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Biology and descriptions of the larva and pupa of

Anopheles (Cellia) elegans James (1903).

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ABSTRACT. Descriptions and illustrations of the pupa and larva of Anopheles (Cellia) elegans are presented for the first time. The bionomics and medical significance of this species are reviewed and updated.

INTRODUCTION

Anopheles (Cel.) elegans James whose distribution is limited to southwest India and Sri Lanka (Colless 1956; Reid 1968) has been incriminated as the vector of simian malaria in these areas (Choudhury, Wattal and Ramakrishnan 1963; Nelson, Jayasuriya and Bandarawatta 1971). During the course of studies in establishing laboratory models of simian malarias indigenous to Sri Lanka, we find that descriptive accounts of the larva and pupa of *elegans* have not to date, been documented.

In the last major revision of the Leucosphyrus Group by Colless (1956), the egg and adult stages of *elegans* are described; it is mentioned that the larva of *elegans* is unknown, and scanty reference is made to its pupa, based on a single unconfirmed specimen in the collection of the British Museum (Nat. Hist.). Subsequent literature on *Anopheles (Cel.) elegans*, which includes Reid's (1968) account of the Leucosphyrus Group and a report by Harrison et al. (1974), confirms this stage of knowledge.

Over the past two years we have reared adult *elegans* for the purpose of transmitting simian malaria in the laboratory from first to third instar larvae collected biweekly from a forest reserve, Udawattekelle, in the Kandy district of Sri Lanka. Collections were temporarily suspended from December to March, owing to the drought. We have now ceased this collecting because a large colony of *elegans* is being maintained successfully in our laboratory in

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Sri Lanka by artificial mating (Mendis and Nanayakkara, unpublished data). We have therefore, had ample opportunity to examine over 100 specimens of larvae and pupae of *elegans*, whose morphology and bionomics we describe in this paper. Specimens used for the descriptions are larvae and pupae from the colony maintained in the laboratory by artificial mating, and exuviae of larvae and pupae reared from wild-caught early larval instars, the associated adult specimens of which provided the identity of the species. Although the descriptions are based on specimens from Sri Lanka, comparison was made with associated pupal and larval exuviae of known adults of *elegans* from localities in southwest India. Reid (1949) and Colless (1956) discuss in great detail the problems associated with the name elegans. The question of a type for elegans has not been resolved. Both of these authors question Theobald's (1903) supposed designation of a specimen in the BMNH as the "type" since the specimen was never labeled as such and the accompanying description and illustration of *elegans* do not completely agree with the specimen in question, nor is it conspecific with the species treated here. We therefore make clear here that we are treating the species *elegans* as recognized by Colless (1956) and Reid (1949; 1968) and leave the more complicated problem of a type for elegans for a later, more comprehensive review of the Leucosphyrus Group. Terminology used follows that of Harrison and Scanlon (1975), except for the toothed margin (TM) index of the pupal paddle which follows that of Colless (1956) for ease of comparison with the TM index given for other members of the Leucosphyrus Group in that publication.

DESCRIPTIONS

PUPA (Fig. 1). Modal condition of chaetotaxy as figured. Diagnostic features as follows: *Abdomen*. Seta 1-II brush-like with 12-25 branches; 1-III with 3-8 branches, 5-III with 5-9 branches; 1-IV with 2-6 branches, 5-IV with 4-7 branches, 9-IV of short type, length 0.031-0.056 mm (\bar{x} = 0.043), ratios of length of seta 9, IV/III 1.40-3.00 (\bar{x} = 2.19), IV/V 0.29-0.52 (\bar{x} = 0.40); 1-V usually with 2,3 branches, occasionally single, 5-V with 3-7 branches, 9-V-VII usually with few lateral spicules; 1-VI usually single, occasionally double, rarely (3/20) double on both sides, 5-VI with 4-7 branches; 1-VII usually single, rarely double or triple, 5-VII with 4-7 branches; toothed margin index of paddle 0.83-0.89 (\bar{x} = 0.85).

LARVA (Figs. 1, 2). Living larva light brown. Modal condition of chaetotaxy as figured. Diagnostic features as follows: *Head*: Prominent dark brown collar; seta 2-C simple, not frayed; 4-C posterolaterad of 2-C, usually single, rarely bifid or trifid (4/35 specimens), far back from both 2-C and 3-C, bases of the 3 setae forming more or less an isosceles triangle; distance between insertions of 2-C and $\overline{4}$ -C, 0.065-0.116 mm (\overline{x} = 0.089); length of 4-C, 0.103-0.163 mm (\bar{x} = 0.122), reaching as far as 0.33 length of 2-C from its base or extending forward 0.105-2.04 distance between the insertions of 2-C and 4-C; 2-C wide apart (0.073-0.103 mm), twice or more distance between 2-C and 3-C on one side; 13-C with 3-6 branches; dorsomentum with 4 teeth on a side. Thorax. Seta 1-P with 15-21 branches, stem strong, flattened and slightly shorter than stem of 2-P, basal sclerotized tubercles of setae 1-P and 2-P large, fused basally, both with strong, pointed or rounded apical tooth projecting forward over base of each; 14-P with 6-12 branches; 14-M with 5-12 branches; 3-T with 3-9 filiform branches or weak, narrow lanceolate leaflets. Abdomen. Seta 1-I only slightly developed with 3-7 undifferentiated rigid branches, 3-I single or bifid, 9-I with 3-6 branches; 1-II weakly developed with 8-12 very narrow, lanceolate, unpigmented leaflets; 2-IV with 3-5 branches, 6-IV with 2,3 branches, 13-IV with 3,4 branches, about 0.85-1.00 length of 10-IV; 2-V with 3-6 branches, 6-V with 2,3 branches; 1-VII smaller than 1-VI with 10-16 lanceolate leaflets; most leaflets of palmate setae 1-III-VI with a few apicolateral serrations; both filament and blade of palmate setae III-VI equally pigmented; pecten with 3-5 long teeth and 6-8 shorter ones of varied lengths.

DISTRIBUTION

Anopheles elegans is known to occur only in southwest India and Sri Lanka (Colless 1956, Reid 1968); the only other member of the Leucosphyrus Group reported from Sri Lanka is balabacensis balabacensis Baisas (Kalra and Wattal 1962). The record of balabacensis from Sri Lanka was based on a single specimen in the collection of the Malaria Institute of India, Delhi (Central Institute for Communicable Diseases) which was listed from Ceylon with "unknown" locality and without additional data. Reid (1968:299) repeats this record without further comment. We have not seen specimens resembling balabacensis from Sri Lanka and we are unaware of any other reported occurrences of this species in the country. If a second species resembling balabacensis does occur in Sri Lanka, it would appear that it is extremely rare considering the number of mosquito collections made in recent years.

The collection of specimens for this study was confined to a restricted area of a natural reserve for flora and fauna, Udawattekelle, in the city of Kandy, Kandy District, Central Province. Previous studies have also recorded *elegans* breeding in Kandy (Udawattekelle) and Hantane (Nelson, Jayasuriya and Bandarawatta 1971) and Wakarawatta (Harrison et al. 1974). A few adults of *elegans* have also been collected from the North Central Province, Maho, in the dry zone where monkey malaria is endemic (A.S. Dissanaika, personal communication). In 1975, E.L. Peyton and Yiau-Min Huang (unpublished data) made several larval collections of *elegans* and reared a large number of adults from the following localities. Central Province: Kandy District, Udawattekelle; Matale District, Matale and Imbulpitiya. Sabaragamuwa Province: Ratnapura District, Vaddagala. Southern Province: Galle District, Kanneliya. Western Province: Colombo District, Labugama; Kalutara District, Morapitiya.

DISCUSSION

The chief characters of the pupa of *elegans* are as follows. Seta 1-II brushlike with 12-25 branches. Seta 9-IV of the short type. Mean ratios of length of 9 IV/III and 9 IV/V are 2.19 and 0.40, respectively. Seta 1-V usually with 2,3 branches, occasionally single. Seta 1-VI usually single and 1-VII usually single, rarely double or triple. The larva of *elegans* is distinctive in the length and placement of seta 4-C. Seta 4-C is longer than that of *balabacensis*, having a mean length of 0.122 mm, whereas the mean lengths of 4-C in *dirus* and *takasagoensis* are 0.077 mm and 0.062 mm, respectively. The length of 4-C in *balabacensis* is similar to that of *dirus*. Seta 4-C in *elegans* is placed far back with a mean distance of 0.089 mm between the bases of 2-C and 4-C, yet, on account of its length, it reaches as far as 0.33 the length of 2-C from its base. Mosquito Systematics

BIONOMICS

Udawattekelle, where material was collected for this study, is a small forest bordering the city of Kandy in the Central Province of Sri Lanka. It is situated among the central hills of the country at an elevation of 518 m above sea level and has an extent of 104 hectares. The vegetation includes tall trees with intervening dense undergrowth. The breeding places of elegans are small collections of muddy water in cart tracks, tire marks and similar depressions on an infrequently used gravel road. Having explored the entire length of the road we found that 4 to 5 such mud pools consistently formed the habitat of the immature stages of *elegans* throughout most of the year other than during a period of drought which extends from December to April. Though muddy, these pools were clean except for a few fallen leaves. The breeding pools were heavily shaded from direct sunlight by the overhanging branches of trees on either side of the road. Although larvae of several culicine species were obtained from these breeding places, *elegans* was the only anopheline larva encountered throughout the rainy season. However, at a time when the drought was approaching, and *elegans* larvae were declining in numbers, a few specimens of Anopheles (Ano.) barbirostris Van der Wulp were also recovered from these mud pools. The bionomic data obtained in this study tally closely with the findings of Nelson, Jayasuriya and Bandarawatta (1971) in a study carried out at the same location to find the natural vector of simian malaria in Sri Lanka. Choudhury, Wattal and Ramakrishnan (1963) describe the resting places of the adults of *elegans* in southwest India, but do not clearly identify the habitat of the immature stages.

MEDICAL SIGNIFICANCE

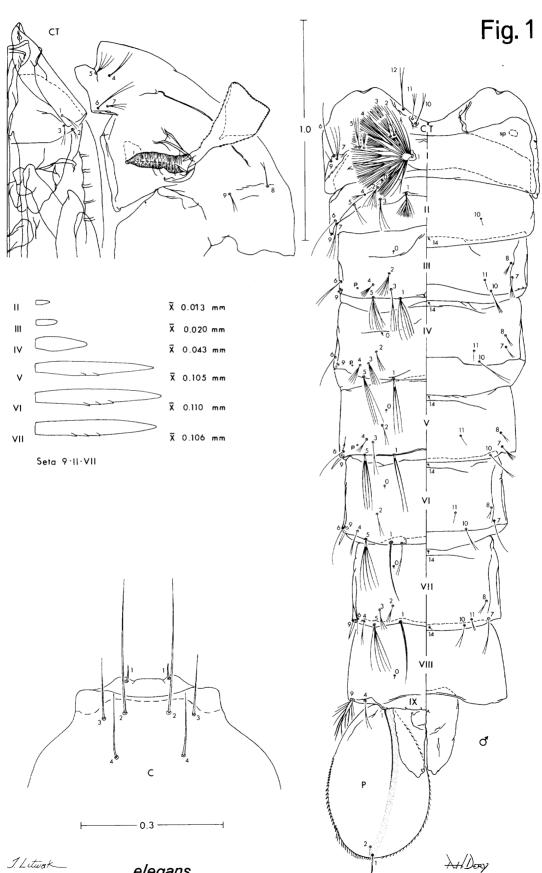
Anopheles elegans has been incriminated as the natural vector of simian malaria in southwest India (Choudhury, Wattal and Ramakrishnan 1963) and Sri Lanka (Nelson, Jayasuriya and Bandarawatta 1971). These authors found wildcaught adults infected with sporozoites, which when injected to uninfected monkeys proved to be sporozoites of *Plasmodium inui* Halberstaedter and von Prowazek, P. cynomolgi Mayer and P. fragile Dissanaike, Nelson and Garnham. In another study (Choudhury, Mohan, Prakash and Ramakrishnan 1963), laboratory-reared *elegans* were infected by feeding on infected monkeys. However, in none of their studies was the entire cycle of transmission from an infected to a clean monkey completed, due, as the authors explain, to the nonavailability of sufficient numbers of elegans. In this laboratory, we have established the entire cycle of transmission with P. inui shortti Bray and P. cynomolgi from naturally-infected toque monkeys (Macaca sinica Linnaeus) to uninfected ones (Mendis and Munesinghe, unpublished data), thus confirming its role as a natural and an efficient laboratory vector of simian malarial parasites of Sri Lanka. Due possibly to the restricted distribution and relative non-availability of *elegans*, the species has received considerably less attention than the other members of the Leucosphyrus Group, such as dirus Peyton and Harrison, whose role as a vector of simian malarias has long been established, and is being used extensively as an experimental vector in research. A large colony of *elegans* is now being maintained successfully in this laboratory by artificial mating, and we use *elegans* as an experimental vector in immunological studies on transmission blocking vaccines in simian malaria.

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