

The Importance of an Integrated
Approach in Solving a Problem in
Mosquito Systematics^{1,2}

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ABSTRACT. Systematic data consist of any information about the diversity of organisms, which may include aspects of their morphology, behavior, genetics, biochemistry, ecology, physiology and distribution. In working with species complexes, such as those of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*, it is important that all available data from each of the latter disciplines be correlated (integrated approach) and used in delimiting the sibling species within those complexes. This idea of a multidisciplinary approach to mosquito systematics is dependent on the interaction and cooperation among investigators in their various fields, as is illustrated by *Anopheles (Nyssorhynchus) nuneztovari* Gabaldon 1940 and *An. (Nys.) trinkae* Faran 1979.

It will be attempted in this presentation to emphasize, and hopefully demonstrate, the importance of using an integrated or multidisciplinary approach to solve a systematic problem. For this, several aspects of the systematics of *Anopheles (Nyssorhynchus) nuneztovari* Gabaldon 1940 will be compared with those of the newly described species, *An. (Nys.) trinkae* Faran 1979.

Anopheles nuneztovari and *trinkae* are in the Albimanus Section of the subgenus *Nyssorhynchus*. The species within the Albimanus Section, or "Tarsimaculatus" Complex as it was formerly known, form a very compact well-defined group of which several members such as *An. albimanus* Wiedemann 1820, *An. aquasalis* Curry 1932 and *nuneztovari* are important vectors of malaria. The entire section is restricted to the Neotropics except for *albimanus* which extends into the Nearctic. Many of the species within the group are morphologically extremely similar, particularly in the adult female. This has resulted in considerable confusion and difficulty for malariologists and medical entomologists, and great systematic problems to taxonomists.

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Based on the male genitalia, Gabaldon (1940) described *nuneztovari* from San Carlos, Cojedes, Venezuela. One year later Rozeboom and Gabaldon (1941) described a similar form of *Nyssorhynchus* from the Amazon Basin which was named *goeldii*, and stated that *goeldii* and *nuneztovari* were distinguished by differences in the apex of the "mesosome" [phallosome] and the "fused dorsal lobes" [ventral claspettes] of the male genitalia. Again, based on the apex of the phallosome of the male genitalia which was considered to be without leaflets and broader than in *goeldii*, Causey (1945) described *dunhami* from Tefe in the upper Amazon Basin. Floch and Abonnenc (1946:1), after comparison of the male genitalia of *goeldii* with several specimens of *nuneztovari*, synonymized *goeldii* with *nuneztovari*. Lane in his "Neotropical Culicidae" (1953:268) listed *dunhami* as a synonym of *nuneztovari*, so *nuneztovari* became the valid name for the above 3 nominal species.

In 1950 Rey and Renjifo reported encountering adult females of *nuneztovari* naturally infected with *Plasmodium* sp. in Norte de Santander, Columbia, and said that *nuneztovari* was abundant inside houses entering between 2200-2400 h. In 3 months, from September to November, they collected 3,301 anophelines inside houses and stables, of which 2,722 were identified as a *nuneztovari-rangeli* mixture. A study of the eggs from 501 of those females showed that 95% were *nuneztovari*. The discovery of *nuneztovari* being strongly endophagous and a vector of malaria in western Venezuela and northern Colombia greatly stimulated interest in the ecology and behavior of this species.

Elliott (1968, 1972) studied the relationship between the biting activity of *nuneztovari* indoors and outdoors with regard to human activity in Colombia. He stated that in northern Colombia biting of *nuneztovari* reached a peak between 2200-2400 h during periods of high density. Since most blood feeding of this species takes place late in the evening (as in western Venezuela) when few people are outdoors, feeding on humans occurs "mainly inside houses" (Elliott 1972).

Interestingly, in Para, Brazil, where *nuneztovari* has not been considered important as a vector, the females seem to be primarily exophagous and exophilic. Deane, Causey and Deane (1948:895-6) reported that of the 21,967 adults of *nuneztovari* collected, only 411 or 1.9% were captured inside houses. Recent studies in Palestina, Para, also indicate that *nuneztovari* is exophilic and most active at sunset (Dixon and Roberts, personal communication). Likewise, in Surinam, Panday (1977) reported a unimodal distribution of biting activity for *nuneztovari* occurring from 1800-1900 h beginning at the termination of twilight. Elliott (1972:758) stated, "In Brazil and Surinam, in Ecuador, and almost certainly in Bolivia and Peru, there is a species [Elliott considered =*dunhami*] that bites at sunset, and therefore mainly outside houses, and preferentially on animals."

Kitzmilller, Kreutzer and Tallaferro (1973) studied the salivary chromosomes of *nuneztovari* from 3 populations in Brazil, 2 in western Venezuela and one in northern Colombia. They reported the Colombian and Venezuelan populations consistently differ from the Brazilian populations by a homozygous inversion in the X-chromosome.

These studies and others have demonstrated differences in the vector potential, behavior and X-chromosome of *nuneztovari* from different localities, and have reinforced the belief among many entomologists that *nuneztovari* is a complex of subspecies or species analogous to the *Gambiae*, *Maculipennis* and *Balabacensis* Complexes.

On examining material collected from the vicinity of Villavicencio, Colombia, that had been previously labelled *rangeli*, I noticed several peculiarities. It seemed that although the pupae looked very much like those of *An. (Nys.) rangeli* Gabaldon, Cova Garcia and Lopez 1940, the larvae resembled those of *nuneztovari*. Many of the adult females appeared like those of *nuneztovari* while a few would be identified as *rangeli* in the key. The male genitalia of these specimens were superficially very similar to those of *nuneztovari*. After detailed study of all the stages (Faran 1979) it became apparent that these specimens represented a new and different species, *trinkae*. It was found that this species can be distinguished in all stages except occasionally in the adult female from either *nuneztovari* (Table 1) or *rangeli*.

Recently, Yiau-Min Huang, of the Medical Entomology Project, Smithsonian Institution, collected and reared about 400 additional specimens of *trinkae* from the provinces of Pastaza and Napo in Ecuador. The diagnostic characters were consistent for all of these specimens.

After studying super-species or complexes such as *nuneztovari-trinkae*, where many important questions remain unanswered, it becomes apparent that a cooperative effort among investigators is essential to our understanding of these taxa. For many of the groups that have been revised in recent years using modern techniques, a very large proportion of the systematic data that is being collected has not been available to the taxonomist.

According to Simpson's definition (1961:7), "systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them." Therefore, we are all systematists even though we look for or at separate sets of characters. Attributes such as biting activity, vector potential, electrophoretic enzyme banding patterns, chromosomal banding patterns, genetics, ecology, distribution and physiology all yield a particular set of taxonomic characters. Together these characters form what Hennig (1966:24-6) has termed the holomorph of an organism (Fig. 1).

Paraphrasing Hennig, we may start from the concept or basic premise of life being a "multidimensional diversity." We may think of the dimensions in which the differences lie as comprising 3 main axes. Hennig called these the morphological, physiological and psychological dimensions. These 3 main axes are inclusive for all the various subdisciplines of each of these dimensions.

Hennig points out that organisms also differ in their position in space and time, and in the time of their origin as individuals and groups of individuals (Fig. 2). Zoogeography, ecology and genetics are disciplines that fall within these 2 new dimensions, chorologic relationships and holo-genetic relationships. Hologenetic relationships include ontogenetic

TABLE 1

Comparison of diagnostic (key) characters
in larvae, pupae, male genitalia and adult females
between *An. trinkae* and *An. nuneztovari*

	<i>An. trinkae</i>	<i>An. nuneztovari</i>
LARVAE	Seta 0-II very short, about 0.5 or less length of leaflets of 1-II, often indistinct with 1-3 branches; 3-C 0.48-0.80 length of 2-C; 4-C long, 0.7-1.0 length of 3-C; 13-IV moderately large, branches 1.5-2.0 length of leaflets of 1-IV	Seta 0-II moderately long, subequal to or longer than length of leaflets of 1-II, conspicuous, usually with 5-8 branches (4-10); 3-C 0.76-0.90 length of 2-C; 4-C moderately long, about 0.3-0.6 length of 3-C; 13-IV usually with 4-6 (3-6) branches equal to or slightly longer than leaflets of 1-IV
PUPAE	Seta 9-VII long, about 0.5 or more length of segment VII; pinna of trumpet not tapering toward apex	Seta 9-VII moderately short, about 0.33 length of segment VII; pinna of trumpet tapering toward apex
MALE GENITALIA	Ratio of length of aedeagus to length of ventral claspette usually 1.0-1.2 (1.00-1.33); ventral claspette moderately long, 0.4-0.5 length of sidepiece, width of apex from 0.4-0.5 length of claspette	Ratio of length of aedeagus to length of ventral claspette usually 1.33-1.60 (1.31-1.89); ventral claspette short, 0.25-0.40 length of sidepiece, width of apex from 0.5-0.6 length of claspette
ADULT FEMALES	Light spots on wing usually very light, white or light cream; vein C with humeral light spot 1.3-2.5 (1.3-4.0) length of basal dark spot	Light spots on wing usually cream at least on anterior veins; vein C with humeral light spot 0.7-1.3 (0.7-1.7) of basal dark spot

relationships of the different life-stages of individuals, genealogical relations of the individual and phylogenetic relationships of the species.

I believe it is clear that if it were possible to know and associate the attributes of a species along each of these axes, we could very carefully and accurately define that species. What in reality taxonomists are forced to work with is, at best, only a very limited data set or character set from one or more of these dimensions. From that they form a hypothesis about a particular group of individuals or populations that share these characters and designate all those included as belonging to a particular species.

Regrettably, we usually work independently. We each develop a certain set of characters that will fit into the above scheme, which belong to individuals or populations of a real species, but we are unable to correlate our set of characters with the sets of characters reported by other investigators. When plotted (Fig. 3) these sets of characters align on the 3 axes as before, however, the axes are not congruent, and therefore, the characters are uncorrelated. This is exactly the case so often with information concerning species complexes where morphology alone, without supporting evidence, will not delimit or identify a species. The taxonomist who has not had the opportunity to study voucher specimens that refer to a particular investigation often does not know which species, subspecies or population that investigation concerned. For this reason, much of the literature on the distribution, biology and medical importance of some species is extremely confusing.

The same is true for medical entomologists. Eldridge (1974:125) stated, "Most taxonomic problems facing a medical entomologist . . . concern attempts to match a sample of a population of insects to a published description, and knowing whether all such samples which do match are conspecific with the species described, and if samples which do not are contraspecific." This problem can be eliminated by collaboration between taxonomist and medical entomologist.

It cannot be overemphasized that the proper correlation of epidemiological and biological data aid in delimiting existing species and elucidating new species. The best example of this is the joint study currently being conducted on the Balabacensis Complex of *Anopheles* by the Medical Entomology Project, Smithsonian Institution and the Department of Medical Entomology, U. S. Armed Forces Research Institute of Medical Sciences, Thailand. These two organizations have made a coordinated effort to study the morphology, behavior, ecology, vector potential and cytogenetics of members of the Balabacensis Complex from the same localities. Their endeavors have resulted in the description of a new species, *Anopheles (Cellia) dirus* Peyton and Harrison 1979, a confirmed vector of malaria in Thailand, and have uncovered many new systematic problems.

To summarize, in order to maximize the usefulness of information that can be extracted from any data concerning a species complex, it is imperative that the data can be correlated with those of others. This will necessitate an increase in communication, interaction and cooperation among investigators, and foresight in the planning of research programs so that studies can be interfaced.

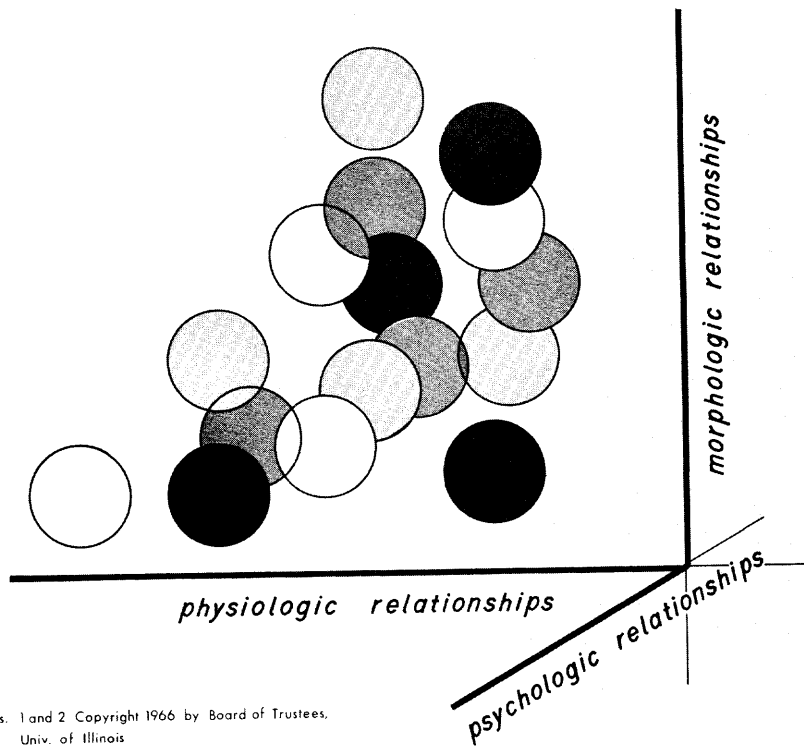
At a time when research funds are scarce and many of the arthropod-borne diseases are on the increase, it is vital that we do all we can to insure that the results of our work can be used by others. Our data will become that much more powerful. We may then be able to say with some assurance that this phenotype with this behavior and ecology, cytotype, electrophoretic banding pattern and genetics is, for example, *nuneztovari*.

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Figure 1. Parameters of the Holomorph of an Organism



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Figure 2. Parameters of an Organism or a Species

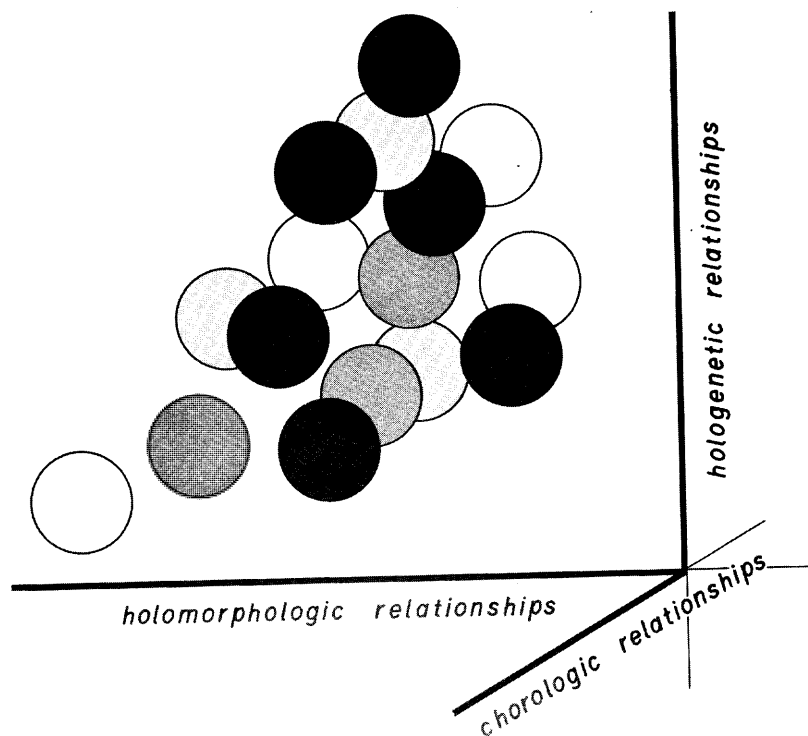


Figure 3. Taxonomic Characters of an Organism or a Species

