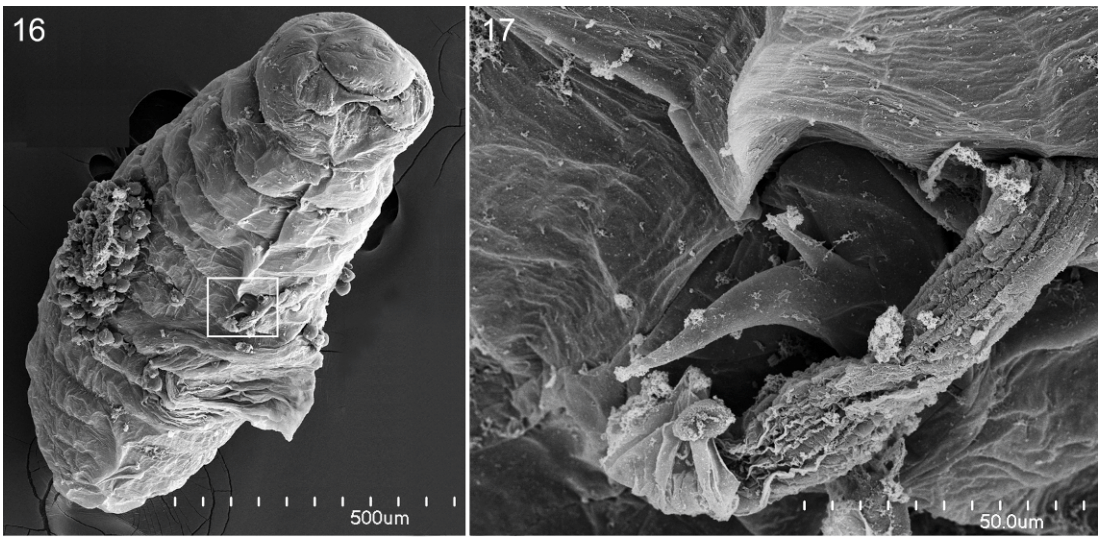
[3]This question is based on the assumption that *Coelioxys* is monophyletic. If it were discovered that *Radoszkowskiana* arose from within *Coelioxys*, then the polarity of behavioral character state might change, implying that egg deposition of the cleptoparasite on the host egg was the derived condition. This seems unlikely since it would involve a reversal of the specialized anatomical features of third and presumably second instars to the plesiomorphic condition.



Figs. 11–15. SEM micrographs of first larval instar of *Coelioxys coturnix*. **11**. Head, covered by chorion, biting egg of *Megachile minutissima*, frontolateral view. **12**. Close-up of front of head, showing micropylar sculpturing of chorion. **13**. Head of larva, now removed from host egg, showing mouthparts, near lateral view. **14**. Same, approximate ventral view. **15**. Mouthparts, with egg chorion and lateral part of parietal now removed, approximate frontal view.

At this point we lack concrete evidence as to how this might have happened, but we can speculate on the selection pressure that might account for this diversification in modes of cleptoparasitism in *Coelioxys*. First, we note that there is a very limited time period for a cleptoparasite to enter an open host cell: between when the female host has deposited her egg and when she returns to seal the cell. There is presumably an advantage if a parasitic female can oviposit in an open cell since such a cell is accessible for a longer time period than the short period between host egg laying and cell closure (although there is admittedly a disadvantage in terms of the threat of a returning host female detecting a cleptoparasitic egg). Furthermore, many potential host bees do not have to depart from the nest to gather closure material since they can close the cells with material on hand (such as soil). Such potential hosts do not provide even a narrow window of opportunity to have their completed cells visited by such cleptoparasites as *Radoszkowskiana*, *C. coturnix*, and *C. afra*.[4] It could be argued that one reason that *Coelioxys* with its more than 300

[4]These two species belong to the subgenus *Allocoelioxys*, as does *C. echinata*, the species presumably studied by Carré and Py (1981) as *C. rufocaudata*. If one assumes that *Allocoelioxys* is monophyletic, then either one can conclude that the shift from one type of egg deposition to the other took place in that clade or one becomes skeptical that *C. rufocaudata* of Carré and Py is a synonym of *C. echinata*.



Figs. 16, 17. SEM micrograph of larval instars of *Coelioxys coturnix*. **16**. Second instar showing skin of first instar attached to venter. **17**. Close-up of first-instar mandible (identified by rectangle in fig. 16).

species (Michener, 2007) is so successful is that it was able to expand its roster of potential host taxa because it evolved another mode of cleptoparasitism that permitted it to attack hosts that sealed their cells immediately after ovipositing.

There is a preexisting condition that might be regarded as a step toward ability to attack host larvae rather than host eggs, i.e., an evolutionary step toward the presumed derived mode of cleptoparasitism in *Coelioxys*: larvae of *Coelioxys* beyond the first stadium tend to be combative when teased with a probe, more so than many non-cleptoparasitic larvae. Such a combative behavior may be the result of having to compete with other cleptoparasitic individuals particularly during the early instars.[5] We can envision a hypothetical situation whereby a parasitic first instar should have killed the host egg but failed to do so for whatever reason. When it encounters the host larva later, it is preadapted to kill it. Thus, there is a shift in function of its combative ability from killing competitors to killing the host (which is, of course, also a competitor) at a later time.

[5]In support of this idea, the first instar of *Exaerete smaragdina* (Guérin-Méneville) (Apidae) has curved, sharply pointed mandibles although the cleptoparasitic female normally kills the host offspring (Garófalo and Rozen, 2001).

DESCRIPTIONS OF LARVAL INSTARS OF *COELIOXYS COTURNIX*

In the following accounts comparisons with *Coelioxys sayi* and *C. octodentata* are based on the descriptions and illustrations of Baker (1971) and with *C. decipiens* on the paper by Rozen and Kamel (2006).

First Instar

Figures 11–17

Although many of the details of the anatomy of the first instar are unknown because of its small size and chorion-shrouded body, it can be immediately distinguished from subsequent instars by its mandible. This structure is strongly curved, fanglike, and subapically bears a long, slender, sharply pointed, dorsal, pronglike process, which is more or less a continuation of the long axis of the base of the mandible (figs. 14, 15). Beyond the branching of the prong, the mandibular apex curves strongly, so that its extreme apex is at a right angle to the long axis of the mandibular base and, when both mandibles are closed, their extreme apices are almost parallel (figs. 13, 15). The prong is presumably the modified dorsal tooth of the typical megachilid bidentate mandible. Its function

is unknown: It may stabilize or make taut the chorion (either that covering the first instar, that of the host, or both) while the curved point of the opposing mandible tries to penetrate the chorion. Alternatively, its sharp apex and the fact that its apex extends beyond the curved mandibular apex suggest that it might be a tearing devise to perforate chorion.

The conspicuous apical labral tubercles of subsequent instars (figs. 20, 22, 15) are not in evidence on the first instar (figs. 14–16). Cast skins do not reveal any pigmentation to the cuticle, and their spiracles were too small to be detected. Parietals of first-instar skins collapse and internal head ridges cannot be detected with a compound microscope, all suggesting weak sclerotization, whereas parietals of subsequent instars are distinctly sclerotized and do not collapse after shedding, and internal head ridges are quite evident. For that reason (and the fact that they are small) first instar skins can be easily overlooked whereas head capsules of subsequent instars flag the presence of the body exuviae that adhere to the preserved instar. In contrast to the rest of the head, mandibular apices including the dorsal prong are more heavily sclerotized on cast skins of first instars and consequently can often be identified with a compound microscope (figs. 16, 17). Maxillae of first instars are represented by low mounds posterior and mesad of the mandibular bases and lack obvious palpi or sensilla, as revealed by SEM (figs. 14, 15). The labium (figs. 14, 15) is a shallow, median, longitudinal trough separating the maxillae and also lacks obvious palpi and sensilla.

The dorsal mandibular prong is possibly homologous with the ''dorsal spine'' identified by Baker (1971) on the mandibles of *Coelioxys sayi* and *C. octodentata*, which are depicted as very short. These structures are tentatively considered homologous to the dorsal mandibular tooth found on subsequent instars of these taxa as well as on *Radoszkowskiana rufiventris*. Carré and Py (1981) also refer to a ''épine dorsale'' on the mandible of *C. echinata*, but it is impossible to interpret from their diagram the length of this structure.

Material Examined: One first instar feeding on host egg: Egypt: Ismailia, Suez Canal University, IV-30-2007 (J.G. Rozen); same except two cast skins, IV-29-2007; same except one cast skin, IV-27-2007.

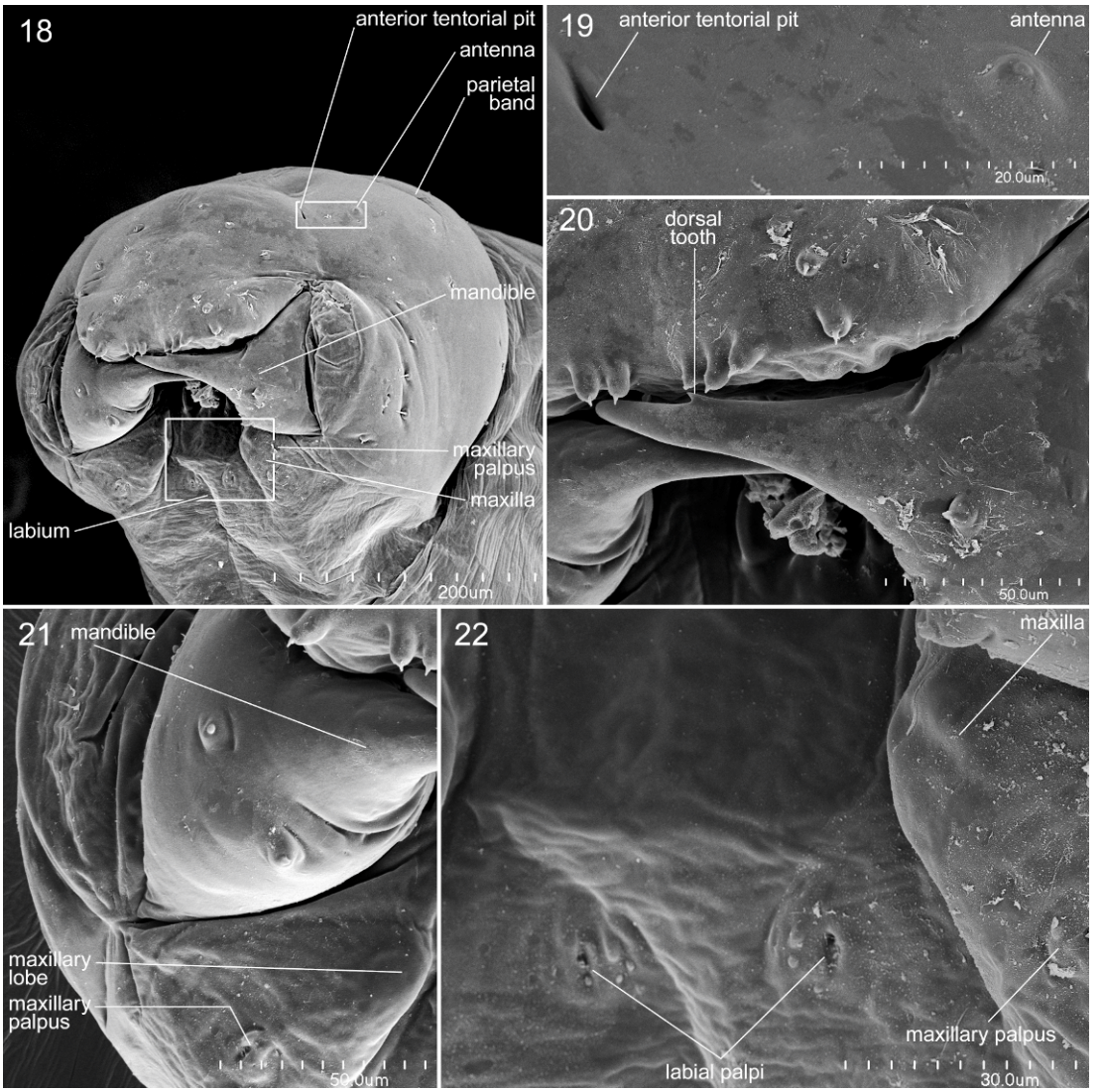
Instars Two to Four

Figures 18–27

Head: The integument of the parietals of the second instar of *Coelioxys coturnix* is distinctly sclerotized but only moderately pigmented, and the mandibles are perhaps slightly more pigmented than the parietals. The maxillary and labial sclerites are unpigmented and presumably nonsclerotized. The head capsule, labrum, and mandibles exhibit sensilla, some of which are setiform and sometimes arise from small tubercles. Spicules are entirely absent from the head.

The head of the second instar is more or less hypognathous (fig. 18) as is the case for all subsequent instars and almost certainly for the first instar as well. The parietals of all instars are not enlarged, and their posterior boundaries are only slightly constricted, so that the width of each foramen magnum is only a little smaller than the maximum head width. Paired hypostomal tubercles as found in other known *Coelioxys* larvae (Baker, 1971; Rozen and Kamel, 2006) are totally absent. Sclerotization of the parietals ends ventrally at the hypostomal ridges, and thus does not invade the labiomaxillary area; sclerotization posteriorly ends at the postoccipital ridge. The tentorium is complete and thin in the second instar and continues to be present in subsequent instars; by the fourth instar it has become moderately robust. The anterior and posterior tentorial pits are in the normal position and are moderate in size in all instars.

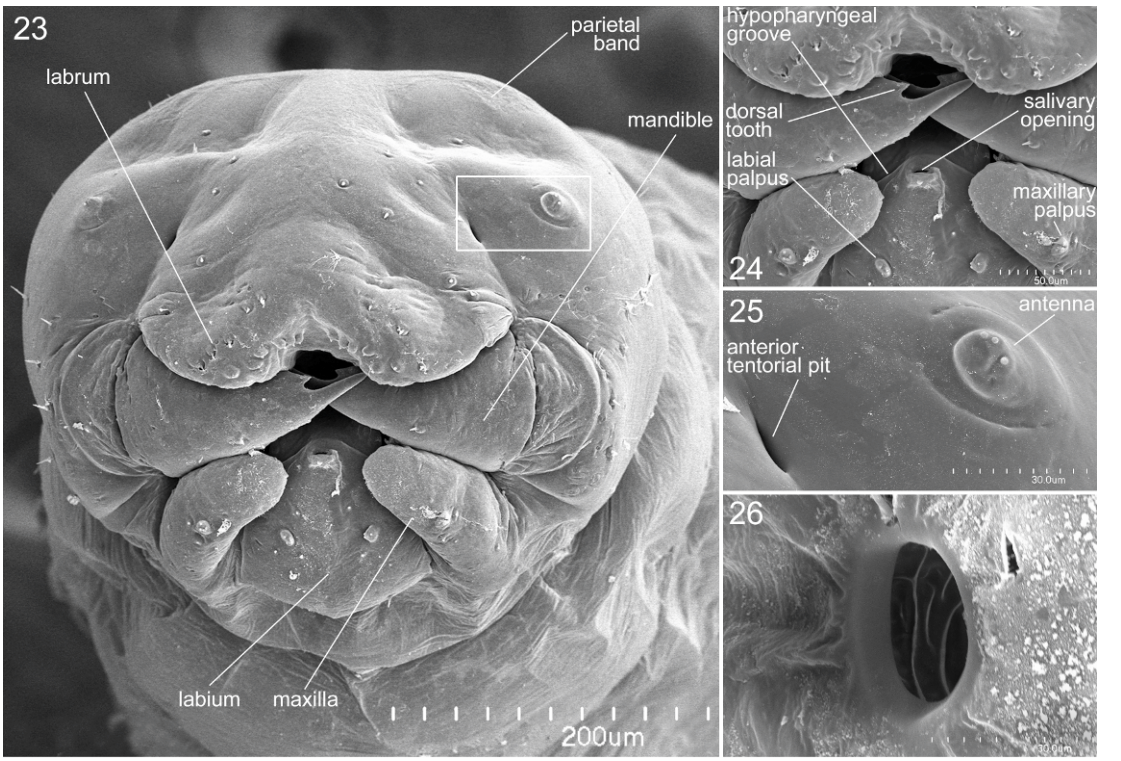
The postoccipital ridge is pronounced but narrow, whereas the hypostomal ridge is strongly developed and wide in all instars. Similarly the pleurostomal ridge is moderately developed, as is the epistomal ridge below (laterad of) the anterior tentorial pits but is absent between the anterior pits. Parietal bands seemed absent in the first instar, but they appeared on SEM micrographs of the instars 2–4, as suggested by a depression on each parietal (figs. 18, 23, 27). The antennal projection (fig. 19) is weak (far less than one-half its presumed basal diameter) in the second instar and lacks a distinct papilla and basal



Figs. 18–22. SEM micrographs of second instar of *Coelioxys coturnix*. **18**. Head, frontolateral view; upper rectangle refers to fig. 19; lower rectangle refers to fig. 22. **19**. Close-up of left antenna and anterior tentorial pit. **20**. Close-up of mandible, showing small subapical dorsal tooth, and labral apex with apical row of pronounced, sensilla-bearing tubercles. **21**. Base of right mandible, showing small tubercles on outer surface, and right maxilla. **22**. Close-up of labiomaxillary region, showing labial palpi and left maxilla and palpus.

ring. In subsequent instars the antennal papilla gradually increases (figs. 25, 29) in size and the basal ring becomes evident. By the fourth instar (fig. 28) it projects slightly less than its basal diameter. In larval instars 2–5 (figs. 19, 25, 29) (unknown for first instar), each antenna possesses 2–3 nonsetiform sensilla.

The labrum of the second instar is broad, apically subtruncate when seen in front (fig. 18), but when viewed adorally (i.e., from somewhat below), one can see its median emargination that becomes more apparent in subsequent instars (figs. 23, 27). Apically the labrum of the second and third instars bears short setiform sensilla borne on small tubercles and an apical transverse row of conspicuous seta-bearing tubercles (fig. 20). On the second instar, this row tends to be on the



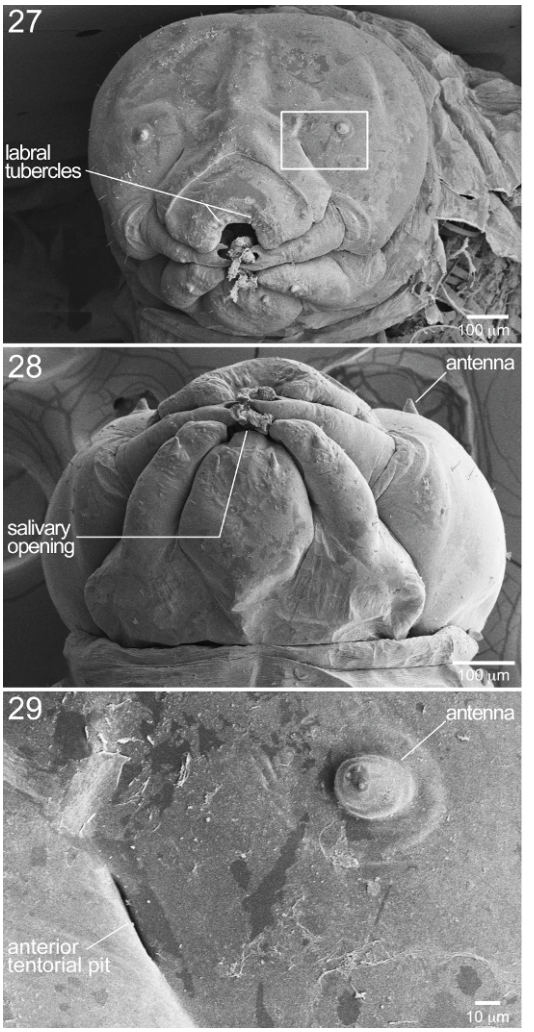
Figs. 23–26. SEM micrographs of third larval instar of *Coelioxys coturnix*. **23**. Head, mostly frontal view. **24**. Close-up of mouthparts, showing dorsal mandibular tooth larger than on previous instar and showing larger palpi than on previous instar. **25**. Close-up of left antenna and anterior tentorial pit (as identified by rectangle, fig. 23), showing two sensilla. **26**. Spiracle, abdominal segment 3, right side.

leading edge of the labrum (fig. 20), but in the third instar this row points downward (fig. 24), and by the fourth instar these tubercles are smaller and far less pronounced relative to the head size (fig. 27). Paired labral tubercles are absent in all instars. The boundary between the labrum and lower end of clypeus is scarcely defined in the second instar but becomes a transverse integumental fold by the fourth instar (fig. 25).

Although the mandible of the second instar of *Coelioxys coturnix* continues to be curved, sharply pointed, and fanglike, as in the first instar, the curvature of the apex is less pronounced and the dorsal tooth is so small that it is not visible with a compound stereomicroscope. It is, however, clearly visible on an SEM micrograph (fig. 20) as a sharply pointed, subapical tooth. The mandible (figs. 20, 21) is also heavily sclerotized, moderately pigmented, and basally stout. It has two, small, sensilla-bearing tubercles on the outer aspect and another on its ventral surface. Its inner surface has a cuspal area that is swollen and somewhat irregular but lacks teeth or projections.

The mandible (figs. 23, 24) of the third instar is perhaps more heavily sclerotized than that of the second and is moderately pigmented. Its apex is curved and ends in a sharp, slender point. The subapical dorsal tooth is now clearly visible (fig. 24) with a stereomicroscope, and the curved long axis of this tooth is parallel to that of the rest of the mandible as seen from above. The inner mandibular surface bears a shallow apical concavity; the outer surface has several setae arising from small tubercles (fig. 24).

In the fourth instar the apex of mandible is far more darkly pigmented than any other feature on the head capsule, which is otherwise scarcely pigmented. The mandible (fig. 25) is



Figs. 27–29. SEM micrographs of fourth larval instar of *Coelioxys coturnix*. **27**. Head, frontal view, showing enlarged dorsal mandibular teeth. **28**. Same, ventral view, showing longer antennal papillae and palpi compared with earlier instars. **29**. Close-up of left antenna with three sensilla and anterior tentorial pit (identified by rectangle in fig. 27).

now robust, has two apical teeth, the ventral one being finely pointed and still somewhat longer than the dorsal tooth. The apical concavity is well developed with the dorsal apical edge projecting and seemingly irregularly jagged, as in the fifth instar. The mandible bears several tubercles on its outer aspect.

The labiomaxillary region (fig. 23) of the second instar is recessed, projecting a little downward and not projecting forward beyond the base of the partly opened mandibles in ventral view. It forms a continuous, greatly fused, seemingly membranous, and nearly featureless surface except for a circular salivary opening and two, slightly projecting maxillary lobes. The maxillary and labial sclerites are not defined. Under SEM examination, the maxillary and labial palpi are nonpapillate, each consisting of an indistinctly defined cluster of nonsetiform sensilla (figs. 20, 21).

In the third instar, the labiomaxillary region is now less recessed, and the maxillae and labium are more distinctly separated. The apices of maxillae project well beyond the mandibular base, much ahead of the labium. The prementum and postmentum are differentiated (fig. 23). A transverse groove behind the opening suggests that the articulating arms of the stipites might be present, thus forming the hypopharyngeal groove. The cardo and stipes are sclerotized but not pigmented (best observed on a cast skin). The cardo is not fused with the parietal as in *Coelioxys decipiens*. The premental sclerite is visible but unpigmented, and the maxillary and labial palpi are papillate, each about as long as its basal diameter (fig. 24).

In the fourth instar (figs. 27–29), the labiomaxillary region has become even larger and less recessed, the labial and maxillary palpi are longer than their basal diameters, and the hypopharynx, which could not be identified earlier, is now defined as the area behind the now-visible articulating arms of the stipites.

The salivary opening of the second instar is a small, circular hole and the salivary duct is evident on a cleared specimen. In the third instar the salivary opening is slightly projecting and slightly transverse (fig. 24). In the fourth stage, the opening has now become a transverse slit on the apex of the somewhat projecting labium.

BODY: The integument of all instars is without conspicuous setae but with fine spicules, but the patterning of spiculate patches is unknown. The body form of the second instar is linear (figs. 9, 16), but later it and that of subsequent instars becomes more physogastric. The intersegmental lines tend to be moderately deeply incised, and abdominal segment 10 is rounded posteriorly. The anus is

apical. On instars 2–5, spiracles are present and large, with the two thoracic pairs and the last abdominal pair smaller than the first seven abdominal pairs. The spiracles of instars 2–4 are funnellike and apparently flush with the body wall (fig. 26). They appear to lack peritremes (but if present the peritremes are very narrow). The atrial wall is indistinctly, concentrically, and somewhat irregularly ridged. The subatrium is narrow and without chambers in these instars.

MATERIAL EXAMINED: One second instar: Egypt: Ismailia, Suez Canal University, IV-27-2007 (J.G. Rozen); two second instars, same except IV-29-2009. One third instar: same locality, V-03-2007 (J.G. Rozen); one cast skin, same except IV-29-2007; one cast skin, same except V-10-2007. One fourth instar: same locality, IV-28-2007 (J.G. Rozen); one fourth instar molting to fifth, same except IV-28-2007; one cast skin of fourth instar, same except IV-30-2007.

REMARKS: There are no features of the second and third instars of this species that suggest the specialized features of these instars in *Coelioxys octodentata*, *C. sayi*, or *C. decipiens*. In *C. coturnix*, the head is hypognathous, the mandibles bear two apical teeth and are not elongate, the labiomaxillary sclerites are not fused either with one another or with the parietals, and the sclerotization of the parietal does not extend posteriorly beyond the postoccipital ridge. All features seem to be the normal ontogenetic sequence of a developing non-cleptoparasitic megachilid; none suggest an evolutionary reversal from hospicidal second- and third-instar anatomies.

ACTIVE FIFTH INSTAR

The postdefecating larva was described, illustrated, and compared with that of *Coelioxys decipiens* Spinola by Rozen and Kamel (2007). The larval form of the fifth instar before it spins a cocoon and enters diapause[6] was not available at that time and so is treated here.

[6]In bees in which defecation occurs after the fifth instar has completed feeding, this form is called the "predefecating" form of the fifth instar. Since this species commences defecation just as it becomes a fifth instar and while it is still feeding, the term "predefecating" is not appropriate.

The fifth instar can easily be distinguished from the fourth in that the salivary lips are thin and projecting. Furthermore, although head setae are long on preceding instars, only the last larval instar has body setae that are long and conspicuous. The sudden appearance of body setae in the last instar alone has apparently not been reported before for bee larvae, and we wonder whether it may not be a universal feature of the Megachilidae.

The active fifth instar differs from the postdefecating form in that its head capsule and mouthparts are less darkly pigmented. The dark median spot found on the labrum of the postdefecating form is absent, as was also the case for the early fifth instar of *C. decipiens* (Rozen and Kamel, 2007). Thus a feature that may be characteristic of other members of the genus will hold only for postdefecating larvae. In the case of *C. coturnix* this is unfortunate since it does not exhibit hypostomal tubercles, a reliable generic feature of many *Coelioxys*. Nonetheless, head integument of *C. coturnix* before diapause is more darkly pigmented than that of the host in which only the mandibular apices are pigmented. With *C. coturnix*, not only the tips of the mandibles are dark, but the entire structure is tinted, as are the internal head ridges, the labral sclerites, the premental sclerite, and the maxillary sclerites.

MATERIAL EXAMINED: Three active fifth instars, Egypt: Ismailia, Suez Canal University, V-09-2007 (J.G. Rozen).

CONCLUSIONS

When all larval instars of *Coelioxys coturnix* are considered together, anatomical transformation from one instar to the next is incremental. The single exception is the elongate dorsal mandibular tooth in the first instar followed by a minute dorsal tooth in the second that gradually grows and shift to an apical position in the following three instars.

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