

## Biological and Taxonomic Studies of *Chartocerus subaeneus* (Hymenoptera: Signiphoridae), a Hyperparasite of Mealybugs

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**Abstract.**— *Chartocerus subaeneus* (Foerster), an obligatory direct hyperparasite of mealybugs, is redescribed and its developmental stages are described and illustrated. A lectotype is designated. This deuterotokous species develops ectoparasitically on fully developed larvae and pupae of various primary encyrtid parasites in mummified mealybugs. When reared on *Tetracnemoidea peregrina* (Compere) in the long-tailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti) at 28°C, females lived for 25 days and oviposited throughout their lifetime. Fecundity was high, averaging 163 eggs per female or 6.8 eggs per day. Adult females host-fed regularly, and this prolonged their life span considerably. They were susceptible to low relative humidity. Development from egg to adult emergence took 16.4 days. The developmental threshold was 14.8°C, and the thermal constant was 221.5 days-degrees.

The biology of hyperparasitic Hymenoptera has been relatively little studied (see Rosen 1981, Sullivan 1987 and Viggiani 1990 for recent reviews). Of the Signiphoridae known as hyperparasites, only two species have been studied in any detail: *Chartocerus elongatus* (Girault) by Clausen (1924) and *Signiphora coquilletti* Ashmead by Woolley and Vet (1981). Somewhat more information is available on the biology of Signiphoridae acting as primary parasites, all of which are species of *Signiphora* that attack armored scale insects. Quezada et al. (1973) provide the most complete biological information for any signiphorid in their study of *Signiphora borinquensis* Quezada, DeBach and Rosen, DeBach et al. (1958) and Agekyan (1968) provide details for *Signiphora merceti* Malenotti, and Woolley (1988, 1990) reviews the remaining available information.

A little-known hyperparasitic signiphorid, *Chartocerus subaeneus* (Foerster), was found to overwhelm mass cultures of *Tetracnemoidea peregrina* (Compere) (Hymenoptera: Encyrtidae) during a highly successful campaign for biological control of the long-tailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti) (Homoptera: Pseudococcidae), which was the cornerstone of an effective integrated pest management program on avocado in Israel (Swirski et al. 1980, Swirski and Wysoki 1988). In field samples, on the other hand, this hyperparasite was quite rare. A study of its taxonomy and biology was undertaken in order to

contribute to our knowledge of hyperparasites and signiphorids.

### MATERIALS AND METHODS

*Chartocerus subaeneus* was originally obtained from a laboratory mass culture at the Volcani Center, Bet Dagan, Israel. Cultures of the hyperparasite were maintained at 28±1°C and >50% RH, in ventilated plastic cages, 15 x 25 x 35 cm or so, with *Tetracnemoidea peregrina* serving as primary host and the long-tailed mealybug, *Pseudococcus longispinus*, infesting sprouting potatoes, as secondary host. Both the primary and secondary (mealybug) hosts were reared at the Volcani Center on sprouting potatoes, at 26±2°C and >65%.

Material for description of adults and developmental stages was obtained from the laboratory cultures. In biological experiments, parasitized mealybug mummies were glued to small cards (2.5 x 3 cm) with neutral glue (Sachinata Mucilage Glue, Japan) and presented to newly-emerged wasps in small plastic cups. Unless stated otherwise, all experiments were held at 28±1°C and >50% RH, and the wasps were provided with honey streaks for food.

### TAXONOMY

The genera of Signiphoridae have recently been reviewed by Woolley (1988), who provides a key to

genera and species groups, diagnostic characteristics, discussions of various anatomical characters, and hypotheses for phylogenetic relationships. Rozanov (1965) treated *Chartocerus* as containing three subgenera, and referred *C. subaeneus* to the subgenus *Signiphorina*. Woolley (1988) provided evidence for monophyly of *Chartocerus* and the nominate subgenus, but was unable to find evidence for monophyly of the other two subgenera. *Chartocerus* is in need of revision on a worldwide basis, and consequently most species are difficult to identify. *Chartocerus subaeneus* is most closely related to *elongatus* (Girault), *novitzkyi* Domenichini, *fimbriae* Hayat, and *intermedius* Hayat. Novitzky (1954) redescribed and figured *subaeneus*, Domenichini (1955) compared *subaeneus* with *elongatus* and *novitzkyi*, and Hayat (1970, 1976) compared *subaeneus* with *fimbriae* and *intermedius*. We have determined the identity of our material based on examination of Foerster's types, the literature, and comparison with specimens determined by Sugonyaev and Ferrière. In addition to Israel, *Chartocerus subaeneus* is reported from Western Europe, the European part of the U.S.S.R., Turkey and Soviet Central Asia.

In view of the inadequate state of the systematics of this group, a detailed redescription is presented for future reference.

#### ADULT MORPHOLOGY

Terminology for anatomical structures follows Woolley (1988). In particular, mesosoma refers to thorax plus propodeum, metasoma refers to the abdomen posterior to the propodeum, and numbering of terga and sterna (e.g., T2, S2) refers to metasomal terga and sterna. The apparent ninth tergum in females is called the epiproct for reasons discussed by Woolley (1988).

#### *Chartocerus subaeneus* (Foerster)

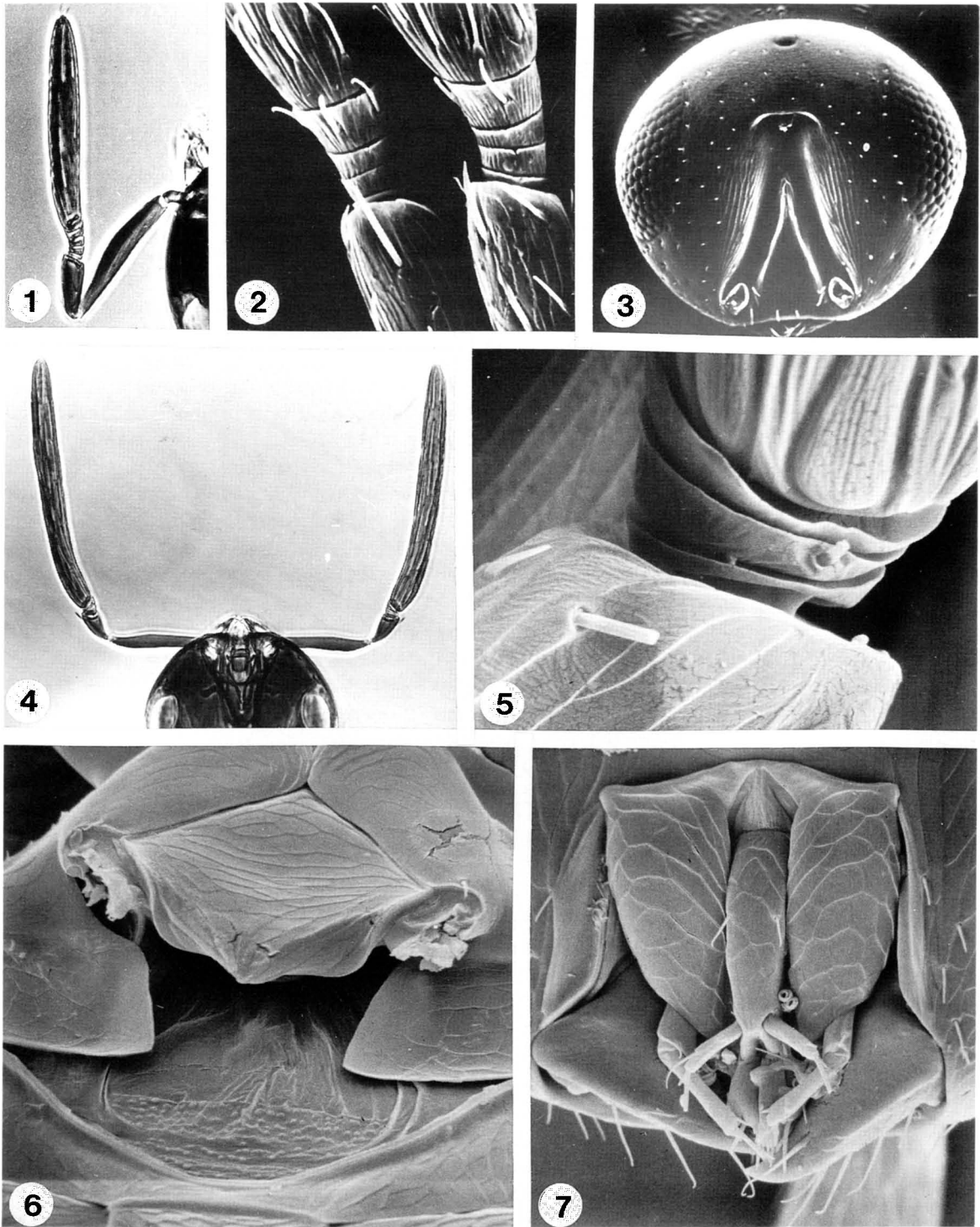
- Plastocharis subaenea* Foerster 1878, Verh. Nat. Ver. Preuss. Rheinl. 35: 69.  
*Thysanus subaenea*: Dalla Torre 1898, Catalogus Hymenopterorum, Lipsiae 5: 223.  
*Signiphorina mala* Nikol'skaya 1950, Dokl. Akad. Nauk SSSR 75: 19-21.  
*Signiphorina subaenea*: Novitzky 1954, Ann. Fac. Agr. (N.S.) 2: 245-255.  
*Signiphora* (*Signiphorina*) *subaenea*: Peck et al. 1964, Mem. Entomol. Soc. Can. 34: 90-91.  
*Chartocerus* (*Signiphorina*) *subaeneus*: Rosanov 1965, Entomol. Obozr. 44: 878.

*Diagnosis*.— Inasmuch as *Chartocerus* is in need of comprehensive revision, it is no doubt premature to attempt to distinguish *subaeneus* from all species with which it might be confused. It appears to be most similar to *C. elongatus*, *C. novitzkyi*, *C. fimbriae* and *C. intermedius*. In *subaeneus*, the medial denticles of the male genitalia are robust, curved and long, extending approximately half the length of the digiti, and they are inserted on a strongly sclerotized region between the bases of the digiti (Fig. 26). The medial denticles in *elongatus* are less robust, straight, and shorter (about 1/3 the length of a digitus), and they are not inserted on a sclerotized region (cf. Fig. 4.8, Domenichini 1955). In *novitzkyi*, the medial denticles are long and straight, almost the length of a digitus, and a sclerotized region is not apparent at their bases (cf. Fig. 4.9, Domenichini 1955). According to Hayat (1970, 1976), *fimbriae* and *intermedius* can be distinguished from *subaeneus* by the longer marginal fringe on both fore and hind wings, and the yellow middle tibiae of all legs (middle and hind tibiae are black in proximal half in *subaeneus*).

*Female*.— General coloration shining black; antennae dark brown; eyes and ocelli black; all tarsi yellowish, fore tibiae pale, middle and hind tibiae black in proximal half and pale distally, other segments of all legs black. Fore wing with alternating, broad hyaline and dark bands (Fig. 13). Hind wing hyaline.

Length 0.95-1.15 mm. Head (Figs. 3, 8) with frontovertex broad, wider than long, transversely striate, bearing sparse short setae. Cheek length not exceeding 2/3 length of eye. Face longitudinally striate, bearing sparse short setae. Eyes with sparse, short inter-ommatidial setae. Ocelli in an obtuse triangle; the posterior pair at about their own diameter from inner orbits. Antennal toruli (Fig. 3) about their own diameter from oral margin; scrobes convergent, the area between them not elevated.

Mandibles bearing long setae, the two denticles subequal in length, the dorsal one somewhat truncate. Maxillary palpi (Fig. 7) 2-segmented, apical segment the longest, bearing an apical spine; labial palpi 1-segmented, bearing an apical spine and a subapical seta. Antenna (Figs. 1, 2) 7-segmented (1141), all but the first 3 funicular segments bearing short setae. Radicle bearing a few sensory setae at base. Scape 4.8 - 6.5 times as long as wide. Pedicel 2.0 - 2.75 times as long as wide, usually 1.5 - 2.0 times as long as funicle. First funicular seg-



Figs. 1-7. *Chartocerus subaeneus*. 1, Female antenna. 2, Female antennae: pedicel, funicle and base of club (SEM). 3, Head, frontal view; antennae removed to show toruli and scrobes (SEM). 4, Male head and antennae. 5, Male antennal funicle (SEM). 6, Female prosternum, ventral pronotum, prepectus, and mesepisternum in ventral view; fore coxae removed (SEM). 7, Female mouthparts, ventral aspect, showing maxillae and labium (SEM).

ment minute, 6-7 times as wide as long; second and third segments subequal in length, twice as long as the first, 2-4 times as wide as long; fourth segment about 1/3 as long as the third, 1.7 - 3.0 times as wide as long. Club 5.0 - 9.3 times as long as wide, 1.5 - 1.6 times as long as scape and about 6 times as long as funicle, bearing 4-6 longitudinal sensilla, 0.26 - 0.32 length of club, and several fingerlike sensilla at tip.

Mesosoma (Figs. 8-10) transversely imbricate, bearing sparse short setae. Pronotum usually 2/5 length of mesoscutum in dorsal view, bearing a transverse row of short setae along posterior margin. Mesoscutum 2.3 - 3.0 times as long as scutellum, bearing 6-19 setae, those in postero-lateral corners larger. Scutellum bearing a row of 7-12 short setae along posterior margin, with a pair of submedian discoid sensilla. A pair of lateral internal costulae on scutellum, visible only in cleared slide mounts (compare Figs. 8 and 9), set off the triangular apparent axillae, each of which bears a single large seta. Metanotum with medial portion faintly striate and devoid of setae; lateral lobes smooth except for some reticulation near margins, each bearing a pair of minute setae. Propodeum (Figs. 9, 10) length 0.6 - 0.8 greatest width, 0.55 - 0.72 length of mesoscutum, 1.66 - 1.75 times as long as scutellum, 2.5 - 3.5 times as long as metanotum; smooth laterally, rather faintly reticulate mesad of spiracles, with medial sclerite triangular and strongly reticulate, apex not quite reaching posterior propodeal margin. Prepectus fused with ventral mesepisternum; the membrane between it and the prosternum, underneath the fore coxae, bearing numerous papillae (Fig. 6).

Metasoma (Figs. 9, 12) subequal in length to the head and mesosoma combined. First tergum (= second abdominal) (Figs. 9, 10) short, smooth, weakly bilobed, not overlapped by propodeum; T2-T6 reticulate, bearing 4-9 setae on each side, with a discoid sensillum anterad of each setiferous area; T2 the longest; T7 (Fig. 25) bearing a transverse row of 8 setae, with a pair of submedian discoid sensilla between the spiracles; T8 (Fig. 25) represented by a narrow transverse plate and two lateral lobes bearing the cerci, each with two long setae and one short seta; epiproct rhomboid. Sixth sternum almost at apex of metasoma and distinctly bilobed medially. Second phragma (Fig. 12) 1.53 - 1.87 times as long as wide, reaching well beyond base of ovipositor to level of fifth tergum. Ovipositor nearly twice as long as middle tibia (1.80 - 1.98); ovipositor sheaths longitudinally striate.

Tarsi of all legs pentamerous (see Fig. 12); strigil (Fig. 15) well developed; middle femur (Fig. 16) bearing 2 or 3 long spines postero-apically, one short apical spine, and a row of short setae dorsally; middle tibia bearing 2 long setae and 2 shorter setae dorsally, in addition to numerous short setae; mid-tibial spur (Figs. 16, 17) 4/5 length of corresponding basitarsus or more (0.78 - 0.93), bearing a row of 4-5 teeth.

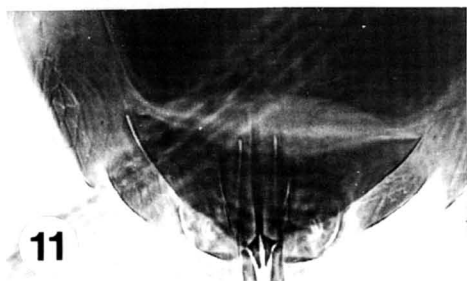
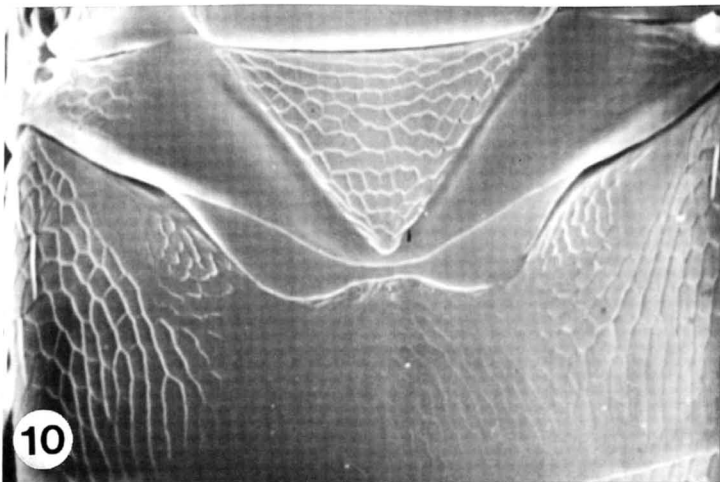
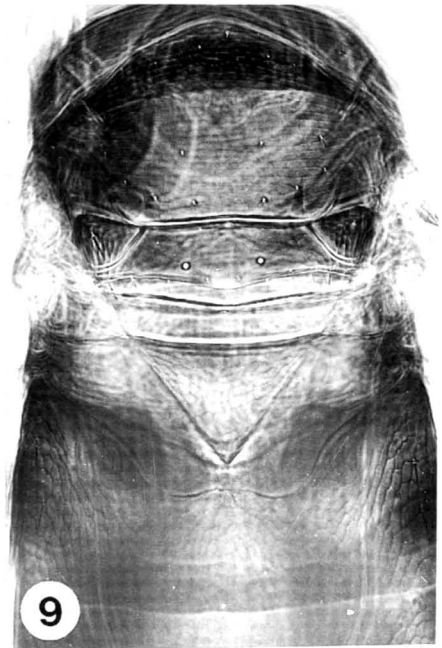
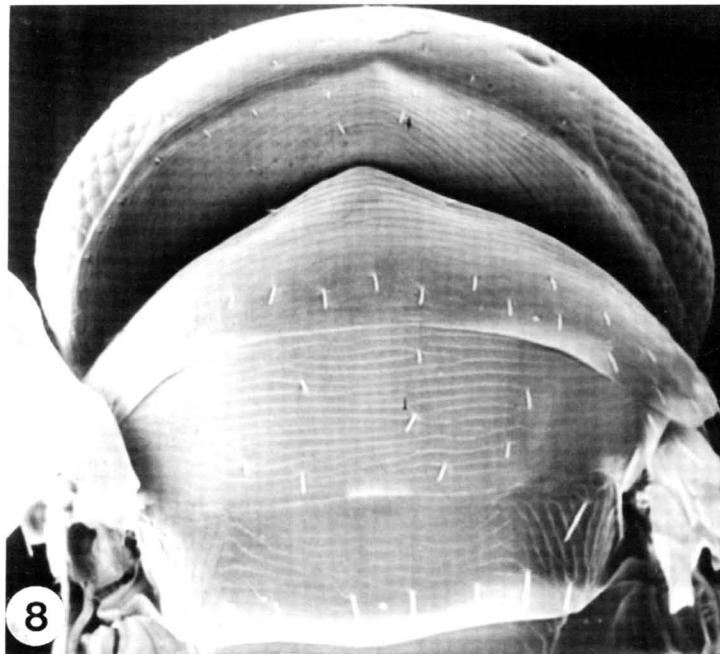
Fore wing (Fig. 13) about 3 times as long as wide (2.8 - 3.1); longest marginal cilia 1/2 - 2/3 width of disk. Submarginal vein 3/4 to nearly as long as marginal vein, bearing 2 setae and 13 bullae; marginal vein bearing 4 long setae along anterior margin and 4-5 short ventral setae; stigmal vein about 1/5 length of marginal vein, bearing a single seta and 3 discoid sensilla. Costal cell bearing a single dorsal seta; disk otherwise bare except for 5-7 minute ventral setae below junction of submarginal and marginal veins. An oblique fold near center of disk, beginning below apex of venation.

Hind wing (Fig. 14) about 5 times as long as wide (4.1 - 5.5); longest marginal cilia 3/4 width of disk or more (0.75 - 0.93); disk bare except for one short seta below apex of marginal vein. Marginal vein bearing one long proximal seta followed by 2-5 shorter setae, 2 hamuli and 3 straight spines at apex.

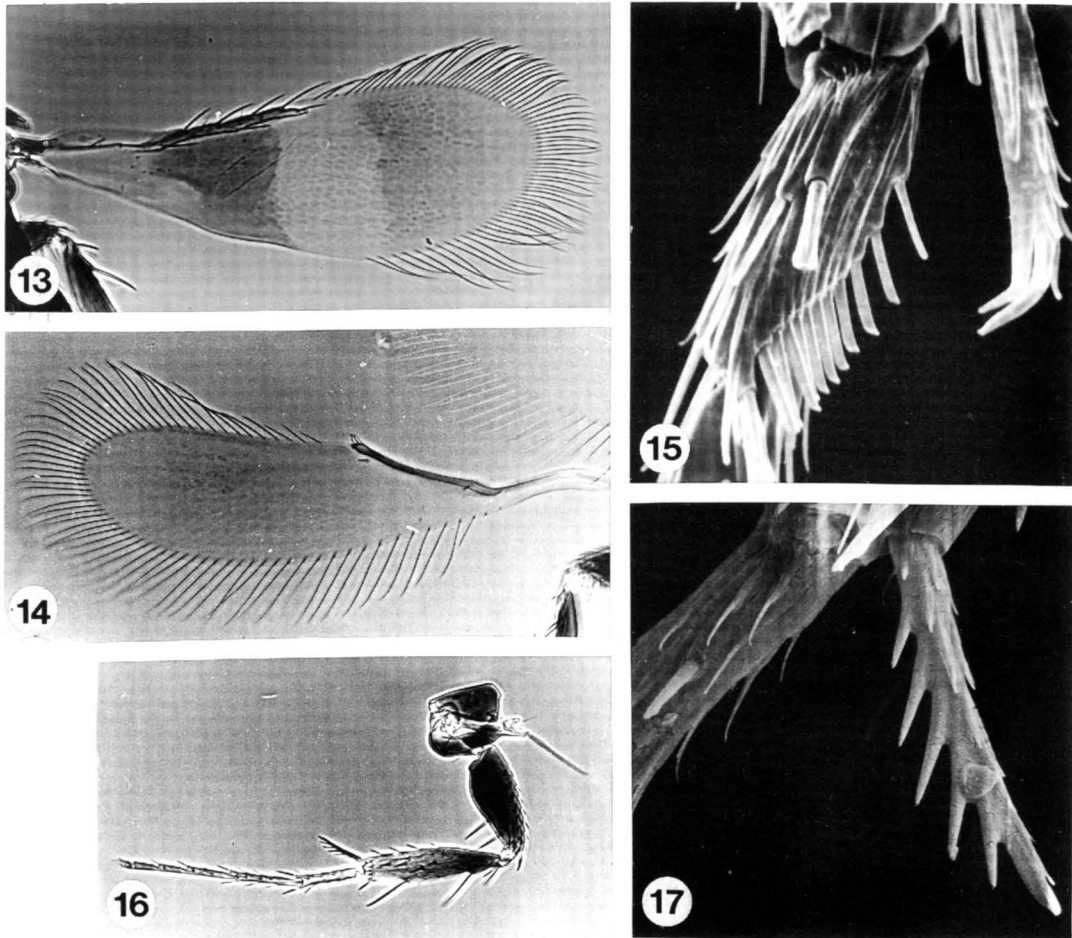
*Male*.—Similar to female in structure, chaetotaxis, sculpture and coloration, differing mainly in antennal and genital characters.

Antenna (Fig. 4) six-segmented (1131). Scape as in female; pedicel more pyriform, 2.0 - 2.5 times as long as funicle; funicular segments (Fig. 5) ring-like, subequal in width, the first 4 times as wide as long, the second 3 times as wide as long and twice as long as the first, the third twice as wide as long and twice as long as the second; club longer than in female, 11-14 times as long as wide, 15 times as long as funicle, bearing about 20 longitudinal sensilla.

Mesoscutum and scutellum each bearing 7-9 setae. Eighth sternum (Fig. 26) broadly crescent-shaped and rounded posteriorly, 3 times as wide as long at midline, about 1.2 times as wide as seventh sternum. Genitalia (Fig. 26) 1/2 - 2/3 length of middle tibia, ventral surface of phallobase with distinct longitudinal thickening at midline, running from between bases of digiti almost to apex, digitus with apical denticle about 1/3 its length, medial denticles slightly curved and 1/3 - 1/2 length of digitus (excluding apical denticle), phallobase sclerotized at base of medial denticles



Figs. 8-12. *Chartocerus subaeneus*. 8, Female head, pronotum, mesoscutum and scutellum (SEM). 9, Female mesosoma and base of metasoma. 10, Female propodeum and base of metasoma (SEM). 11, Male, apex of metasoma and genitalia, ventral aspect. 12, Female mesosoma and metasoma, showing second phragma and ovipositor.



Figs. 13-17. *Chartocerus subaeneus*, female: 13, Fore wing. 14, Hind wing. 15, Calcar and strigil on fore leg (SEM). 16, Middle leg. 17, Mid-tibial spur (SEM).

and bearing a pair of lateral setae, slightly longer than medial denticles. Our material agrees well with the Fig. of Domenichini (1955), but the medial and apical denticles are somewhat longer relative to the digiti in our specimens.

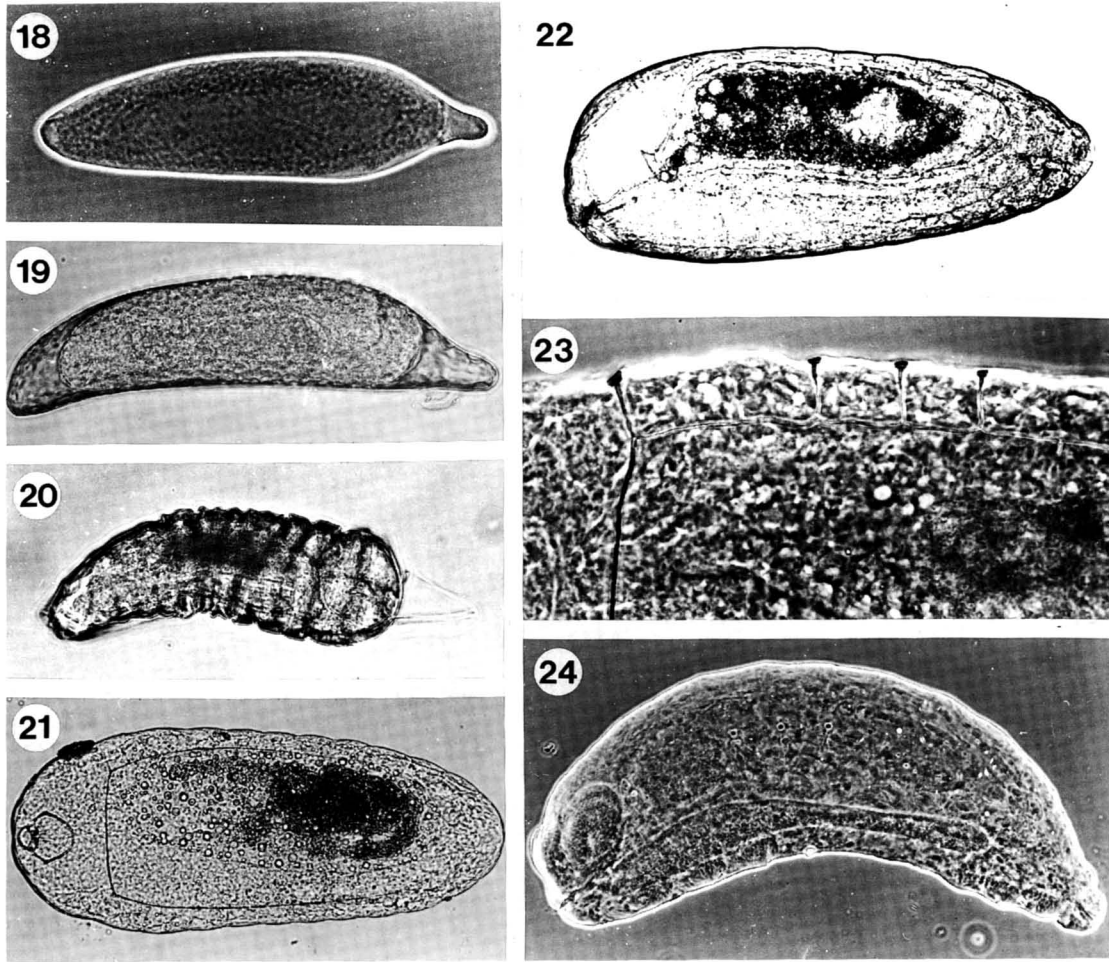
*Material examined.*— Redescribed from 20 females and 13 males, reared in laboratory culture on *Tetracnemoidea peregrina* in the long-tailed mealybug, *Pseudococcus longispinus*, on potato sprouts, Rehovot, Israel.

*Types.*— Lectotype male (present designation). "17/610, Frst, *Plastocharis subaeneus* Frt., Zool. Mus. Berlin." Mounted on a pinned card between small pieces of glass cemented across an opening. The mountant has partially dried out and the specimen is crushed and flattened but otherwise in reasonably good condition. One fore wing and the head are dissected and only the antennal scapes and a single club remain. This specimen is here

designated lectotype and has been labelled accordingly. It is in the Museum für Naturkunde der Humboldt Universität zu Berlin. Paralectotype. "17/584, Aachen, Frst, *Plastocharis subaeneus* Frst, Zool. Mus. Berlin." Mounted in a manner similar to the lectotype but the mountant is more badly dried out. Foerster (1878) stated that this species was described from male and female material; however, as Novitzky (1954) pointed out, this specimen is a male *Thysanus*, probably *ater* Haliday. Also housed in the Museum für Naturkunde.

#### DEVELOPMENTAL STAGES

Mummies of the long-tailed mealybug containing advanced larvae or pupae of *T. peregrina* were exposed to females of *C. subaeneus* for 24 hours, and were then kept at 32°C. At daily intervals, 30 mummies were dissected in saline solution under



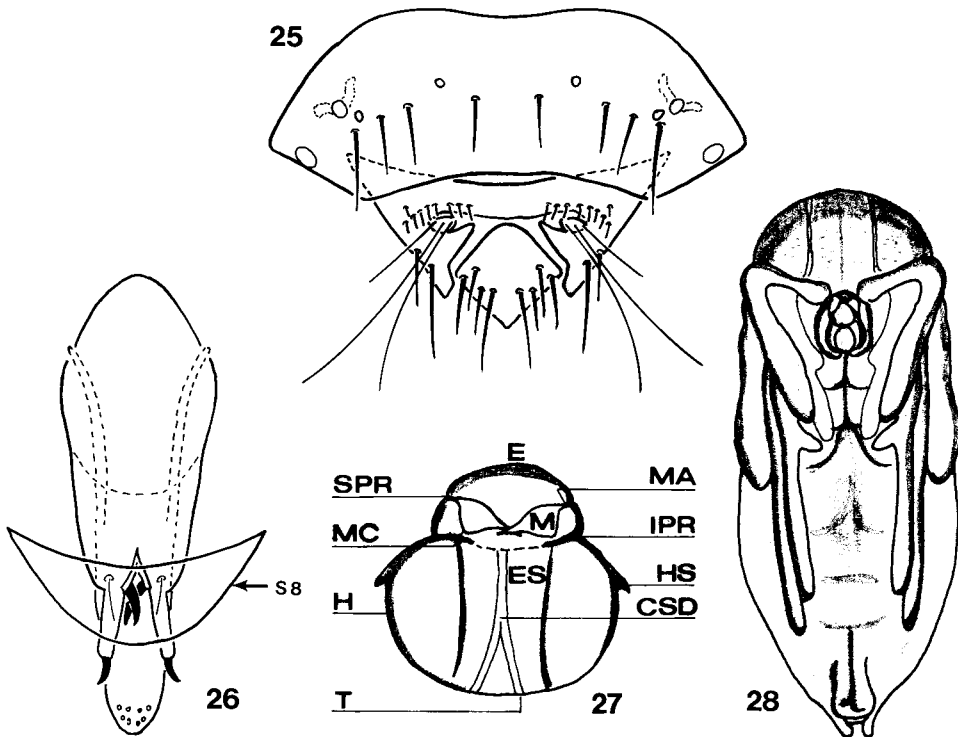
Figs. 18-24. *Chartocerus subaeneus*, developmental stages. 18, Egg, several hours after oviposition. 19, Egg, one day after oviposition. 20, First instar larva. 21, Second instar larva, showing the cephalic skeleton and respiratory system. 22, Second instar larva, showing the oesophagus, midgut and salivary gland. 23, Second instar larva: spiracles. 24, Third instar larva.

a stereoscopic microscope. Eggs and first-instar larvae were mounted directly in Hoyer's medium. Later developmental stages were soaked in chlorophenol solution for 2-7 days prior to mounting in Hoyer's medium. For observation of respiratory systems, live larvae were immersed in bromophenol solution on a slide, covered with a cover slip and studied immediately under a phase-contrast microscope.

The egg (Figs. 18, 19) is hymenopteriform, banana-shaped, with an elongate projection (or short stalk), grayish white, 265-300  $\mu$  long, and 75-110  $\mu$  wide. During the first few hours its contents appear as a mottled mass surrounded by a membrane, then the mouthparts and midgut become visible.

The larva (Figs. 20-24) develops through 4 instars. These are distinguishable by their general dimensions and by the shape and size of their

mandibles. All instars are hymenopteriform, with the head and 13 body segments evident, and all have 4 pairs of spiracles - in the mesothorax and first 3 abdominal segments (Fig. 23). First instar larva (Fig. 20) transparent white, elongate, tapering posteriorly, 215-260  $\mu$  long and 60-75  $\mu$  wide; mandibular denticle somewhat curved, 4  $\mu$  long. Second instar (Figs. 21-23) of typical chalcidoid form, with the head partially withdrawn into the thorax, 325-480  $\mu$  long and 125-200  $\mu$  wide; mandibles beak-like, 6  $\mu$  long, with a broader base than in the first instar. The oesophagus, midgut and salivary gland are clearly evident at this stage (Fig. 22), as are the cephalic skeleton and respiratory system (Figs. 21, 23). The latter, as in the first instar, consists of two lateral longitudinal trunks, connected by transverse commissures in the prothorax and eighth abdominal segment; the mesothoracic



Figs. 25-28. *Chartocerus subaeneus*. 25, Female metasoma: T7, T8 and epiproct. 26, Male genitalia and eighth sternum. 27, Fourth instar larva: cephalic skeleton. CSD = common salivary duct; E = epistoma; ES = oesophagus; H = hypostoma; HS = hypostomal spur; IPR = inferior pleurostomal ramus; M = mandible; MA = mandibular acron; MC = mandibular condyle; SPR = superior pleurostomal ramus; T = tentorium (terminology after Vance and Smith 1933). 28, Female pupa, ventral view.

spiracles are larger than the other pairs. The midgut occupies the first 7 abdominal segments; the beginning of a hind-intestinal invagination can also be discerned. Third instar (Fig. 24) 800-900  $\mu$  long and 370-450  $\mu$  wide; mandibular denticle 10  $\mu$  long. The midgut now occupies most of the body cavity, and the hind gut is clearly evident but still disconnected. Respiratory system as in the preceding instars. A pair of large glands can be seen in the prothoracic region. Fourth instar 1000  $\mu$  long and 450  $\mu$  wide; mandibular denticle 14-16  $\mu$  long. It is otherwise similar to the preceding instar. The cephalic skeleton is best seen in cleared specimens of this instar (Fig. 27).

At the end of the fourth instar, the larva emits meconial pellets. The prepupa is milky white, somewhat smaller than the full-grown fourth instar. At 32°C, pupation takes place on the 7th day after oviposition.

The pupa (Fig. 28) is of typical chalcidoid form. On the first day it is entirely pale, sometimes with beginnings of pigmentation on the vertex. On the second day pigmentation appears on the abdomen, and the propodeal triangle becomes evident.

On the third day pigmentation spreads to all parts of the body, and all imaginal organs can be discerned. On the fourth day the pupa is entirely black. Adult emergence occurs 6 or 7 days after pupation.

#### BIOLOGY

*Chartocerus subaeneus* is a direct hyperparasite, attacking only parasitized, mummified mealybugs. It develops ectoparasitically upon well-developed larvae or pupae of its primary hosts within the mummified mealybug. Although occasional mummies were found to contain two *Chartocerus* eggs, development is always solitary and only one adult hyperparasite emerges from each secondary host. Prior to parasitization, the female usually host-feeds on its primary hosts. Reproduction is deuterotokous: virgin females give rise to female progeny, and males are rather rare. Random sampling of lab cultures yielded 5.25% males. However, males were observed to court females and tried to mate with them.



### Adult Behavior: Predatory Host-Feeding and Oviposition

Female *C. subaeneus* wasps are positively phototactic, negatively geotactic, and positively thigmotactic, tending to enter crevices, folded leaves, mealybug egg masses and empty mummies.

Upon encountering a mummified mealybug, the female wasp walks around and over it and taps it with the tips of her antennal clubs. Some hosts are accepted rapidly (1-2 min.), but for others more prolonged examination is needed before a decision to accept or reject the host is reached. When a mummy is found acceptable, the wasp turns around, with her caudal end directed towards it, and examines it with her ovipositor. If all is well, this again may take only 2 minutes or less; otherwise, the examination is prolonged until the host is accepted or rejected. When the site of attack is selected, the wasp appears to lean with her antennae upon the substrate, her body pressed hard to the mummy, and starts drilling a host-feeding hole. During drilling, the entire body is seen to tremble, and the ovipositor may be partly withdrawn and re-inserted. An inexperienced wasp may drill for 12-23 min., whereas wasps with several days' experience accomplish the task in 10-14 min. After the ovipositor is withdrawn, the wasp examines the mummy for a few seconds with her antennae, locates the hole, and commences feeding. Immediately after feeding is completed, the wasp turns around and drills again in the same area, searches with her ovipositor inside the mummy, and lays an egg. Drilling and oviposition take 8-13 min., after which the wasp moves on to another mummy, examines it with her antennae and ovipositor, drills and oviposits in it without further host-feeding. In two hours a wasp may construct a feeding hole, host-feed, and lay about 3 eggs in as many mummies.

Most wasps went through this cycle of host-feeding and oviposition. However, a few departed from it and laid an egg before host-feeding or, when offered honey, fed upon it before laying an egg.

For successful drilling by *C. subaeneus*, the secondary host mummy has to be secured to the substrate. In nature, parasitized mealybugs tend to hide in crevices and adhere to the substrate prior to mummification.

Two types of host-feeding were observed: a short feeding period of 1-4 min., and a longer one

lasting 12.5-22 min. Both were always followed by oviposition upon the same host. It appears that the short host-feeding period serves mostly as a stimulant to oviposition, whereas the longer period serves to satisfy the wasp's nutritional requirements for continued oviposition. Extensive feeding may result in death of the primary host. Indeed, some of the mealybug mummies in our *C. subaeneus* cultures did not yield any parasite, primary or secondary, and comparison with mummies exposed to *T. peregrina* alone indicated that the incidence of such mortality was significantly higher in the presence of the hyperparasite than in its absence (19.1% vs. 7.3%, respectively,  $p=0.05$ ).

### Host Range

In single-choice laboratory experiments, females of *C. subaeneus* readily examined, host-fed and oviposited upon the following encyrtid primary hosts, on which their progeny developed to maturity: *Anagyrus fusciventris* (Girault), *Anagyrus pseudococci* (Girault), *Leptomastidea rubra* Tachikawa and *Tetracnemoidea peregrina* (Compere) in the long-tailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti); *Anagyrus pseudococci* (Girault), *Clausenia josefi* Rosen and *Leptomastix dactylopii* Howard in the grape mealybug, *Planococcus vitis* (Niedzielski); *Anagyrus pseudococci* (Girault) in the citrus mealybug, *Planococcus citri* (Risso); and *Clausenia purpurea* Ishii in the citriculus mealybug, *Pseudococcus citriculus* Green.

The wasps examined, inserted their ovipositor into and host-fed upon the encyrtid *Microterys flavus* (Howard) in the brown soft scale, *Coccus hesperidum* L. (Homoptera: Coccidae), and *Aphidius* sp. (Hymenoptera: Aphidiidae) in mummies of the oleander aphid, *Aphis nerii* Boyer de Fonscolombe (Homoptera: Aphididae), but no eggs were laid upon these hosts. Puparia of *Drosophila melanogaster* were ignored by the wasps.

### Fecundity

*Materials and methods.*— In order to determine whether the wasps were pro- or synovigenic, several newly-emerged females were dissected before they had the chance to either feed or oviposit, and their ovaries were examined.

Cards bearing 20-30 mummies of the long-tailed mealybug, containing *T. peregrina*, were placed in small plastic cups. A silk cover was fastened to the cup by means of a plastic lid in which a large hole was bored for ventilation, and some honey was

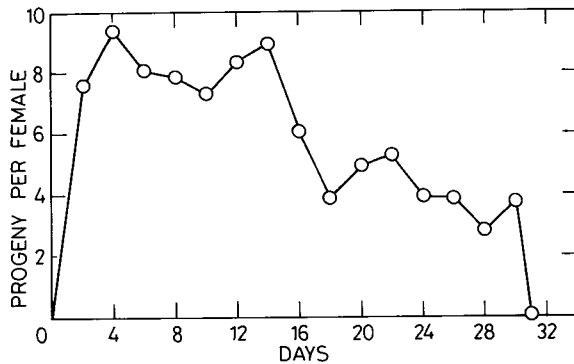


Fig. 29. *Chartocerus subaeneus*, daily progeny production at 28°C, >50% RH (initial n = 6).

streaked on the inside of the cup. Six newly-emerged female wasps were placed singly in these oviposition cups, at 28±1°C, >50% RH and a photoperiod of 12L:12D, and were transferred to new cups, without anaesthetization, at daily intervals throughout their life. Following oviposition, the cups were kept at 28±1°C, and emergence of *C. subaeneus* progeny was recorded daily.

**Results.**— Dissection of 10 newly-emerged females revealed that each ovary comprised 4 ovarioles, each containing one fully-developed egg.

Oviposition commenced upon the day of emergence and continued evenly throughout the female's life span, declining somewhat in the last days (Figs. 29, 30). On average, a female laid 57% of her eggs during the first half of her life.

Fecundity was rather high. The females lived for a mean of 24.9 days (17-30), and oviposited

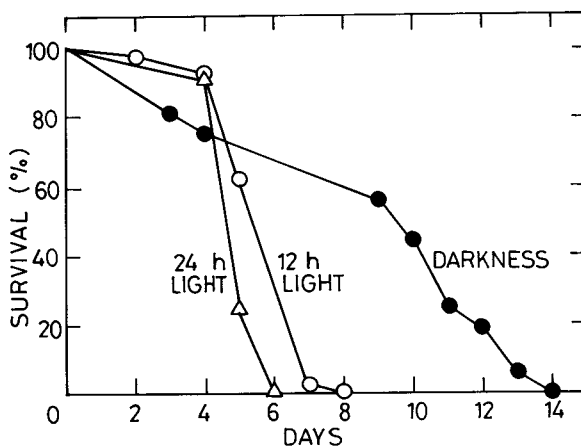


Fig. 31. *Chartocerus subaeneus*, effect of photoperiod on survival of adult females at 28°C, >50% RH, with honey as food.

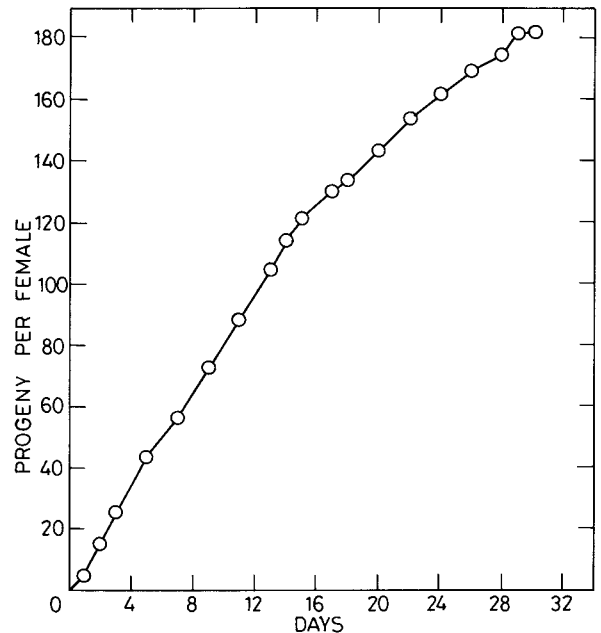


Fig. 30. *Chartocerus subaeneus*, cumulative progeny production at 28°C, >50% RH (initial n = 6).

throughout that period (average 24.3 days). Daily progeny production averaged 6.8, and mean total fecundity was 163.3 progeny per female (range 125-192).

#### FACTORS AFFECTING ADULT LONGEVITY

##### Photoperiod

**Materials and methods.**— Batches of 15 or more newly-emerged female wasps were kept in small plastic cups (as in Fecundity, above), with some honey as food, at 28±1°C and >50% RH, under 24D, 12L:12D and 24L. Mortality was recorded daily.

**Results.**— As shown in Fig. 31, the longevity of adult females was similar at 12L:12D and 24L, but their longevity was prolonged considerably at 24D. This may have resulted from reduced activity in darkness.

##### Relative humidity

**Materials and methods.**— To assess the moisture requirements of *C. subaeneus*, the longevity of female wasps was compared under 0% and 50% RH, as suggested by Bartlett (1962). Two small plastic cups, each with a silk cover held in place by a plastic lid with a large hole in it, were sealed and fastened to one another, lid to lid, with Permagem® cords (Virginia Chemicals, U.S.A.) so that they

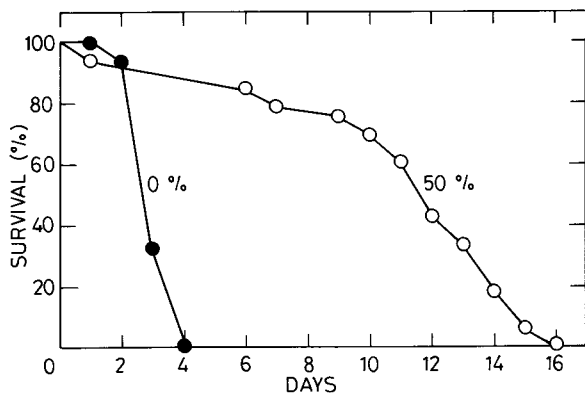


Fig. 32. *Chartocerus subaeneus*, effect of relative humidity on survival of adult females (black circle n = 47; open circle n = 45).

formed a closed unit. Relative humidity was controlled by placing in the bottom cup dry P<sub>2</sub>O<sub>5</sub> for 0% RH or a solution of 62.5 gr KOH in 100 cc water for 50% RH (Peterson 1964). Some honey was streaked in the upper cup, and after the system was allowed to reach an equilibrium for 48 hours at 28±1°C, it was opened momentarily and several newly-emerged female wasps were placed in the upper cup. Forty-seven wasps were used at 0% RH, 45 at 50% RH. The units were kept in the dark, at 28±1°C, and mortality was recorded daily.

**Results.**— *C. subaeneus* is rather susceptible to low relative humidity (Fig. 32). At 0% RH, 50% mortality was reached on the third day and the last survivors died on the fourth day; whereas at 50% RH, 50% mortality was reached on the 12th day and the last survivors died on the 16th day.

**Nutrition**

When batches of 10 or more newly-emerged female wasps were kept at 28±1°C and >50% RH, with no food or water and in the absence of hosts, they all died within 3 days. Provision of water for drinking did not prolong their life span.

Provision of pollen in addition to honey, at 12L:12D and in the absence of hosts, shortened the longevity of the wasps considerably in comparison to that on honey alone: 50% mortality was reached on the 4th day, and the last survivors died on the 6th day (compare with Fig. 31, 12L:12D for survival on honey alone).

In all the experiments with honey in the absence of hosts, the longevity of adult female wasps was

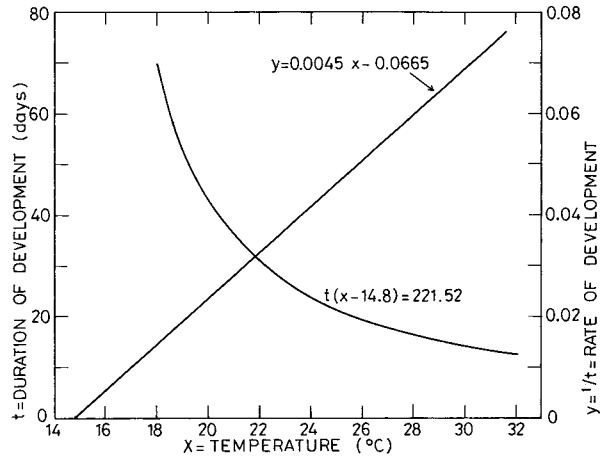


Fig. 33. *Chartocerus subaeneus*, effect of temperature on rate and duration of development.

considerably lower than in the presence of hosts. The 6 females used in the fecundity experiment (Fig. 29) had a mean life span of 24.9 days, whereas under similar conditions but without hosts their mean life span was 6.9 days and they never survived for more than 16 days (see Figs. 31, 32). Predatory host-feeding must have a pronounced effect in prolonging the life span of *C. subaeneus*.

**Effect of Temperature on Rate of Development**

**Materials and methods.**— Cards bearing long-tailed mealybug mummies, containing *T. peregrina*, were exposed to females of *C. subaeneus* for 24 hours at room temperature, and were then placed in incubators at 24°, 25°, 28° and 32°C. Emergence of adult *C. subaeneus* progeny was recorded daily.

The equilateral hyperbola equation, based on the assumption that the product of developmental time and temperature above a certain threshold is a constant for any given species, is a convenient way of expressing the effects of temperature on the duration of development of insects (Bodenheimer

Table 1. Effect of temperature on duration of development of *C. subaeneus*.

Temperature (°C)	Duration of development (days)		
	N	Range	Average ± SD
24	11	23-25	23.92 ± 0.67
25	34	18-27	21.77 ± 2.30
28	52	15-19	16.39 ± 0.71
32	43	12-14	12.86 ± 0.64

1958). The hyperbola equation for *C. subaeneus* was calculated as follows: rates of development at the various constant temperatures tested were obtained as the reciprocals of the developmental periods; the linear regression ( $y = a + bx$ ) of developmental rate on temperature was then calculated by the method of least squares; the parameters of the equilateral hyperbola (thermal constant, ThC, and the developmental threshold, c), were then obtained from the identities  $ThC = 1/b$ ;  $c = a/b$ .

*Results.*— The results are presented in Table 1. The regression of the rate of development on temperature and the corresponding equilateral hyperbola for *C. subaeneus* are presented in Fig. 33. The calculated developmental threshold was 14.8°C and the thermal constant was 221.52 days-degrees. Since *C. subaeneus* developed normally at 32°C, the upper temperature threshold must have been rather high.

#### DISCUSSION

The oviposition behavior of *C. subaeneus* is similar to that of other Signiphoridae. Other species have been observed to require lengthy periods for oviposition (Woolley and Vet 1981, Agekyan 1968, DeBach et al. 1958, Clausen 1924), and other species appear to require a period of host-feeding before oviposition can occur (see Woolley and Vet 1981, Quezada et al. 1973). Oviposition upon the same individual hosts which were used for host-feeding was observed in all of these cases, and in *C. subaeneus* it is the most common behavioral sequence.

The biology of only one other species of *Chartocerus*, *C. elongatus*, has been reported in any detail (Clausen 1924). Like this closely-related species, *C. subaeneus* is an obligate hyperparasite. In fact, we know of no cases (published or unpublished) in which it is clear that a *Chartocerus* species develops as a primary parasite. This is in contrast to *Signiphora*, in which some species are primary and others are hyperparasitic. In our experiments, *C. subaeneus* was strictly a solitary parasite, but Clausen (1924) found *C. elongatus* to be gregarious in attacking primary parasites of *Pseudococcus maritimus* (Ehrhorn).

The *C. subaeneus* female emerges with several well-developed eggs that she may deposit on the first day of her adult life, and develops the rest continually during her lifetime. Predatory host-feeding or other sources of proteinaceous nutrition are not required, other than as a stimulant for the

beginning of oviposition, but are presumed to be necessary for continuous egg production in later stages.

As has been reported for other ectophagous hyperparasites (Sullivan 1987), *Chartocerus subaeneus* appears to be rather polyphagous, capable of attacking various encyrtid primary parasites within various mummified mealybugs. It may utilize other primary parasites developing in yet other homopterous hosts for host-feeding. This may enhance its survivorship in the absence of suitable hosts for oviposition.

We were intrigued to find that the egg of *C. subaeneus* bears an elongate projection similar to that reported for *C. elongatus* by Clausen (1924). Such a projection has not been reported for any *Signiphora* species to our knowledge, and it may have systematic significance.

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