The Value of the Pupal Stage to Anopheline Taxonomy, With Notes on Anomalous Setae (Diptera: Culicidae)[1]

by

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ABSTRACT. The importance of the pupal stage of anophelines in taxonomic studies is highlighted, including a review of some advantages and disadvantages for using this stage, a listing of commonly encountered taxonomic characters found on the pupal stage and their significance, and an indication of the world anopheline taxa with poorly known pupae. Certain anomalous setae on anopheline pupae are discussed and illustrated, and homologies are suggested for these setae within the Culicidae and with some other Nematocera.

INTRODUCTION

The pupal stage is a "neglected stage" for many mosquito collectors, and is often ignored in mosquito taxonomic studies, e.g., Wood et al. (1979). During taxonomic studies over the last 20 years we have examined thousands of pupae of *Anopheles* Meigen from many regions of the world. With time it has become increasingly apparent that beside possessing supportive characters to be used with characters on other stages, the pupal stage frequently offers the best characters for differentiating many anopheline categories, e.g., series, species groups, and species (including sibling species). In this paper we present advantages/disadvantages for collecting and using anopheline pupae in taxonomic studies. Also, certain abnormal or anomalous setae observed on anopheline pupae are discussed.

Only a few early mosquito workers demonstrated an interest in the pupal stage of anophelines, e.g., Senevet (1930), Christophers (1933), Baisas (1936), Crawford (1938), and they generated only minimal interest among their peers. One reason for this disinterest was the inability to homologize the pupal setae with those found on the larvae. By the late 1940's several elaborate alphabetical and/or numerical schemes had been proposed for pupal setae (see review by Harbach and Knight 1980). Although these schemes were different, collectively confusing and did not resolve the homology problem, they did demonstrate the value of pupal characters for differentiating species. Belkin (1952, 1953, 1960, 1962), following the lead of Baisas and

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[1] The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

Ubaldo-Pagayon (1949), discovered that homologies of the larval and pupal setae could be established by following the sensory neuron connections in prepupae. This significant discovery opened the door for direct comparisons of the chaetotaxy of larvae and pupae via a unified numerical system and stimulated more interest in the taxonomic usefulness of pupal characters. These studies further demonstrated the value of reared adults with associated immature exuviae.[1] The pupa is best studied from slide mounted exuviae.

Simultaneous with Belkin's studies, Reid (1950, 1953, 1962, 1965) followed Crawford (1938) and established that the pupal stage of *Anopheles* was important for differentiating species within difficult species complexes in Southeast Asia. In revisions of the Umbrosus, Hyrcanus, Barbirostris and Aitkenii Groups, Reid relied heavily on pupal characters. Reid and Knight (1961) continued the emphasis on the pupal stage with the publication of a system of classification for the subgenus *Anopheles* based on very distinct differences in the structure of the trumpet. This system, which utilizes infrasubgeneric categories called "Sections" and "Series," is simple and very useful in segregating Asian *Anopheles (Anopheles)*. Unfortunately, the categories, "Sections" and "Series," as defined by Reid (1968) for Asian *Anopheles*, are not synonymous with the "Series" and "Sections" used for African *Anopheles* by Gillies and de Meillon (1968). The disparities in these 2 systems need to be resolved so that a unified system of classification can be developed for infrasubgeneric categories within the genus *Anopheles*.

ADVANTAGES AND DISADVANTAGES OF USING THE PUPAL STAGE

Advantages for collecting and using the pupal stage in taxonomic studies of anophelines far outweight the disadvantages.

Advantages:

(1) The pupal stage requires no food and can be reared in clean water.

(2) The pupa or its exuviae usually remain clean, because they are not in the water long enough to accumulate debris on the setae.

(3) The pupal exuviae is sturdier than a larval exuviae and is easier to preserve and mount on a slide without the loss of setae.

(4) The pupal exuviae has fewer setae than a larval exuviae and is easier to scan for characters.

(5) The pupal stage is easily sexed.

(6) Pupae of certain species do not submerge for long periods, and are therefore easier to capture than the larvae of those species.

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[1] The word, exuviae, encompasses both the singular and pleural, as found in Latin dictionaries.

(7) Duration of the pupal stage is usually short, making it an ideal target during short collecting trips.

(8) Pupae may exhibit inherited color patterns which are excellent genetic markers.

(9) Male mitotic metaphase chromosomes are easily obtained from pupal testes.

Disadvantages:

(1) There is a shortage of keys and adequate descriptions for the pupal stage of many species.

(2) Pupae are more difficult to find because they tend to hide in dark secretive spots and they have a short duration.

(3) Pupae are less able to withstand transportation conditions than are adults and larvae. However, techniques such as keeping pupae chilled on wet filter paper in vials or chilled in small air tight plastic bags can significantly reduce their mortality.

(4) The pupal stage cannot be used to differentiate *Bironella* Theobald from *Anopheles*.

COMMON ANOPHELINE PUPAL CHARACTERS OF TAXONOMIC VALUE

The taxonomic characters on pupae usually are easily seen, and characters of value seem to be more common on pupae of anophelines than on pupae of most culicines. There are certain characters that occur frequently on anopheline pupae and are of value to taxonomists.

Character State	Level of Value
CEPHALOTHORAX	
- color patterns on wing and antenna	Species
- presence/absence of pigmented knobs below trumpet or on wing	Species Group, Species
- branching of setae 10-12-CT	Species
TRUMPET	
- presence/absence of tragus	Species Group, Species
- depth of meatal cleft	Species
- presence/absence of secondary meatal cleft	Species Group, Species
- sculpturing, ridges and rim structure	Species
- shape	Section, Series, Species Group

Character State	Level of Value
ABDOMEN	
- color patterns on terga	Species
- presence/absence of denticles on terga	Species Group
- shape of ♂ genital lobe	Subgenus, Species
- development and position of setae 0-III-VII	Species
- development and branching of setae 1,5-III-VII	Series, Species Group, Species
- sum of branches of setae 2-VI-VII	Species
- sterna length in relation to setae 7-VI,VII length	Species Group
- structure and length of setae 9-I-VII	Series, Species Group, Species
- shape of seta 9-IV tip	Series, Species
PADDLE	
- broad or elongate	Subgeneric, Section, Series
- apex emarginate or convex	Species
- presence/absence of stout denticles on lateral margin	Species Group, Species
- refractile index	Species
- length and structure of lateral and apical fringe	Section, Series, Species Group, Species
- presence/absence of fringe mesad of seta 1-P	Species
- structure, length and shape of seta 1-P	Subgeneric, Section Series, Species

GENERAL DISCUSSION

Despite an abundance of good morphological characters such as listed above, the pupae of most anopheline species, including many important vectors of human pathogens, have received only superficial morphological examination and usually are considered unimportant taxonomically. This general perception has been used to justify, at times, the abandoning of the morphological approach and the use of other approaches, such as cytogenetic and electrophoretic techniques. In many instances, the target species received only a partial morphological study and the pupal stage remains very poorly known. This suggests a lack of faith in morphological taxonomy or an unwillingness on the part of many researchers to attempt thorough morphological studies. Based on our experience with *Anopheles* pupae, this attitude in many instances is totally unjustified. While there are a few groups, complexes and species with the pupal stage well known, we feel that thorough morphological studies of the pupae of the following taxa will reveal excellent characters for differentiating many species, including sibling species: Palaearctic Maculipennis Complex, Palaearctic Hyrcanus Complex, Asian Palaearctic *Anopheles*, Mediterranean and Ethiopian *Anopheles*, Australasian *Anopheles*, Neotropical *Anopheles (Anopheles)*, Neotropical Myzorhynchella of *Anopheles (Nyssorhynchus)*, Neotropical *Anopheles (Stethomyia)*, Southeast Asian Gigas and Lindesayi Complexes, many species complexes and groups of Southeast Asian subgenus *Cellia*, Southeast Asian Culiciformis Group, Nearctic Punctipennis Complex, and the Nearctic Maculipennis Complex.

ANOMALIES

Belkin (1952) states: "Anomalies, particularly those produced through developmental arrest or excess, furnish some of the most convincing evidence for the homology of structures and are widely used in the field of comparative anatomy." Anomalous setae seem to occur more frequently on anopheline pupae than on many culicine pupae. Belkin capitalized on this, using the pupae of at least 4 different species of *Anopheles* during his studies of larval-pupal setal homologies. During our studies, anomalous pupal setae of anophelines were detected which not only reflect homologies within the family Culicidae, but also with certain setae in other Nematocera.

The dorsal apotome (DAp) of mosquito pupae previously has never been illustrated or described with setae. A single pupal exuviae of *Anopheles (Ano.) lesteri* Baisas & Hu was found with a pair of branched setae on this area of the cephalothorax (Fig. 1). Neither seta has an alveolus, and the seta on the right has unusual branches and rootlike structures. Although this seta is almost certainly homologous with a larval head seta, we designate it seta 0-CT, following the current cephalothoracic setal numbering system. This pupal exuviae, "17065, A-1916-5, Japan, Hokkaido, Engaru, 28.VIII.1974, (ground pool), K. Mizusawa & B.D. Hall leg.", is mounted on a slide with the 4th instar larval exuviae and deposited in the National Museum of Natural History (NMNH), Smithsonian Institution. Other specimens in the NMNH of *An. lesteri* from Japan, Ryukyu Islands and other Asian and Southeast Asian countries are without seta 0-CT. We have been unable to find published reports or illustrations showing this seta on culicid, dixid or chaoborid pupae.

A partial review of the morphology of dipteran pupae revealed a similar pair of setae in approximately the same position in several other families of Nematocera. Illustrations in Johannsen (1934) show these setae in Ptychopteridae and Tipulidae. Saether (1980, Fig. 52) illustrates 2 setae on the "Frontal apotome" of Chironomidae. Thomsen (1937) and Jones (1961) clearly show 2 cephalothoracic tubercles, each with a stout bristle, on the "operculum" of pupae of Ceratopogonidae. Apparently these "anteromarginal" bristles are not present on all ceratopogonids. Although we cannot be certain that this pair of setae is homologous in all of these families, it seems likely that a pair of setae in this location on pupae is an ancestral character in Tipuloidea, Psychodoidea and Culicoidea. It is a very rare ancestral anomaly in mosquitoes.

Species of the genus *Toxorhynchites* Theobald are usually characterized as the only culicids possessing a paired seta on the ♀ cercus or ♂ proctiger of the pupa (paired seta of "Anal Segment" of Edwards 1941; 1-X of Mattingly 1971). However, Belkin (1962:529) noted that while seta 1-XI (as 1-X) is strongly developed in *Toxorhynchites*, it also "...is usually represented by an alveolus or minute hair in *Culiseta* Felt, and has been reported as an anomaly in *Aedes (O.) monticola* Belkin & McDonald, 1959 [sic 1957]." More recently, Belkin et al. (1970) found that seta 1-XI (as 1-X) appeared as a fixed anomaly on 5 pupae of *Anopheles (Ano.) vestitipennis* Dyar & Knab from Jamaica. They felt the seta was a fixed anomaly because it did not have an alveolus. We recently examined a series of 9 pupal exuviae of *An. vestitipennis* from Puerto Rico. These specimens (6 ♀ and 3♂) are mounted on 8 slides that are deposited in the NMNH and have the following label data: "Pupa · Anopheles vestitipennis D & K · Carolina, P.R. · VII-5-42 · Hoffman · Pratt." Seta 1-XI was present only on 3♀ (paired on 2 specimens, single on one). An alveolus was not present regardless of whether a seta was present or absent. The transient occurrence of this seta on the Puerto Rican specimens indicates that it is not a fixed anomaly on *An. vestitipennis* and an examination of additional pupae from Jamaica will probably confirm this.

A partial survey of pupae belonging to other species in the Arribalzagia Series of *Anopheles (Anopheles)*, that are deposited in the NMNH, revealed 2 additional species with seta 1-XI. The first species, *Anopheles (Ano.) mediopunctatus* (Theobald) (Fig. 1), is represented by 18 pupal exuviae or whole pupae (11♂, 7♀) that are mounted on 18 slides, with or without an associated 4th instar larval exuviae, from 4 collections (BRZ-16, BRZ-19, BRZ-27, BRZ-28) made in Floresta, Amazonas State, Brazil in May and June 1979. All 18 specimens have a paired, 1- or 2-branched seta 1-XI which apparently lacks an alveolus. The second species, an undetermined *An. (Ano.)*, is represented by 6 pupal exuviae mounted on 6 slides, each with an associated 4th instar larval exuviae. These specimens came from 2 collections: Coll. 41, from the Maraba Area, Para State, Brazil, October 1974; and from Balconcito, Restrepo, Colombia, August 1935. All 6 specimens (3♀, 3♂) possess a paired, 1- to 4-branched seta 1-XI, which lacks an alveolus.

Although at least 3 species in the Neotropical Arribalzagia Series of *Anopheles (Anopheles)* may possess the anomalous pupal seta 1-XI, it was not found on pupae of several other species in the series. Thus, it apparently cannot be used as a diagnostic character for the series, and it still must be determined if it is a fixed anomaly in *An. mediopunctatus* and the undetermined species of *Anopheles*.

Three pupal specimens of *Anopheles (Cellia) jeyporiensis* James were encountered which possess at least one extra seta on the mesothoracic region of the cephalothorax (Fig. 1). These specimens are mounted (with associated 4th instar larval exuviae) on 3 slides that are deposited in the NMNH. All 3 specimens came from collection 05633, seepage marsh, Ban Don Kaeo, Mae Rim, Chiang Mai, Thailand, 29 November 1969. Specimen 05633-3 has an extra seta and an empty alveolus. Because of similarities in the development and length of the extra seta on the 3 specimens, this seta is designated "9a"; the alveolus on 05633-3 is designated "9b."

Homologies of pupal cephalothoracic setae with larval thoracic setae are still unresolved, hence the artificial anterior-posterior numbering system. Belkin (1952) indicated that seta 8-CT appeared to be homologous with 1-M. The origin of 9-CT is uncertain, although he thought it might be homologous with 4-M. Belkin (1962:94) described and illustrated an extra seta "x" on the pupal mesothorax of the subfamily Dixinae. This extra seta was later illustrated on all the dixid species treated by Belkin (1968) and Belkin et al. (1970). It was also illustrated as an alveolus on several species of *Corethrella* Coquillett (subfamily Chaoborinae) in the latter paper. Actually, the positions of the "x" seta on all illustrated species suggest the possibility of 2 transient anomalous setae. This situation is indicated for *Paradixa fuscinervis* (Tonnoir) where 2 "x" setae are illustrated (Belkin 1962, Fig. 10). A comparison of the locations of the 2 extra setae on the dixid and chaoborid pupae with those found on *An. jeyporiensis* leaves little doubt that they are homologous. In fact, the 2 anomalous setae on *Paradixa fuscinervis* are positioned (in relation to 9-CT) nearly identically with "9a" and "9b" on *An. jeyporiensis*. Accordingly, we feel that seta "x", or alveoli, on the dixids and chaoborids should have the following designations. (1) Belkin (1962) - *Dixina solomonis* Belkin, Fig. 35, "x" = 9b. (2) Belkin (1968) - *Nothodixa campbelli* (Alexander), Fig. 5, "x" = 9a; *Nothodixa septentrionalis* (Tonnoir), Fig. 6, "x" = 9a; *Paradixa neozelandica* (Tonnoir), Fig. 9, "x" = 9b; *Paradixa fuscinervis* (Tonnoir), Fig. 10, 2 "x's" = 9a (seta "x" closest to 9-CT) and 9b (seta "x" most distant from 9-CT); *Paradixa tonnoiri* Belkin, Fig. 11, "x" = 9b; and *Paradixa harrisi* (Tonnoir), Fig. 12, "x" = 9b. (3) Belkin et al. (1970) - *Corethrella appendiculata* Grabham, Fig. 119, alveolus = 9b; *Corethrella longitubus* Belkin, Heinemann & Page, Fig. 120, alveolus = 9b; *Corethrella librata* Belkin, Heinemann & Page, Fig. 123, alveolus = 9b; *Dixella scitula* Belkin, Heinemann & Page, Fig. 125, "x" = 9b; and *Mesodixa biambulacra* Belkin, Heinemann & Page, Fig. 126, "x" = 9b.

Although 4 setae (2 anomalous) are now known for the mesothoracic area of the pupal cephalothorax, we cannot be certain of their homologies with larval setae. We suspect that 8-CT, 9-CT, 9a-CT and 9b-CT are homologous with setae 1-4-M on the larva, but homologies between the individual setae cannot be determined at present.

Reinert (1980) reviewed the occurrence of the anomalous seta 13-CT in the family Culicidae, including its occurrence on species of *Bironella* Theobald, *Anopheles (Kertezia)* and *Anopheles (Nyssorhynchus)*. During our studies we have noted 13-CT on species in 2 other subgenera of *Anopheles*. Figure 1 illustrates several different structural variations of 13-CT observed on pupal exuviae of *Anopheles (Anopheles) lesteri*, *Anopheles (Anopheles) pollicaris* Reid and *Anopheles (Cellia) culicifacies* Giles (cytogenetic species unknown) that are deposited in the NMNH. We also noted that Belkin (1968, Fig. 10) illustrated an extra unlabelled seta on the metanotum of *Paradixa fuscinervis* that is probably homologous with seta 13-CT.

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UNUSUAL ANOPHELINE PUPAL SETAE Fig. 1