Journal of the American Mosquito Control Association, 13(Supplement):1–102, 1997

ANALYSIS OF THE *ANOPHELES (ANOPHELES) QUADRIMACULATUS* COMPLEX OF SIBLING SPECIES (DIPTERA: CULICIDAE) USING MORPHOLOGICAL, CYTOLOGICAL, MOLECULAR, GENETIC, BIOCHEMICAL, AND ECOLOGICAL TECHNIQUES IN AN INTEGRATED APPROACH[1]

JOHN F. REINERT, P. E. KAISER[2] AND JACK A. SEAWRIGHT

Center for Medical, Agricultural and Veterinary Entomology (CMAVE), USDA, Agricultural Research Service, 1600/1700 SW 23rd Drive, Gainesville, FL 32604

CONTENTS

[1] Mention of a commercial or proprietary product does not constitute an endorsement by the U.S. Department of Agriculture (USDA).

[2] Current address: USDA-APHIS-IS, P. O. Box 3149, Laredo, TX 78044.

ABSTRACT. The *Anopheles quadrimaculatus* complex of 5 cryptic species (i.e., *An. diluvialis* Reinert, new species; *An. inundatus* Reinert, new species; *An. maverlius* Reinert, new species; *An. quadrimaculatus* Say; *An. smaragdinus* Reinert, new species) is analyzed using multiple techniques, including morphological, cytological, molecular, genetic, biochemical, and ecological procedures. All life stages (egg, 4th-instar larva, pupa, and female and male adults) are described using morphological features, and pertinent stages or structures are illustrated. A neotype for *An. quadrimaculatus* is designated, and the synonymy of *An. annulimanus* Van der Wulp is confirmed. Several new morphological features are described. New and summarized data from published literature on hybridization, cytological, electrophoretic, molecular, and cuticular hydrocarbon studies are included. Immature and adult bionomics are given. The geographic distribution for each species is listed and shown on maps. Procedures for collecting, processing, and rearing specimens are described. Keys using morphological characters are included for the eggs, 4th-instar larvae, pupae, adult females, and male genitalia. Also, a biochemical key for the 5 species is included. Color and pattern variations of larvae and pupae are discussed.

INTRODUCTION

Anopheles quadrimaculatus sensu lato (*s.l.*) has been historically considered the principal vector of malaria in the eastern half of the United States in addition to being a pest to humans and domesticated animals. There have been recent reports of malaria transmission in the United States attributed to *An. quadrimaculatus s.l.* (e.g., in Florida, 1990 [Centers for Disease Control and Prevention (CDCP) 1991]; in New Jersey, 1991 [Brook et al. 1994]; in New York, 1993 [Layton et al. 1995]; in Texas, 1994 [CDCP 1995, World Health Organization 1995]; in Michigan, 1995 [CDCP 1996]; and in Florida, 1996 [Nayar, personal communication]). In addition to being a vector for species of *Plasmodium, An. quadrimaculatus s.l.* also has been incriminated in laboratory and/or field studies as a potential vector of the following viruses and filariae: Cache Valley virus (Kokernot et al. 1969, Wong et al. 1971, Saliba et al. 1973, Calisher et al. 1986), eastern equine encephalitis virus (Chamberlain et al. 1954, Morris 1992), Jamestown Canyon virus (Boromisa and Grimstad 1986, DeFoliart et al. 1986), Saint Louis encephalitis virus (Webster et al. 1935, Sudia et al. 1967), Semliki Forest virus (Collins and Chester 1963, Collins et al. 1964), Sindbis virus (Schiefer and Smith 1974), Tensaw virus (Chamberlain 1963, Chamberlain et al. 1969, Sudia et al. 1969, Calisher et al. 1986), *Brugia malayi* (Buckley) (Nayar et al. 1990, Nayar and Knight 1991), *Brugia pahangi* (Buckley and Edesen) (Schacher 1962, Nayar et al. 1990), *Brugia patei* Buckley, Nelson, and Heisch (Nayar et al. 1984), *Dirofilaria immitis* Leidy (Weiner and Bradley 1970; Nayar and Sauerman 1973, 1975; Todaro et al. 1977), *Dirofilaria tenuis* Chandler (Nayar and Sauerman 1973), and *Dirofilaria uniformis* Price (Duxbury et al. 1961, Bray and Walton 1961). *Anopheles quadrimaculatus* Say *sensu stricto* (*s.s.*) (as *An. quadrimaculatus* sp. A) was reported to be widespread, common, and the main mosquito pest in Mississippi (Mallet and Fritzius 1993). Also, *An. quadrimaculatus s.l.* was recently included among the top 10 mosquito pests of northern and southern New Jersey (Crans 1996a, 1996b).

In most early records for North America (during the 1800s and early 1900s), this taxon was reported as *Anopheles maculipennis* Meigen by authors who regarded their specimens to be conspecific with European populations of this species, even though Say in 1824 had described the new species *An. quadrimaculatus* (as *4-maculatus*) from the Northwest Territory, and Van der Wulp (1867) had described *An. annulimanus* (now considered a synonym of *An. quadrimaculatus s.s.*) from Wisconsin. See Reinert (1997) for a bibliography of *An. quadrimaculatus s.l.* that includes citations to early as well as recent articles covering studies from throughout its geographical range (southeastern Canada, the eastern portion of the United States, and northeastern Mexico).

During the mid- to late 1980s and early 1990s, personnel working at the United States Department of Agriculture's Insects Affecting Man and Animals Research Laboratory (IAMARL), later renamed Medical and Veterinary Entomology Research Laboratory, Gainesville, FL (now part of the Center for Medical, Agricultural and Veterinary Entomology), recognized *An. quadrimaculatus* to be a complex of 5 cryptic species that were designated A, B, C_1, C_2, and D (Lanzaro 1987; Kaiser et al. 1988b, 1988c; Lanzaro et al. 1988; Narang et al. 1989a, 1990a) based on a variety of genetic, biochemical, and molecular techniques.

In October 1994, work was initiated on a morphological taxonomic evaluation of the Quadrimaculatus Complex by the senior author, who was solely responsible for the morphological taxonomic portions of this paper. This research initially included the collection and laboratory rearing of numerous isofemale progeny broods (IPBs) of the 5 species from as many geographic localities as funding and time permitted. These IPBs were first identified by starch gel electrophoresis and then reared, producing numerous adults (females and males), with their associated pupal and 4th-instar larval exuviae, which were of the high quality necessary for conducting an analysis of a mosquito species complex. During the morphological taxonomic analysis, 141 IPBs were reared for study. A total of 21,684 specimens were examined during this study as follows: 3,282 females and 2,455 males with their associated pupal and 4th-instar larval exuviae; 169 male genitalia; 2,974 4th-instar larvae; and 1,335 nonassociated adults, pupae, and pupal and 4th-instar larval exuviae. This material was mounted and

examined for morphological characters useful in distinguishing the 5 species (*An. diluvialis* Reinert, new species; *An. inundatus* Reinert, new species; *An. maverlius* Reinert, new species; *An. quadrimaculatus* Say; and *An. smaragdinus* Reinert, new species). During the study of this material, the variability of some morphological characters was noted and reported in the keys, descriptions, and discussions for the species of the Quadrimaculatus Complex. An explanation of this variability within a species may be provided by Narang et al. (1993) as follows: "Population genetic studies on a number of insect species have shown that there is no single population that represents the entire gene pool of a species. A species is an assemblage of variable populations dynamically evolving in response to geographical, temporal, clinal, and other ecologically related patterns, that is genetically distinct from other species. The genetic structure of each population is a unique response to a local environment." These authors further state: "Because of the complexity of population analysis, the resolution possible with a single technique can be unsatisfactory, and analysis of individuals of populations using a combination of different techniques becomes desirable." During the present study, data for the type series of each species of the complex were characterized by, and provided to include: morphological analysis of females, males, male genitalia, pupal exuviae, and 4th-instar larvae and larval exuviae; starch gel electrophorsis of 15 adult enzymes; examination of salivary gland polytene X chromosomes; and analysis of cuticular hydrocarbons of females. Nontype series specimens of the species also were used to provide data on hybridization, other molecular evaluations, and ecology.

This paper represents the culmination of a multiyear, multiphase, and multidiscipline research project involving numerous investigators that was directed at elucidating the *An. quadrimaculatus* species complex. Previous portions of the project emphasized the characterization of the molecular and genetic composition of the complex, the ecology of the species, and sampled specimens from a broad geographic range (see Seawright et al. [1992] and the following distribution section). The focus of the present phase of the project was, for the species of the complex, to provide: 1) an analysis, descriptions, and illustrations of the morphological features of the adults (both sexes) to include male genitalia, pupae, 4th-instar larvae, and eggs; 2) formal names for the 4 new species of the complex and selection of a neotype for *An. quadrimaculatus s.s.*; 3) keys for all life stages using morphological features; 4) known bionomics and distribution; 5) summarized molecular, cytological, genetic, and biochemical data concerning the species; 6) an analysis of female cuticular hydrocarbons; and 7) detailed information on collection, rearing, and preparation procedures of specimens.

Numerous reports have been published on *An. quadrimaculatus s.l.* (see Reinert 1997) concerning the bionomics, distribution, and ability to transmit disease pathogens, but, in comparison, little has been published on these subjects for the specific members of the complex. For the 5 species, the available information on these topics is included here. New as well as summarized information is provided concerning studies on hybridization, cytology, electrophoresis, molecular evaluation, cuticular hydrocarbons, and bionomics.

Even though considerable data are known for the 5 species of the Quadrimaculatus Complex, on reflection, much information remains unknown concerning the ecology of adult and immature stages, maximum geographical ranges of each species, adult (both female and male) feeding behavior, and ability to transmit disease pathogens. It is recommended that the establishment of new distributional records, bionomic data, and other studies be based on identified material from reared isofemale progeny broods; the use of a molecular, biochemical, or genetic technique; identified material from multiple specimens of individually reared adults with their linked pupal and 4th-instar larval exuviae; or the use of a combination of procedures.

MATERIALS AND METHODS

Morphological taxonomic procedures: Initially, only specimens that were individually reared with associated adult and immature exuviae obtained from feral IPBs that had been identified by starch gel electrophoresis were examined for features that would distinguish each of the life stages of the 5 sibling species. Once useful characters were identified from the samples of the selected IPBs, they were checked in the remaining material of the IPBs from all geographic localities to determine their stability and variability. Some characters could not be observed on every specimen due to distortion resulting from mounting. In some localities and in some IPBs, a character was uniformly consistent, but when it was examined in specimens from other localities, it displayed considerable variability and was thus eliminated from further consideration. After the electrophoretically identified IPBs had been examined for the consistent characters, all remaining material was identified based on these features. In the final analysis, and based on the material available, only features that were consistent throughout the known geographic range of the species were selected for use. Finally, based on the aforementioned evaluations, characters were selected for construction of the keys, inclusion as primary features in the discussion sections, development of the descriptions, and illustrations.

Adults were examined using a Wild M5 binocular stereomicroscope with a 4-step magnification changer, fitted with 10× wide field eyepieces, a 1.25× phototube, and a 1.50× supplementary objective. Total magnification provided with the 4

steps was 11.25, 22.50, 46.88, and 93.75×. Measurements were made with an ocular micrometer having a linear scale of 100 divisions that had been calibrated using a stage micrometer. The lighting source used bifurcated gooseneck fiberoptic illumination tubes and a 75-W xenon lamp, which provided a precisely oriented cold white light. Pinned adults were studied using an adjustable examination stage with biaxes rotation capability that provided observation and lighting of the specimen at any angle. Light was directed onto the upper surface of the specimen at an angle (*ca.* 40° and 140°) by the 2 illumination tubes. Most observations concerning details of the setae were made at a magnification of 93.75×, while those of scale patterns and measurements of structures were accomplished at 46.88×.

Slide-mounted immature specimens and male genitalia preparations were examined using an Olympus BH2-PC phase contrast microscope with wide field eyepieces (10 and 15×) and 10, 20, 40, and 60× objectives, and were illuminated with a 100-W xenon lamp. Most observations were made at 200 and 400×, but some counts of very finely branched setae were made at 600×. Measurements were made with an ocular micrometer having a linear scale of 100 divisions that had been calibrated using a stage micrometer.

Chaetotaxy and anatomical nomenclature used follow Harbach and Knight (1980, 1982) except for terms proposed by Reinert (1990) and as noted below. Some terms used for the eggs follow Linley et al. (1993). Several new terms and/or modifications to previous methods of measuring or describing and/or interpreting structures are provided in the structure and terminology section below.

Observations on the condition of the cephalothoracic postscutal area (intact or split by the dorsal ecdysial opening; see Reinert et al. 1996) were made on the pupal exuviae prior to mounting on microscope slides. However, in most cases, slide-mounted specimens of *An. diluvialis* and *An. inundatus* displayed a jagged tear to one side of the midline, indicating that the prescutal area was intact prior to mounting. Length of pupal seta 1-VII was compared to tergum VIII length at the point of attachment of 1-VII. The length of the refractile border (Fig. 7D) is measured as the direct length along a line drawn perpendicular to the paddle length (not measured along the curve).

Careful, exacting measurements of the 4th-instar larval antenna (Fig. 7) were found to be important in separating some of the species. Measurements of the antenna were taken at 400× magnification. When counting the number of long spines of the larval pecten plate, the posterior 1 or 2 spines were included if they were at least 0.6 the length of the other long spines.

In the taxonomic treatment section, *An. quadrimaculatus s.s.* (the nominal species of the Quadrimaculatus Complex) has all life stages or structures fully described and the other species of the complex are compared to it; therefore, those features that were identical were not included, for the most part, in the other species descriptions. The word "usually," when used in the morphological descriptions, refers to a character state with an occurrence of 80% or greater (e.g., proepisternum usually with 2–6 upper setae [98.6%]) and the word "often" is used to denote characters with a percentage of 51 to 79. When the word "both" is used in keys, descriptions, and tables, it refers to the character on both sides of the specimen (e.g., sum of setae on both scutal fossal areas usually 20–40). Descriptions are based on laboratory-reared specimens that produced mint condition adults with all setae and scales intact. Field-collected adults are usually in poorer condition, and some diagnostic characters may be missing. Therefore, the use of the additional characters in the primary features list, provided in the discussion section for each species, should make these specimens identifiable.

In the discussion section for each species, the list of additional features used for identification contains important characters of that species, presented in decreasing order of percentage. The list of primary features, given in decreasing order of separation for species of the complex, uses numbers corresponding to those mentioned directly above (in parentheses) in the list of additional features. The decreasing sequence of numbers in the latter list was derived by summing the percentages for the same character (in the list of additional features) for the 2 compared species and dividing by 2. A similar procedure is followed for characters used in the keys, and these data are provided in footnotes to provide a measure of the feature's reliability.

Keys using morphological characters are included for all life stages (i.e., females, male genitalia, pupae, 4th-instar larvae, and eggs). Each character used in the first 4 keys includes a footnote denoting, for each species, the percentage of specimens correctly identified from specimens available during the study. Commonly, in many, if not most, published taxonomic keys, not all specimens can be keyed correctly with the characters used, and this is especially true for species complexes. The keys prepared for the Quadrimaculatus Complex, with the exception of the male genitalia key, provided, for the most part, a very high percentage of correct identifications. Additionally, a list of primary features is provided in the discussion section of each species that can be used for making identifications (see above). Persons using the illustrated key by Darsie and Ward (1981) can key females of all 5 species of the Quadrimaculatus Complex to couplet 12, page 83, and 4th-instar larvae to couplet 12, page 188, at which point they can use the keys presented here for making a specific identification. In the key to larvae by Darsie and Ward (1981), some difficulty may be experienced with couplet 8, page 185, in that for some specimens of *An. dilu-*

vialis, An. inundatus, An. maverlius, and *An. smaragdinus,* the branching of seta 1-P occurs more proximal than 0.5 of the length. The key to eggs is based on data published by Linley et al. (1993). Even though eggs of each species of the complex can be separated by the characters outlined in the key, it should be noted that the data reported by Linley et al. (1993) were based on small numbers of eggs from only a few specimens and from a very limited portion of the known geographic range of the species; therefore, variability of the characters for other populations is unknown. Also, eggs from different seasons were not examined to determine seasonal variation. The biochemical key to the species of the complex is modified from Narang et al. (1989a) and includes data given by Narang et al. (1990a) and in Table 32.

Abbreviations or symbols used in figures, synonymies, literature references, and distribution sections for the various life stages and structures follow: ♀ = female; ♂ = male; ♂g = male genitalia; p = pupal exuviae; P = pupa; l = 4th-instar larval exuviae; L = 4th-instar larva; and E = egg. In literature references sections, an asterisk following an abbreviation or symbol indicates that at least part of the stage or structure was illustrated. Abbreviations of states on specimen labels and in the references cited section follow the list in Publication 201, January 1995 edition, of the U.S. Postal Service's *A Consumer's Guide to Postal Services and Products.* Appendix 2 lists abbreviations used for the names of collectors of specimens.

Illustrations are provided that display pertinent characters of the adults, male genitalia, pupae, 4th-instar larvae, and eggs. The entire chaetotaxy of the pupa and 4th-instar larva is figured for the 5 species of the complex. Salivary gland polytene X chromosomes (Fig. 20) and electromorphs of enzyme loci (Figs. 21 and 22) are provided for specimens from the type series IPBs of each species of the complex. Figures 18 and 19 of the eggs are modified from Linley et al. (1993). The figures are preceded by a list of abbreviations used in the illustrations and a listing of the figures. Scale lines used in Figs. 1–17 are in millmeters.

Tables are included that provide listings for numbers of setae (adults) or setal branching (pupae and 4th-instar larvae). When percentages are used, the total for all setal conditions may not always equal 100% due to rounding error (e.g., 0.05 rounded up to 0.10). For each seta in Tables 6–10 and 18–22, the range is given and followed by the mode in parentheses. Several tables include the sum of a seta on both sides of the specimen, and others include the sum of 2 setae on both sides.

Distributions of the 5 species are plotted in Figs. 23–27, in which each county or parish is blackened to represent one or more collections. Only material positively identified as belonging to a specific species of the complex by current understanding is included on the maps. Collection data for all specimens examined in the morphological study are listed for each species. Additionally, data on all specimens examined by biochemical, molecular, or genetic techniques are given separately in the distribution section. Literature records containing positive identifications of the species are also provided.

The voluminous literature concerning *An. quadrimaculatus s.l.* could not, for the most part, be ascribed to a specific species of the Quadrimaculatus Complex; therefore, only those articles in which the species could be positively identified are included in the synonymy, literature references, bionomics, and distribution sections. In the literature references sections, citations are listed chronologically. An extensive listing of the literature (over 1,900 citations) dealing with *An. quadrimaculatus s.l.* was provided by Reinert (1997) and included all aspects (e.g., identification, morphology, physiology, cytology, ecology, behavior, surveillance, and distribution) except those dealing strictly with control.

Field collection and handling procedures: Immatures were collected using a white enameled (or plastic) pint dipper with a *ca.* 1.52-m fiberglass pole inserted in the handle. The white background of the dipper provided for easy observation of any larvae or pupae collected, and the long handle enabled an extended reach into the immature habitat with minimal effort. Larvae and pupae were transferred with a small polyethelene pipette from the dipper to a polyethylene Whirl-Pak® bag with an attached puncture-proof covered wire tab on the upper lip used for sealing the bag. Either a 177.4- or a 532.3-ml bag was used, depending on the size of the sample collected. The bag was filled *ca.* 0.6 full of habitat water (the remaining *ca.* 0.4 contained atmospheric air), labeled with the collection data using a permanent ink marker, sealed with the wire tab, and placed in a chilled drink cooler (see below) for transportation to the laboratory.

During the morphological study, efforts were concentrated on collecting females for rearing progeny broods. Adults were aspirated from daytime resting sites with a battery-powered mechanical aspirator (a modified Black & Decker Dustbuster® described by Meek et al. [1985]). The aspirator was further modified by using 2 extension tubes (*ca.* 46 and 86 cm long) fabricated from 3.3-cm (outside diam) white polyvinyl chloride (PVC) pipe. By using 1 or a combination of the 2 PVC tubes, most adult resting sites (e.g., in large rot cavities of trees, livestock barns, wooden resting boxes, and outdoor shelters, and under bridges, culverts, and eaves of buildings) could be easily reached. Light traps were not used for collections; however, light traps, some baited with CO_2 and/or octenol as an attractant, have shown mixed results in capturing adults of *An. quadrimaculatus s.l.* (e.g., Takken and Kline 1989, Kline et al. 1991, Jensen et al. 1993). During the current investigations, light traps were not used, primarily because of the logistical problems of trapping at several sites per night, the difficulty and

time required to sort unwanted species, the poor condition of trapped specimens, the chance of inclement weather, and the mixed results observed in collection of the different species of the complex. It was found that, when sampling at locations distant from our laboratory, the most efficient method of collecting adults that were mated and/or gravid and in good condition was by aspiration from daytime resting sites. By using this procedure, collections from several sites could be completed each day. This adult collection procedure, therefore, was somewhat biased and may not have provided an accurate measurement of population size or species frequencies; however, in Florida, some collections included 4 species (*An. inundatus, An. maverlius, An. quadrimaculatus,* and *An. smaragdinus*) that were taken together resting in the same site (e.g., in a rot cavity of a tree, Blountstown, Calhoun Co.; under a bridge, Westville, Holmes Co.; under a bridge, Chattahoochee, Jackson Co.; under eaves of a house, Bruce, Walton Co.; and in a rot cavity of a tree, Red Bay, Walton Co.).

In the field, living adults that had been aspirated from resting sites were transferred to 1.89-liter paper cans fitted with a nylon screen on the top end and a cotton muslin sock on the other end, covered with a wet paper towel on the screened end, and placed upright in a large plastic drink cooler chilled inside with a 2.54–5.08-cm layer of ice cubes enclosed in plastic bags that was situated beneath a 2.54-cm-thick sheet of Styrofoam®. The cooler was then transported by car or airplane to the laboratory at Gainesville, FL. However, field-collected adults to be examined solely by starch gel electrophoresis or by another molecular technique were placed in a liquid nitrogen container that was transported to the laboratory where the specimens were sorted and transferred to an ultralow freezer (maintained at −80°C) for storage.

Laboratory and isofemale progeny brood rearing procedures: On arrival at the laboratory, adults were lightly anesthetized with CO_2, transferred to a white enameled pan placed on a chill table, tentatively identified, and each gravid female was isolated in an oviposition chamber (labeled with an identification number) if a progeny brood was to be reared. If the specimen was to be examined by electrophoresis, it was placed in a labeled polypropylene microtube with a snap cap and stored in an ultralow freezer at −80°C. The F_1 progeny were used for morphological taxonomic, cytological, hybridization, molecular, and cuticular hydrocarbon studies.

On the day of arrival at the laboratory, nongravid females were transferred to 0.28-liter paper cans (35 mm deep) with screen bottoms, placed under a black cloth, and fed on guinea pigs or, in some cases, on volunteers (the authors). Animals were utilized and maintained in accordance with the guidelines outlined in the National Research Council's 1985 edition of *Guide for Laboratory Animal Facilities and Care* and the current 1996 edition of *Guide for the Care and Use of Laboratory Animals.* Engorgement rate of field-collected anophelines was enhanced by holding the females in the 0.28-liter paper cans for 1 h in a darkened room prior to attempting to feed. For cytological studies, the engorged females were held for 28 h at 25°C and then dissected. For studies requiring F_1 progeny broods, the engorged females were maintained for 72 h at 25°C and traumatized by removal of a wing, and each adult was isolated in an oviposition chamber consisting of an opaque plastic vial (4.5 cm diam × 6.5 cm high) lined inside with a strip of filter paper (4 × 15 cm), containing *ca.* 26 ml tap water, and capped with a screen-covered lid. Females that oviposited were assigned an identification number, frozen at −80°C, and held in an ultralow freezer for isozyme analysis. The eggs were incubated in the oviposition chamber at 27°C, and hatching normally started after *ca.* 48 h. Eggs of *An. diluvialis* and *An. inundatus* usually needed to be held for 48 h and then placed under vacuum for *ca.* 5 min to stimulate hatching.

Progeny broods of newly emerged 1st-instar larvae were transferred to polystyrene tissue culture dishes (15 cm diam × 2.5 cm deep, with covers) containing 125 ml tap water (warmed to 27°C) and labeled with the identification number, and 0.5 ml of food was added. First-instar larval food consisted of a 2% aqueous suspension of 2 parts TetraMin® Baby "E" fish food and 1 part brewer's yeast (2:1 food). Twenty-four hours later, each brood was transferred to a white enameled pan (24 × 40 × 6 cm deep, containing *ca.* 0.8 liters of tap water and 2.5 ml of 2:1 food) in the case of small broods or to an opaque whitish Nalgene® tray (38 × 46 × 12.5 cm deep, containing *ca.* 1.5 liters of tap water and 3.5 ml of 2:1 food) for larger broods. Subsequently, food for 2nd- through 4th-instar larvae was provided by lightly dusting onto the water surface, 1 or 2 times daily, a finely ground, dry mixture of 1 part Baby "E" fish food, 1 part brewer's yeast, and 1 part hog chow. All stages of the larvae (and adults) were reared at a temperature of 26 ± 1.0°C, 80 ± 5.0 relative humidity, and a 14 h light–10 h dark photoperiod (provided by 4 light banks that turned on/off at 15-min intervals in the morning and evening, respectively).

Individual rearing procedures: Detailed procedures, outlined in Appendix 1, were used to obtain individually reared adults, with their associated pupal and 4th-instar larval exuviae, for use in the taxonomic analysis. Most of these specimens were from isofemale progeny broods. Many broods resulted in long series of reared specimens that were useful in evaluating variability of morphological characters.

Specimen preparation and preservation procedures: Appendix 1 provides techniques for preparing and preserving adults, pupal and 4th-instar larval exuviae, larvae, and genitalia used for speci-

mens examined in the morphological taxonomic studies.

STRUCTURE AND TERMINOLOGY

During the morphological taxonomic analysis, it became necessary to describe several new terms and/or modifications to previous methods of measuring or describing and/or interpreting structures; therefore, the following treatment is provided (by J.F.R.). Mounting procedures for specimens used in the descriptions follow those outlined in Appendix 1.

When examining adults of the Quadrimaculatus Complex with a stereomicroscope, some difficulty may be experienced initially in separating scales from setae on the scutum and scutellum. The following features usually can be used to distinguish the two: setae were attenuated, simple, longer, dark brown (occasionally some were pale), and erect or semierect, while scales were piliform, shorter, golden, and decumbent. Also, when slide-mounted adults were viewed under high magnification with a phase contrast microscope, the setae were smooth and arose from larger alveoli, while the scales possessed longitudinal ridges and arose from small alveoli. The number of setae on the median and posterior scutal fossal areas formed a continuous patch (combined median and posterior patches referred to as **scutal fossal setae** in this study) and was found to be important in separating some species of the complex. This scutal fossal group of setae was usually separated by a small bare space from the small patch of setae on the anterior scutal fossal area (restricted to the anterior margin of the scutum) and the dorsocentral setae and by the prescutal suture from the antealar setae.

For the male genitalia of the Quadrimaculatus Complex, the following terminology is proposed and used. Descriptions and observations were made from genitalia that had been dissected, positioned in a flattened aspect, and mounted in Canada balsam on microscope slides. The claspette of anophelines appeared to be homologous with the basal mesal lobe of other Culicidae, which is in agreement with Belkin (1962). In species of the complex, the claspette (Figs. 3 and 4) is separated into a dorsal, heavily pigmented, nonspiculate lobe bearing seta 5 on the posterior margin, and is broadly fused to the ventromesal, heavily pigmented, spiculate lobe bearing setae 1–4 on its posterior margin. The ventromesal lobe is connected mesally by a narrow band with its bilateral mate. Seta 1 is long, narrow, curved, lanceolate, and situated mesally. Seta 3 is long, flattened, distally curved, and lanceolate with a long, sheathlike basal structure and is situated posterolaterally to seta 1 and ventromesally to seta 4. Seta 4 is moderately long to long, flattened, and lanceolate with a moderately long, sheathlike basal structure (*ca.* 0.5 the length of seta 2 sheathlike basal structure) and is situated between seta 3 and seta 5. Seta 5 is composed of 1–4 (numbered 5a–5d, mesally to laterally) moderately long, flattened setae with apices rounded, often expanded, and usually fused; stems of setae are separated and may be borne on an elevated process. Seta 2 (when present; if 1 or more are present, then they are numbered 2a, 2b, etc.) is short, simple, and situated laterally to seta 1 and mesally to seta 3. The terminology of the claspette proposed above differs from that given by Martini (1933).

Tergum IX (Te-IX) (Fig. 5) of the male genitalia consists of a **median band,** which is heavily pigmented and sclerotized; its length is measured at the midpoint, and its width is measured between the outer margins of the lateral apotomes. The elongate **posterolateral lobes** (PlLs) extend caudally from the median band's posterior lateral margin. The **PlL length** is measured along a perpendicular line from the midpoint of the posterior margin of Te-IX to a line drawn mesally from the apex of the PlL. The **laterobasal band** consists of a heavily pigmented and sclerotized structure extending lateroventrally from the anterolateral margin of the median band and has its base joined to the dorsolateral margin of sternum IX. The **PlL/Te-IX index** is determined by dividing the PlL length by the Te-IX median band length.

The eighth abdominal segment is discussed here with the male genitalia, since it rotates 180° with the genitalia after the adult emerges from the pupal exuviae and is often specially modified. **Basal median setae** (BMS) (Fig. 3) consist of a pair of tiny setae situated near the midline on the anterior, nonspiculate, more heavily pigmented portion of sternum VIII.

New terms proposed for pupal exuviae, which had been mounted in a flattened aspect on a microscope slide, follow. The **anterior angle of scutum** (Fig. 11B) is the angle formed by the anterior and dorsal margins of the scutum. **Lateral line of median keel** (Fig. 11A) is a noticeable creaselike line formed along the lateral margin of the median keel resulting from the straightening effect of the median keel during the flattening process of mounting on a microscope slide. **Trumpet length** (Fig. 7B) is measured from the point of articulation at the base along an angled line to the extreme apex; the angle occurs on the margin of the side opposite the pinna opening and is near the level of the base of the pinna, where the trumpet begins to expand in size. **Trumpet width** (Fig. 7B) is measured along a line extending from the base of the pinna to the angle of the trumpet length. These measurements are used because they gave reproducible values even with the distortion resulting from slide-mounting the flared apical portion of the trumpet. The **male genital lobe index** (Fig. 7C) is the ratio resulting from the median ventral length measured to the apex of the lobe being divided by the ventral width measured at the widest point.

Through a phase contrast microscope, no discernible separation was seen between the fused fla-

gellum and pedicel of the 4th-instar larval antenna; therefore, these 2 structures are treated here as the **flagellum**. The **flagellum length** (Fig. 7A) of the larval antenna is measured on the mesal margin as the straight-line distance from the base (defined by being slightly larger than the diameter of the scape) to the apex minus the apical stout spicules and setae. **Seta 1-A's point of insertion** (Fig. 7A) is measured along the mesal margin from the base of the flagellum to the proximal margin of the alveolus.

TAXONOMIC TREATMENT

The arrangement of higher categories for the species complex treated here follows Harbach (1994), i.e., Genus *Anopheles,* Subgenus *Anopheles,* Angusticorn Section, Anopheles Series, Maculipennis Group, Maculipennis Subgroup, and Quadrimaculatus Complex. This article should be utilized as a review of the internal classification of the genus *Anopheles.*

The morphological taxonomic treatment that follows includes keys to females, male genitalia, pupae, 4th-instar larvae, and eggs; synonymy and/or literature references; descriptions and illustrations of females, males, male genitalia, pupae, 4th-instar larvae, and eggs; discussion and comparison of species; tables listing ranges of setae for adults, pupae, and 4th-instar larvae; information on type material; and bionomics.

KEYS TO SPECIES

In the following keys, characters are used that distinguish the species of the Quadrimaculatus Complex. A footnote for each character used denotes the percentage of specimens correctly identified from material available during the study and thus provides an indication of the reliability of the character. Additional features, not included in the keys, that are useful in identifying the sibling species are provided in the discussion section for each species. Separation of the species in a species complex using morphological features often necessitates the use of a combination of characters, which may require additional effort to discern.

Females

1. Upper proepisternum usually with 2–6 setae (Fig. 2F)[1]; tibiae II (and usually I) with pale scales apically (Fig. 1C)[2] 2
– Upper proepisternum usually with 7–26 setae (Fig. 2G)[1]; tibiae I, II dark-scaled (Fig. 1E)[2] 3
2. Sum of setae on both scutal fossal areas usually 41–75[3]; scutal fossal area usually with 21–45 setae on 1 side (Fig. 2A)[4]; prealar area usually with 6–12 setae[5]; interocular area usually with 7–12 setae[6] . . . . . *quadrimaculatus*
– Sum of setae on both scutal fossal areas usually 17–40[3]; scutal fossal area usually with 8–20 setae on 1 side (Fig. 2B)[4]; prealar area usually with 1–5 setae[5]; interocular area usually with 4–6 setae[6] . . . . . *smaragdinus*
3. Dorsocentral area usually without golden piliform scales on anterior margin (Fig. 2D)[7] . . *diluvialis*
– Dorsocentral area with few to several golden piliform scales on anterior margin (Fig. 2E)[7] 4
4. Femur I usually with pale scales apically (Fig. 1G)[8]; femur II usually with pale scales apically (Fig. 1G)[9]; scutal fossal area usually with 9–20 setae (Fig. 2C)[10]; maxillary palpus often less than 1.0 length of proboscis[11]; interocular area usually with 3–5 setae[12] . . . . . *maverlius*
– Femur I dark-scaled (Fig. 1F)[8]; femur II usually dark-scaled (Fig. 1F)[9]; scutal fossal area usually with 21–32 setae (Fig. 2E)[10]; maxillary palpus 1.0 or greater length of proboscis[11]; interocular area usually with 6–9 setae[12] . . . . . *inundatus*

[1] Feature correctly distinguished 97.3% of specimens (*diluvialis* = 99.0%; *inundatus* = 96.0%; *maverlius* = 94.4%; *quadrimaculatus* = 98.6%; *smaragdinus* = 98.5%).

[2] Feature correctly distinguished 100% of specimens.

[3] Feature correctly distinguished 95.4% of specimens to species (*quadrimaculatus* = 97.1%; *smaragdinus* = 93.7%).

[4] Feature correctly distinguished 93.5% of specimens to species (*quadrimaculatus* = 93.1%; *smaragdinus* = 93.9%).

[5] Feature correctly distinguished 85.8% of specimens to species (*quadrimaculatus* = 83.9%; *smaragdinus* = 87.6%).

[6] Feature correctly distinguished 89.2% of specimens to species (*quadrimaculatus* = 98.0%; *smaragdinus* = 80.4%).

[7] Feature correctly distinguished 97.8% of specimens (*diluvialis* = 96.7%; *inundatus* = 97.5%; *maverlius* = 99.1%).

[8] Feature correctly distinguished 98.7% of specimens to species (*inundatus* = 100%; *maverlius* = 97.3%).

[9] Feature correctly distinguished 92.7% of specimens to species (*inundatus* = 86.2%; *maverlius* = 99.1%).

[10] Feature correctly distinguished 82.4% of specimens to species (*inundatus* = 82.7%; *maverlius* = 82.1%).

[11] Feature correctly distinguished 85.0% of specimens to species (*inundatus* = 100%; *maverlius* = 70.0%).

[12] Feature correctly distinguished 85.0% of specimens to species (*inundatus* = 90.1%; *maverlius* = 79.9%).

Male Genitalia[1]

1. Aedeagus with apical leaflet narrow, pale, and moderately long (Fig. 4C)[2]; tergum VIII usually with apex straight or very gently convex (Fig. 6A)[3]; claspette with seta 2 present (Fig. 4C)[4] 2
– Aedeagus with apical leaflet broader, dark, and long (Fig. 4A)[2]; tergum VIII usually with median depression on apical margin (Fig. 6C)[3]; claspette with seta 2 usually absent (Fig. 4D)[4] . . . 3
2. Claspette with seta 4 usually longer than seta 5 (Fig. 4C)[5]; sum of both seta 5 often with 3,4 stems[6] . . . . . *quadrimaculatus*
– Claspette with seta 4 often shorter than seta 5

(Fig. 4B)[5]; sum of both seta 5 often with 5,6 stems[6] . . . . . *smaragdinus*

3. Apical margin on tergum VIII with moderately deep median depression separating broadly rounded lobes (Fig. 6C)[7]; claspette usually with seta 4 situated dorsally at same level or slightly mesal to seta 3 (Fig. 4A)[8]; gonostylus usually without tiny setae on proximal 0.30–0.33 (Fig. 4A)[9]. . . . . *maverlius*

– Apical margin of tergum VIII with shallow median depression (Figs. 6D, 6E)[7]; claspette with seta 4 situated dorsolaterally to seta 3 (Figs. 4D, 4E)[8]; gonostylus with tiny setae usually extending further proximally (Figs. 4D, 4E)[9]. . . . . *diluvialis, inundatus*

[1] Observations of genitalia made from component parts dissected, positioned in a flattened aspect, and mounted in Canada balsam.

[2] Feature correctly distinguished 100% of specimens.

[3] Feature correctly distinguished 98.6% of specimens (*diluvialis* = 97.5%; *inundatus* = 100%; *maverlius* = 100%; *quadrimaculatus* = 98.0%; *smaragdinus* = 97.5%).

[4] Feature correctly distinguished 91.6% of specimens (*diluvialis* = 92.5%; *inundatus* = 71.4%; *maverlius* = 94.1%; *quadrimaculatus* = 100%; *smaragdinus* = 100%).

[5] Feature correctly distinguished 79.8% of specimens to species (*quadrimaculatus* = 81.6%; *smaragdinus* = 78.0%).

[6] Feature correctly distinguished 69.0% of specimens to species (*quadrimaculatus* = 67.3%; *smaragdinus* = 70.7%).

[7] Feature correctly distinguished 99.2% of specimens (*diluvialis* = 97.5%; *inundatus* = 100%; *maverlius* = 100%).

[8] Feature correctly distinguished 99.0% of specimens (*diluvialis* = 100%; *inundatus* = 100%; *maverlius* = 96.9%).

[9] Feature correctly distinguished 88.0% of specimens (*diluvialis* = 90.0%; *inundatus* = 88.9%; *maverlius* = 85.0%).

Pupae (Exuviae)

1. Dorsal apotome with well-developed median apical projection (Fig. 10D)[1]; scutum with anterior angle broad and approximately 90° (Fig. 10B)[2] . . . . . *maverlius*

– Dorsal apotome without apical projection (Fig. 11D)[1]; scutum with anterior angle acute (Fig. 11B)[2] 2

2. Cephalothorax with lateral line of median keel long, extending posteriorly to seta 8-CT (Fig. 12A)[3]; cephalothorax with postscutal area split by dorsal ecdysial opening (Fig. 12A)[4]; seta 1-VII usually slightly longer than length of tergum VIII (Fig. 12C)[5] 3

– Cephalothorax with lateral line of median keel short, extending posteriorly only to point of trumpet attachment (Fig. 8A)[3]; cephalothorax with postscutal area intact (Fig. 8A)[4]; seta 1-VII usually 0.75 or less length of tergum VIII (Fig. 8C)[5]. 4

3. Sum of branches for both seta 1-Pa usually 4–12[6]; seta 1-Pa usually with 2–6 branches (Fig. 11C)[7]; sum of branches for both seta 9-VIII usually 24–43[8]. . . . . *quadrimaculatus*

– Sum of branches for both seta 1-Pa usually 2,3[6]; seta 1-Pa usually single (Fig. 12C)[7]; sum of branches for both seta 9-VIII usually 12–23[8] . . . . . *smaragdinus*

4. Distribution limited to part of Florida (Fig. 23)[9]; sum of branches for both seta 10-CT usually 2–5[10] . . . . . *diluvialis*

– Distribution otherwise (Fig. 24)[9]; sum of branches for both seta 10-CT usually 6–12[10] . . . . . *inundatus*

[1] Feature correctly distinguished 100% of specimens.

[2] Feature correctly distinguished 99.9% of specimens (*diluvialis* = 100%; *inundatus* = 100%; *maverlius* = 99.6%; *quadrimaculatus* = 100%; *smaragdinus* = 100%).

[3] Feature correctly distinguished 99.9% of specimens; visible only in slide-mounted exuviae (*diluvialis* = 100%; *inundatus* = 100%; *quadrimaculatus* = 100%; *smaragdinus* = 99.6%).

[4] Feature correctly distinguished 100% of specimens; condition of postscutal area best observed while preparing specimen for mounting prior to dissection of cephalothorax from metanotum and abdomen.

[5] Feature correctly distinguished 94.4% of specimens (*diluvialis* = 92.1%; *inundatus* = 93.3%; *quadrimaculatus* = 98.1%; *smaragdinus* = 94.0%).

[6] Feature correctly distinguished 98.0% of specimens to species (*quadrimaculatus* = 97.8%; *smaragdinus* = 98.2%).

[7] Feature correctly distinguished 95.3% of specimens to species (*quadrimaculatus* = 98.3%; *smaragdinus* = 92.3%).

[8] Feature correctly distinguished 97.4% of specimens to species (*quadrimaculatus* = 97.5%; *smaragdinus* = 97.2%).

[9] Feature correctly distinguished 100% of specimens to species.

[10] Feature correctly distinguished 87.3% of specimens to species (*diluvialis* = 91.8%; *inundatus* = 82.8%).

Fourth-Instar Larvae

1. At least 2 of following 3 characters present[1]: sum of both seta 1-A usually with 5–17 branches[2]; sum of both seta 8-III plus both seta 8-VI usually with 8–18 branches[3]; seta 2-C long, 1.30 or greater length of seta 3-C (Fig. 16A)[4] 2

– At least 2 of following 3 characters present[1]: sum of both seta 1-A usually with 18–29 branches[2]; sum of both seta 8-III plus both seta 8-VI usually with 19–28 branches[3]; seta 2-C shorter, 1.29 or less length of seta 3-C (Fig. 15A)[4] . . . . . *maverlius*

2. At least 2 of following 3 characters present[5]: both pecten plates each usually with 6–8 long spines (Fig. 16G)[6]; antennal flagellum length usually

0.31–0.38 mm[7]; seta 1-A usually inserted 0.31–0.56 from base of flagellum (Fig. 16A)[8] 3
– At least 2 of following 3 characters present[5]: one or both pecten plates usually with 9–11 long spines (Fig. 14G)[6]; antennal flagellum length usually 0.24–0.30 mm[7]; seta 1-A usually inserted 0.19–0.30 from base of flagellum (Fig. 14A)[8] 4
3. Sum of both seta 3-C with 25–63 thin branches which are more or less widely separated distally[9]; sum of both seta 8-V usually with 7–10 branches[10]; abdominal segment VIII usually with anteromedian sternal plate (Fig. 16E)[11] . . . . . *quadrimaculatus*
– Sum of both seta 3-C usually with 64–101 thicker, bunched branches[9]; sum of both seta 8-V usually with 4–6 branches[10]; abdominal segment VIII usually without anteromedian sternal plate (Fig. 17E)[11] . . . . . *smaragdinus*
4. At least 2 of following 3 characters present[12]: sum of both seta 8-II plus both seta 9-II usually with 18–25 branches[13]; sum of both seta 2-I plus both seta 9-I usually with 16–24 branches[14]; sum of both seta 14-P often with 17–27 branches[15] . . . . . *diluvialis*
– At least 2 of following 3 characters present[12]: sum of both seta 8-II plus both seta 9-II usually with 26–40 branches[13]; sum of both seta 2-I plus both seta 9-I usually with 25–35 branches[14]; sum of both seta 14-P usually with 8–16 branches[15] . . . . . *inundatus*

[1] Feature correctly distinguished 100% of specimens.
[2] Feature correctly distinguished 99.1% of specimens (*diluvialis* = 98.8%; *inundatus* = 100%; *maverlius* = 98.9%; *quadrimaculatus* = 100%; *smaragdinus* = 98.0%).
[3] Feature correctly distinguished 99.3% of specimens (*diluvialis* = 99.6%; *inundatus* = 97.6%; *maverlius* = 99.5%; *quadrimaculatus* = 99.6%; *smaragdinus* = 100%).
[4] Feature correctly distinguished 94.6% of specimens (*diluvialis* = 93.3%; *inundatus* = 95.6%; *maverlius* = 86.2%; *quadrimaculatus* = 97.8%; *smaragdinus* = 100%).
[5] Feature correctly distinguished 98.8% of specimens (*diluvialis* = 97.4%; *inundatus* = 100%; *quadrimaculatus* = 99.5%; *smaragdinus* = 98.3%).
[6] Feature correctly distinguished 93.2% of specimens (*diluvialis* = 91.3%; *inundatus* = 96.1%; *quadrimaculatus* = 97.4%; *smaragdinus* = 87.8%).
[7] Feature correctly distinguished 95.0% of specimens (*diluvialis* = 95.8%; *inundatus* = 100%; *quadrimaculatus* = 91.9%; *smaragdinus* = 92.3%).
[8] Feature correctly distinguished 92.5% of specimens (*diluvialis* = 86.6%; *inundatus* = 95.2%; *quadrimaculatus* = 98.5%; *smaragdinus* = 89.7%).
[9] Feature correctly distinguished 99.8% of specimens to species (*quadrimaculatus* = 100%; *smaragdinus* = 99.6%).
[10] Feature correctly distinguished 92.8% of specimens to species (*quadrimaculatus* = 93.0%; *smaragdinus* = 92.6%).
[11] Feature correctly distinguished 94.1% of specimens to species (*quadrimaculatus* = 95.3%; *smaragdinus* = 92.9%).
[12] Feature correctly distinguished 98.2% of specimens to species (*diluvialis* = 98.1%; *inundatus* = 98.3%).
[13] Feature correctly distinguished 93.0% of specimens to species (*diluvialis* = 89.5%; *inundatus* = 96.6%).
[14] Feature correctly distinguished 91.3% of specimens to species (*diluvialis* = 96.6%; *inundatus* = 86.0%).
[15] Feature correctly distinguished 81.5% of specimens to species (*diluvialis* = 72.5%; *inundatus* = 90.5%).

Eggs[1]

1. Plastron of ventral area confined to 2 narrow strips adjacent to floats and not dividing deck (Fig. 18D) 2
– Plastron of ventral area developed as single broad strip between floats and dividing deck into anterior and posterior areas (Fig. 18C) 3
2. Floats larger in relation to egg length, mean length of both floats 285 ± 4.5; number of posterior lobed tubercles 7.3 ± 0.3 (Fig. 18E) . . . . . *quadrimaculatus*
– Floats shorter in relation to egg length, mean length of both floats 253.1 ± 5.2; number of posterior lobed tubercles 5.1 ± 0.6 (Fig. 18D) . . . . . *smaragdinus*
3. Plastron of dorsal area with pores large, mean individual pore area at least 2.25 (Fig. 19-1E) . . . . . *maverlius*
– Plastron of dorsal area with pores small, mean individual pore area less than 1.0 (Fig. 19-1C) 4
4. Mean anterior deck tubercle factor 1.95 ± 0.14, tubercles more irregular in outline and with relatively much larger peripheral ridges (Fig. 19-2C); number of posterior lobed tubercles 7.7 ± 0.3 (Fig. 18C) . . . . . *diluvialis*
– Mean anterior deck tubercle factor 1.42 ± 0.05, tubercles relatively regular in outline and with relatively smaller peripheral ridges (Fig. 19-2D); number of posterior lobed tubercles 5.7 ± 0.2 (Fig. 18B) . . . . . *inundatus*

[1] Tentative key based on data from Linley et al. (1993); their data were based on a relatively small number of specimens from a limited geographical area.

Biochemical to Adults[1]

1. *Mpi-1* slow, *Rf* 62 (rarely with 52/62 heterozygotes). . . . . *maverlius*
– *Mpi-1* faster, *Rf* 78 or greater. 2
2. *Idh-1* slow, *Rf* 86; *Idh-2* fast, *Rf* 162 . . . . *smaragdinus*
– *Idh-1* faster, *Rf* 100 or greater (sometimes with 86/100 heterozygotes); *Idh-2* fast or slower, *Rf* 100, 132, 162 (sometimes heterozygotes for 100, 132, 162) 3
3. *Had-3* faster, *Rf* 100 (sometimes with 45/100 heterozygotes); *Pgi-1* faster, *Rf* 100 (rarely with 95/100 heterozygotes) . . . . . *quadrimaculatus*
– *Had-3* slow, *Rf* 45; *Pgi-1* slow, *Rf* 95 4
4. *Acon-1* faster, *Rf* 118; *Me-1* faster, *Rf* 100; *Est-6* slow, *Rf* 94. . . . . *diluvialis*

– *Acon-1* slow, *Rf* 109; *Me-1* slow, *Rf* 92; *Est-6* faster, *Rf* 100 ..... *inundatus*

[1] Biochemical key was modified from Narang et al. (1989a) and includes data from Narang et al. (1990a) and from Table 32. These articles should be consulted for background data.

SPECIES DESCRIPTIONS

Anopheles (*Anopheles*) *diluvialis* Reinert, new species

Literature references

Anopheles quadrimaculatus species C of Cockburn and Seawright 1988:261; Cockburn et al. 1988: 299; Narang and Seawright 1988:303; Kaiser 1988: 311; Kaiser et al. 1988c:494; Cockburn and Mitchell 1989:105; Narang et al. 1989b:508, 1989a:317; Cockburn 1990:191; Narang and Seawright 1991: 59; Nayar et al. 1992:61; Mitchell et al. 1992:939; Jensen et al. 1993:1038.

Anopheles quadrimaculatus species C_1 of Narang et al. 1990a:179; Seawright et al. 1992:289; Linley et al. 1993:124 (E*); Johnson et al. 1993:939; Narang et al. 1993:463; Kaiser 1994:8; Jensen et al. 1994: 1150, 1995:141; Kaiser 1995:32; Cornel et al. 1996:109; Jensen et al. 1996:523; Reinert et al. 1996:310.

Female (Figs. 1E and 2D; Tables 1–5). *Head:* Antennal flagellum 0.69–0.80 length of proboscis, mean 0.74; maxillary palpus 1.01–1.12 length of proboscis, mean 1.06, without or with few semirecumbent scales basally; proboscis 0.91–1.15 length of femur I, mean 1.00; vertex, occiput, and dorsal area of postgena with erect forked scales long and narrow, pale-scaled patch of erect forked scales smaller; interocular area usually with 3–6 dark setae (93.7%), range 3–8. *Thorax:* Anterior promontory with only *ca.* 8–10 golden scales, 3,4 golden or golden-brown setae; acrostichal area with somewhat reduced number of golden piliform scales; dorsocentral area with anterior margin with only dark brown setae, piliform scales usually absent (96.7%) (occasionally with few dark brown piliform scales, very rarely with 1–3 golden piliform scales, 3.3%), remainder of dorsocentral area with only sparse brown or golden piliform scales; prescutellar area with few golden-brown piliform scales on anterior area mesally, moderate number of dark brown setae on anterior and lateral areas and few on posterolateral margin, leaving moderately wide median bare space; scutal fossal area with fewer setae (most long), usually with 9–20 setae (96.7%), range 9–25, sum of setae on both scutal fossal areas usually 20–40 (97.2%) (20–37 setae, 92.1%), range 20–46; supraalar setae all dark brown; parascutellar area often with 1 long dark seta (1 on both sides, 74.1%; 2 on both sides, 7.5%; 1 on 1 side and 2 on other, 18.3%), range 1–2; scutellum with few brown or golden-brown piliform scales, usually only on median 0.5–0.7; proepisternum usually with 7–26 upper setae (99.0%), range 6–26 (often several setae short and fine and difficult to see except when viewed at high magnification and at approximately 70–80° angle); prespiracular area with 1–5 setae; mesokatepisternum often with 1–3 upper (71.0%), range 1–6, and with 2–7 lower setae, sum of upper and lower setae usually 4–8 (80.3%), range 4–11; prealar area usually with 1–5 setae (94.0%), range 1–7; mesepimeron with 4–16 upper setae. *Legs:* Coxa I usually without scales anterodorsally, rarely with 1 scale; femora I,II usually dark-scaled (95.1%), very rarely with few pale scales apically on anterior and/or posterior surfaces (4.9%), dorsal surface dark-scaled, III usually with pale scales apically (with narrow pale-scaled band, 59.0%; with fringe of pale scales, 36.1%; apex dark, 4.9%); tibiae I–III dark-scaled (100%).

Male. Essentially similar to female except for sexual features. *Head:* Antennal flagellum 0.77–0.85 length of proboscis, mean 0.80; maxillary palpus 1.01–1.07 length of proboscis, mean 1.04; proboscis 1.26–1.47 length of femur I, mean 1.34.

Male genitalia (Figs. 4D, 5D, and 6D). *Tergum VIII:* Apical margin usually shallowly concave and gently sloping from lateroapical corners to midline (97.5%) (straight, 2.5%); length 0.33–0.44 mm, mean 0.39 mm; width 0.43–0.55 mm, mean 0.51 mm; VIII-Te index 0.69–0.82, mean 0.77. *Sternum VIII:* Lateral margin with subbasal depression. *Tergum IX:* Median band moderately long, length 0.03–0.05 mm, mean 0.04 mm; width 0.18–0.21 mm, mean 0.19 mm; Te-IX index 0.16–0.27, mean 0.20; posterolateral lobe smooth apically, length 0.06–0.10 mm, mean 0.08 mm; PlL/Te-IX index 1.24–2.67, mean 1.97; laterobasal band with heavily pigmented area relatively narrow (100%), ventrolateral margin relatively smooth (100%). *Phallosome:* Aedeagus length 0.18–0.22 mm, mean 0.20 mm, apex bearing 2–4 (2 on 1 side and 3 on other, 5.0%; 2 on 1 side and 4 on other, 5.0%; 3 on both sides, 52.5%; 3 on 1 side and 4 on other, 22.5%; 4 on both sides, 15.0%) dark leaflets on each side of midline, apical leaflet broader, long and 0.37–0.52 length of aedeagus, other leaflets narrow, shorter, basal 1,2 leaflets often with 1,2 thin spicules anterobasally. *Gonocoxite:* Length 0.29–0.38 mm, mean 0.32 mm; ventral surface with internal seta situated slightly more distal than *An. quadrimaculatus,* apical portion usually recurved (occasionally curved). *Gonostylus:* Moderately pigmented; length 0.35–0.42 mm, mean 0.39 mm; 20–33 tiny setae (95.0%), range 17–33, most in more or less irregular line on distal 0.71–0.86 (90.0%); Gs/Gc index 1.13–1.27, mean 1.21. *Claspette:* Seta 2 usually absent (92.5%); seta 4 noticeably shorter than seta 5 (97.5%) (4 slightly longer than 5, 2.5%), situated dorsolateral to seta 3 (100%); seta 5 formed of 2–4 (2 on both sides, 32.5%; 2 on 1 side and 3 on other, 22.5%; 2 on 1 side and 4 on other, 2.5%; 3 on both sides, 35.0%; 3 on 1 side and 4 on other, 5.0%; 4 on both sides, 2.5%) apically

fused stems, usually not borne on elevated process (92.7%).

Pupa (Fig. 8). Chaetotaxy as figured and recorded in Tables 6 and 11–17. *Cephalothorax:* Integument usually light tan, often with some lightly to moderately pigmented areas; dorsal apotome with apex often broadly rounded (79.1%) (flat or slightly concave, 21.9%), without apical flaplike projection (100%); lateralia with cuticular ocular facets of compound eye absent or very poorly developed; scutum with anterior angle acute, produced into short blunt lobe (100%); median keel with lateral line short, extending posteriorly to approximately base of trumpet (100%); postscutal area intact and not split by dorsal ecdysial opening (100%); mesothoracic wing usually uniformly pale tan, rarely with moderately pigmented indistinct longitudinal lines. *Trumpet:* Similar to *An. quadrimaculatus* except somewhat darker; index 3.22–4.34, mean 3.94; length 0.44–0.55 mm, mean 0.50 mm; width 0.12–0.15 mm, mean 0.13 mm; pinna 0.70–0.86 of trumpet length, mean 0.79. *Metanotum:* Metanotal wing usually pale tan, with or without lightly or moderately pigmented area; area between pair of seta 10-CT with few shallow transverse grooves; sum of both 10-CT usually with 2–6 branches (96.9%) (2–5 branches, 91.8%), range 2–8. *Abdomen:* Tergum I usually uniformly light tan or with lightly pigmented short U-shaped area between pair of seta 1-I (rarely with moderately pigmented long U- or O-shaped area); terga III-VII with well developed curved transverse ridge posterior to seta 1; 1-VII usually 0.75 or less length of tergum VIII (92.1%) (length 0.8–0.9, 6.2%; slightly longer than 1.0, 1.7%); sum of both 5-I usually with 9–17 branches (95.1%), range 7–17; sum of both 6-I usually with 8–15 branches (90.0%), range 5–15; sum of both 6-III usually with 9–18 branches (96.4%), range 6–18; sum of both 7-I usually with 11–24 branches (92.6%), range 8–24; sum of both 7-I plus both 6-III usually with 20–37 branches (97.4%), range 17–37; 9-III-V spiniform, usually broad, flattened, straight (tapered in 1.4% of III, 2.8% of IV, 5.6% of V), 9-VI,VII spiniform, often tapered (54.9% of VI, 67.6% of VII) (curved in 4.2% of VI, 70.4% of VII); ratio of length of 9-III/9-IV 0.45–0.74 (mean 0.57), 9-IV/9-V 0.74–0.97 (mean 0.85), 9-V/9-VI 0.72–0.98 (mean 0.87), 9-VI/9-VII 0.70–1.10 (mean 0.81), 9-III/9-VII 0.28–0.52 (mean 0.37); sum of both 9-VIII usually with 23–37 branches (98.4%) (24–37 branches, 92.3%), range 19–37; 1-IX usually with 2,3 branches, range 1–4; sternal transverse ridges as in *An. quadrimaculatus*; male genital lobe index 1.08–1.25 (one specimen 1.26), mean 1.17. *Paddle:* Seta 1-Pa usually single (98.6%) (with 2 branches, 1.4%), range 1–2; sum of both 1-Pa usually with 2 branches (97.2%) (with 3 branches, 2.8%); index 1.33–1.46, mean 1.39; length 0.71–0.97 mm, mean 0.88 mm; width 0.54–0.71 mm, mean 0.64 mm; refractile border on basal 0.61–0.81 of outer margin, submarginal row of aciculae usually with break near middle; nonrefractile border on apical 0.19–0.39 of outer and apical 0.00–0.15 of inner margins, with sparse short thin aciculae.

4th-instar larva (Fig. 13). Chaetotaxy as figured and recorded in Tables 18 and 23–31. *Head:* Medium brown usually with more darkly pigmented areas on dorsal and ventral surfaces (94.3%), arrangement of darker areas somewhat variable, frontal ecdysial line with broad darkly pigmented area on *ca.* posterior 0.3 of lateralia margin and narrow on dorsal apotome margin, dorsal surface without or with minute spicules; seta 2-C usually 1.3 or greater length of 3-C (93.3%), range 1.26–1.79; 3-C dendritic, often with 19–30 apical, somewhat thicker branches (56.7%), range 19–44, branches more or less bunched together distally, few stout basal branches; sum of both 3-C usually with 46–63 branches (66.7%), range 46–83. *Antenna:* Flagellum length usually 0.23–0.30 mm (95.8%), range 0.23–0.32 mm; seta 1-A normally shorter than apex of flagellum (97.0%) (equal to apex, 3.0%), inserted usually 0.19–0.30 from base (86.6%), range 0.19–0.34, sum of both 1-A usually with 5–17 branches (98.0%), range 5–19; 3-A darkly pigmented, usually with 4–8 small serrations on mesal margin (84.1%) (margin smooth, 15.9%); 4-A with 4–7 branches. *Mouthparts:* Dorsomentum darkly pigmented with 9 teeth. *Thorax:* Seta 1-P often noticeably longer than 3-P (62.1%) (equal in length, 10.5%; 0.78–0.97 length, 27.4%); 1-P usually with 2–5 branches (95.5%) (single, 4.5%), branching often on apical 0.40–0.87 (58.3%) (on apical 0.13–0.39, 37.1%); sum of both 4-P often with 31–39 branches (67.6%), range 25–39; 9-P usually both single (87.3%) (single on 1 side and other with 2 branches, 8.5%; both sides with 2 branches, 3.7%; 2 branchers on 1 side and 3 on other, 0.3%; both sides with 3 branches, 0.3%); sum of both 14-P usually with 16–27 branches (84.1%) (17–27 branches, 72.5%), range 12–27. *Abdomen:* Seta 1-VII palmate, leaflets often relatively narrow (67.9%) (moderately broad, 32.1%); sum of both 2-I often with 7–11 branches (73.4%), range 7–16; sum of both 2-I plus both 2-III usually with 19–31 branches (93.4%), range 16–31; sum of both 2-I plus both 9-I usually with 16–24 branches (96.6%) (20–28 branches, 82.1%), range 16–28; sum of both 2-III usually with 9–17 branches (97.8%) (10–17 branches, 90.5%), range 8–17; sum of both 8-II usually with 8–15 branches (92.0%), range 6–15; sum of both 8-II plus both 8-III usually with 13–20 branches (95.8%) (13–18 branches, 89.6%), range 13–23; sum of both 8-II plus both 9-II usually with 18–28 branches (97.6%) (18–25 branches, 89.5%), range 18–31; sum of both 8-III usually with 5–9 branches (93.6%), range 5–11; sum of both 8-III plus both 8-VI usually with 11–18 branches (99.6%), range 11–19; sum of both 8-V often with 4–6 branches (76.2%), range 4–9; sum of both 8-VI usually with 4–7 branches (87.0%),

range 4–9; sum of both 9-II usually with 10–19 branches (98.8%), range 10–23; sum of both 10-VI with 4–7 branches (100%); sum of both 13-I usually with 10–17 branches (92.0%), range 8–17; segment VIII usually with small sternal plate anteromesally (93.5%) (absent, 6.5%), with 1 or both pecten plates usually with 9–11 long spines (91.3%) (both sides with 8, 6.1%; 1 side with 8 and other with 7, 2.2%; both sides with 7, 0.4%), long spines with well-developed lateral spicules; seta 7-S both usually with 2 branches (89.3%) (single on 1 side and other with 2 branches, 8.7%; both sides single, 2.0%); segment X with saddle having small, more darkly pigmented area posterodorsally, small spicules forming small patch located mainly posterior to point of insertion of seta 1-X (on saddle and ventral and posterior to saddle).

Egg (Figs. 18C, 19-1C, and 19-2C). The following information is taken from Linley et al. (1993). *Linear dimensions:* Mean length 541.6 ± 8.2 μm; mean width 189.9 ± 1.9 μm. *Deck:* Plastron large, separating deck into anterior and posterior areas; number of lobed tubercles, anterior 8.6 ± 0.3, posterior 7.7 ± 0.3; mean anterior deck tubercle area 1.95 ± 0.14 μm, density 81.1 ± 5.9. *Float:* Mean length of both floats 286.3 ± 5.1 μm; mean float length as percent of egg length 52.9 ± 0.6; mean float length/mean number of ribs 13.6 ± 0.1. *Dorsal plastron:* Mean individual pore area 0.95 ± 0.05 μm, small.

Type data. The type series of *An. diluvialis* was reared from a single F_1 progeny brood of a female collected in nature from a large rot cavity (extending from ground level to a height of *ca.* 1 m) in a tree at the margin of a heavily shaded, shallow freshwater swamp on August 31, 1995, by John F. Reinert and Paul E. Kaiser. This site was located in the southwestern portion of Magnolia Camp, Manatee Springs State Park, and the nearest town was Chiefland, Levy County, FL. The holotype (FL95.51–39) consists of a female glued to a small, triangular paper point on an insect pin, and its associated pupal and 4th-instar larval exuviae mounted in Canada balsam on a microscope slide. The remainder of the type series includes the following specimens: paratypes, FL95.51–1 ♂pl, -2 ♂pl, -3 ♂pl, -4 ♂plg, -5 ♂pl, -6 ♂pl, -7 ♂pl, -8 ♂pl, -9 ♂pl, -10 ♂pl, -11 ♂pl, -12 ♂pl, -13 ♂plg, -14 Pl, -15 ♂pl, -16 ♂pl, -17 ♀pl, -18 ♂plg, -19 ♀pl, -20 ♀pl, -21 pl, -22 ♀pl, -23 ♂plg, -24 ♂pl, -25 ♂plg, -26 ♂pl, -27 ♂pl, -28 ♀pl, -29 ♀pl, -30 ♂pl, -31 ♀pl, -32 ♂pl, -33 ♂pl, -34 ♀pl, -35 ♂plg, -36 ♂pl, -37 ♂plg, -38 ♂pl, -40 ♀pl, -41 ♀pl, -42 ♂pl, -43 ♀pl, -44 ♂pl and 13 L.

The holotype and some paratypes with their associated immature exuviae are deposited in the National Museum of Natural History (NMNH), Smithsonian Institution, Washington, DC. Most of the paratypes are deposited in the Florida State Collection of Arthropods (FSCA), Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL. Additional paratypes are deposited in the Natural History Museum (NHM), London, United Kingdom; and l'Institut Francais de Recherche Scientifique pour le Developpment en Cooperation (ORSTOM), Montpellier, France.

Bionomics. Larvae were collected in swamps from temporary pools of water resulting from heavy rains that led to both localized flooding, which occurred in the hardwood hammocks of the Florida Gulf Coast, and regional flooding, which inundated the lowland areas adjacent to rivers (e.g., Suwannee River). Larvae were found only in freshwater although many collections came from swamps within 2–3 km of the Gulf of Mexico coast. All breeding sites had dense overhead canopies that limited growth of submerged vegetation to a few grasses. Immatures were found in floating debris at the water's interface with grasses, logs, shore and the bases of trees; during winter months larvae tended to accumulate in small algal mats in some swamps. Larvae were never collected from sunlit areas of rivers that were adjacent to breeding sites; however, larvae were collected in full sunlight during winter months from pools in deciduous swamps. In the latter site, immatures of *An. quadrimaculatus, An. perplexens* Ludlow, and *An. punctipennis* (Say) also were occasionally found. Early stages of larvae were regularly collected from newly flooded swamps along with early-instar larvae of floodwater species (e.g., *Psorophora ferox* [Von Humboldt] and *Aedes infirmatus* Dyar and Knab). Hatching of eggs from field-collected females after 3 to 4 days' incubation was normally low (20 ± 10%) without an inducement (i.e., deoxygenation or the addition of water collected from the breeding site of the swamp). Breeding in these swamps appeared to be continuous throughout the year until drying occurred. Therefore, it may be possible that unhatched eggs were either stranded by drying up of the habitat or that the females oviposited on the moist substrate of the habitat, or both. Jensen et al. (1994, 1995) recovered eggs from soil samples taken from known breeding sites that had dried up and demonstrated that these eggs, when dried and stored in the laboratory, would hatch after 4 wk. The survival of the eggs allows this species to exploit a niche used by some floodwater mosquito species. Jensen et al. (1994, 1995) and Kaiser (1994) also report the collection of immatures of *An. diluvialis* (as *An. quadrimaculatus* sp. C1) from temporary pools in intermittently flooded swamps in the northern and central parts of Florida.

Daytime-resting adults were collected from rot cavities in trees, from under bridges, and from under the eaves of buildings. Adults tended to be gregarious in these sites. Preferred resting sites included rotted cavities in bald cypress, sweet gum, and water oak trees that were usually found in coastal and riverine freshwater swamps subject to periodic flooding; red maple and bay trees also were domi-

nant in these swamps. A few adults of *An. quadrimaculatus* routinely shared resting sites with *An. diluvialis* adults and occasionally also with a few *An. smaragdinus* adults. Most habitats where adults were collected were not near populated areas or farms with domesticated animals. Jensen et al. (1996), however, collected adults from daytime resting sites at Manatee Springs State Park, Levy Co., FL, and found that 1.2% (4 of 327) of blood-engorged females at a campground area had fed on humans, but none of 159 from a wooded area. Nayar et al. (1992) reported that females of *An. diluvialis* (as *An. quadrimaculatus* sp. C) in laboratory experiments had infection rates of 39.3% with *Brugia pahangi* and 12.6% with *Brugia malayi*. Jensen et al. (1993), using updraft CDC traps baited with dry ice, monitored changes in the abundance and parity rate of this species in an intermittently flooded swamp at Coleman, Sumter Co., FL. They found that of 1,178 *An. quadrimaculatus s.l.* collected in an 18-day period, 94.7% were *An. diluvialis*, 1,135 of 1,138 females had primary follicles at stage II, none appeared to have bloodfed, and 51.4% (586 of 1,139) were parous.

Discussion. In addition to the characters used in the keys, the following features, used in combination, can be used for the identification of most specimens and for their separation from the other members of the complex: females by the combination of (1) tibiae I,II dark-scaled (100%), (2) sum of setae on both scutal fossal areas 20–40 (97.2%), (3) dorsocentral area without golden piliform scales on anterior margin (96.7%), (4) scutal fossal area with 9–20 setae (96.7%), (5) femora I,II dark-scaled (95.1%), (6) prealar area with 1–5 setae (94.0%), (7) interocular area with 3–6 setae (93.7%), and (8) sum of setae on both scutal fossal areas 20–37 (92.1%); males by (9) maxillary palpus length longer than proboscis (100%), male genitalia by the combination of (10) tergum IX with heavily pigmented area of laterobasal band narrow and ventrolateral margin relatively smooth (100%), (11) claspette with seta 4 noticeably shorter than seta 5 (97.5%), (12) gonostylus with 20–33 tiny setae (95.0%), and (13) claspette with seta 5 not borne on short elevated process (92.7%); pupae by the combination of (14) sum of both seta 1-Pa with 2,3 branches (100%), (15) sum of both seta 9-VIII with 23–37 branches (98.4%), (16) sum of both seta 7-I plus both seta 6-III with 20–37 branches (97.4%), (17) sum of both seta 6-III with 9–18 branches (96.4%), (18) sum of both seta 5-I with 9–17 branches (95.1%), (19) sum of both seta 7-I with 11–24 branches (92.6%), (20) sum of both seta 9-VIII with 24–37 branches (92.3%), (21) seta 1-VII 0.75 or less length of tergum VIII (92.1%), and (22) sum of both seta 6-I with 8–15 branches (90.0%); and 4th-instar larvae by the combination of (23) sum of both seta 10-VI with 4–7 branches (100%), (24) sum of both seta 2-I plus both seta 9-I with 20–35 branches (100%), (25) sum of both seta 9-II with 10–19 branches (98.8%), (26) sum of both seta 2-III with 9–17 branches (97.8%), (27) sum of both seta 8-II plus both seta 9-II with 18–28 branches (97.7%), (28) seta 1-A shorter than apex of flagellum (97.0%), (29) sum of both seta 8-II plus both seta 8-III with 13–20 branches (95.8%), (30) sum of both seta 8-III with 5–9 branches (93.6%), (31) sum of both seta 2-I plus both seta 2-III with 19–31 branches (93.4%), (32) sum of both seta 8-II with 8–15 branches (92.0%), (33) sum of both seta 13-I with 10–17 branches (92.0%), (34) sum of both seta 2-III with 10–17 branches (90.5%), (35) sum of both seta 8-II plus both seta 8-III with 13–18 branches (89.6%), (36) seta 7-S both single (89.3%), (37) sum of both seta 8-VI with 4–7 branches (87.0%), (38) sum of both seta 14-P with 16–27 branches (84.1%), (39) seta 3-A with serrations mesally (84.1%), (40) sum of both seta 4-P with 22–30 branches (81.5%), (41) sum of both seta 2-I with 7–11 branches (73.4%), and (42) seta 1-P longer than or equal to 3-P length (72.6%).

The following primary features, given above by numbers in parentheses and listed here in decreasing order of separation, best distinguish specimens of *An. diluvialis* from *An. inundatus*: in females, 3 (97.1%), 8 (93.3%), and 4 (89.7%); and in 4th-instar larvae, 35 (87.6%) and 41 (80.6%); from *An. maverlius*: in females, 3 (97.9%) and 5 (96.7%); in male genitalia, 10 (100%) and 13 (90.5%); in pupae, 18 (95.1%) and 20 (90.5%); and in 4th-instar larvae, 26 (98.7%), 27 (98.6%), 29 (97.9%), 30 (96.6%), 25 (96.0%), 31 (95.9%), 23 (95.8%), 37 (92.3%), 38 (90.6%), 33 (88.9%), 28 (84.5%), and 36 (83.0%); from *An. quadrimaculatus*: in females, 1 (100%), 3 (98.4%), 5 (97.6%), 2 (97.2%), 7 (95.9%), 4 (94.9%), and 6 (90.5%); in males, 9 (100%); in male genitalia, 10 (100%), 13 (96.4%), 11 (89.6%), and 12 (87.5%); in pupae, 14 (98.9%), 21 (95.1%), and 22 (91.3%); and in 4th-instar larvae, 31 (94.3%), 34 (93.1%), 39 (92.1%), 40 (87.8%), 36 (87.2%), and 42 (86.3%); and from *An. smaragdinus*: in females, 1 (100%), 3 (98.4%), and 5 (97.6%); in males, 9 (100%); in male genitalia, 10 (100%) and 12 (93.3%); in pupae, 16 (98.4%), 17 (97.1%), 15 (95.1%), 20 (94.8%), 18 (93.7%), 22 (93.2%), 21 (93.1%), and 19 (92.5%); and in 4th-instar larvae, 31 (96.6%), 26 (96.1%), 33 (96.0%), 24 (94.1%), 32 (92.9%), 39 (91.0%), and 42 (81.7%).

The parascutellar area often had a single seta on both sides in females of *An. diluvialis* (71.4%) and *An. inundatus* (65.4%), while both sides many times had 2 or more setae in *An. maverlius* (45.8%), *An. quadrimaculatus* (65.9%), and *An. smaragdinus* (38.5%).

Pupal exuviae of *An. diluvialis* were most similar to those of *An. inundatus*. In addition to the features separating these species listed above and in the key, another useful character was the pinna length to trumpet length. The postscutal area of the cepha-

lothorax of *An. diluvialis* and *An. inundatus* remains intact during emergence of the adult and was not split by the dorsal ecdysial opening as in *An. maverlius, An. quadrimaculatus* and *An. smaragdinus,* (see Reinert et al. 1996). The integument of the cephalothorax, the metanotum, and tergum I was usually uniformly pale tan; however, in some specimens there were lightly to moderately pigmented areas.

The species name *diluvialis* is of Latin origin and refers to the habitat of this species, i.e., intermittently flooded swamps.

Anopheles (Anopheles) inundatus Reinert, new species

Literature references

Anopheles quadrimaculatus species C_2 of Narang et al. 1990a:179; Seawright et al. 1992:289; Linley et al. 1993:124 (E*); Johnson et al. 1993:939; Narang et al. 1993:463; Kaiser 1994:8; Rutledge and Meek 1994:585; Cornel et al. 1996:109; Reinert et al. 1996:310; Rutledge et al. 1996:952.

Female (Figs. 1F, 2E, and 2G; Tables 1–5). *Head:* Antennal flagellum 0.63–0.89 length of proboscis, mean 0.73; maxillary palpus without or with few semirecumbent scales basally, 1.00–1.12 length of proboscis, mean 1.03; proboscis 0.95–1.07 length of femur I, mean 1.01; vertex, occiput, and dorsal area of postgena with erect forked scales long and narrow, pale-scaled patch of erect forked scales smaller; interocular area usually with 6–9 setae (90.1%), range 5–9, usually dark, rarely 1–3 pale. *Thorax:* Anterior promontory with numerous golden scales, 8–12 golden setae; acrostichal area with numerous golden piliform scales, setae often dark brown; dorsocentral area usually with few to several golden piliform scales on anterior margin (97.5%), rarely with scales brown (2.5%), remainder of dorsocentral area with moderate number of similar scales; prescutellar area with few golden brown piliform scales on anterior area mesally, numerous dark brown setae on anterior and lateral areas and few (rarely specimen also with 1–3 golden) on posterior margin, leaving moderately wide median bare space; scutal fossal area usually with 21–32 setae (82.7%), range 15–32, sum of setae on both scutal fossal areas usually 38–59 (94.4%), (41–59 setae, 86.5%), range 31–59; parascutellar area often with 1 dark seta (1 on both sides, 65.4%; 2 on both sides, 7.5%; 1 on 1 side and 2 on other, 24.5%; 0 on 1 side and 1 on other, 2.2%; 0 on both sides, 0.3%), range 0–3, second seta usually 0.6 or less of other seta; proepisternum usually with 7–15 upper setae (96.0%), range 3–15, setae similar to those of *An. diluvialis*; prespiracular area with 1–6 setae; mesokatepisternum usually with 1–3 upper setae (82.3%), range 1–6, and with 2–7 lower setae, sum of upper and lower setae often 4–8 (78.9%), range 4–13; prealar area usually with 2–5 setae (82.1%), range 2–9; mesepimeron with 5–15 upper setae. *Legs:* Coxa I usually without scales anterodorsally, occasionally with 1 scale; femur I dark-scaled (100%), II usually dark-scaled (86.2%), occasionally with few pale scales apically on anterior and or posterior surface (13.8%), III often with fringe of pale scales apically (60.5%) or with pale-scaled very narrow band apically (35.8%) (entirely dark, 3.7%); tibiae I–III dark-scaled. *Wing:* Four darker scaled spots less distinct.

Male. Essentially similar to female except for sexual features. *Head:* Antennal flagellum 0.73–0.83 length of proboscis, mean 0.78; maxillary palpus 0.98–1.06 length of proboscis, mean 1.02; proboscis 1.28–1.51 length of femur I, mean 1.34.

Male genitalia (Figs. 4E, 5E, and 6E). *Tergum VIII:* Apical margin with shallow depression separating broadly rounded lobes (100%); length 0.35–0.46 mm, mean 0.40 mm; width 0.49–0.61 mm, mean 0.53 mm; VIII-Te index 0.70–0.81, mean 0.75. *Sternum VIII:* Lateral margin often without subbasal depression (71.4%). *Tergum IX:* Median band moderately long; length 0.03–0.04 mm, mean 0.03 mm; width 0.17–0.22 mm, mean 0.20 mm; Te-IX index 0.14–0.25, mean 0.18; posterolateral lobe smooth apically, length 0.07–0.10 mm, mean 0.08 mm; PlL/Te-IX index 1.80–3.08, mean 2.45; laterobasal band with heavily pigmented area relatively narrow (100%), ventrolateral margin relatively smooth (100%). *Phallosome:* Aedeagus similar to *An. diluvialis,* length 0.18–0.23 mm, mean 0.21 mm, apex bearing 3,4 dark leaflets on each side of midline (3 on both sides, 45.7%; 3 on 1 side and 4 on other, 37.1%; 4 on both sides, 17.1%), apical leaflet broader, long, and 0.36–0.54 length of aedeagus. *Gonocoxite:* Length 0.30–0.35 mm, mean 0.33 mm; ventral surface with internal seta situated slightly more distal than in *An. quadrimaculatus,* apical portion recurved. *Gonostylus:* Moderately pigmented; length 0.37–0.41 mm, mean 0.39 mm; 21–34 tiny setae (100%), most usually in more or less irregular row on distal 0.71–0.86 (88.9%); Gs/Gc index 1.08–1.29, mean 1.18. *Claspette:* Seta 2 often absent (71.4%) (present on 1 or both sides, 28.6%); seta 4 noticeably shorter than seta 5 (100%), situated dorsolateral to seta 3 (100%); seta 5 formed of 1–3 (1 on 1 side and 2 on other, 5.7%; 2 on both sides, 40.0%; 2 on 1 side and 3 on other, 25.7%; 3 on both sides, 25.7%; 3 on 1 side and 4 on other, 2.9%) apically fused stems, usually not borne on short elevated process (97.1%).

Pupa (Fig. 9). Chaetotaxy as figured and recorded in Tables 7 and 11–17. Similar to *An. diluvialis* in development of integument, lateralia, anterior angle of scutum, lateral line of median keel, postscutal area intact, development of trumpet, development of metanotum, tergum I pigmentation, and sternal transverse ridges. *Cephalothorax:* Dorsal apotome often with apex flat or slightly concave (73.5%) (broadly rounded, 26.5%), without apical flaplike projection (100%); mesothoracic wing light

tan, very rarely with indistinct moderately pigmented longitudinal lines. *Trumpet:* Index 3.16–4.19, mean 3.63; length 0.44–0.56 mm, mean 0.50 mm; width 0.13–0.16 mm, mean 0.14 mm; pinna 0.75–0.90 of trumpet length, mean 0.81. *Metanotum:* Sum of both 10-CT usually with 6–12 branches (82.8%), range 4–12. *Abdomen:* Tergum I usually uniformly pale tan, very rarely with lightly pigmented short bar on posterior margin between pair of seta 1-I; seta 1-VII usually 0.75 or less length of tergum VIII (93.3%) (length 0.8–0.9, 5.8%; slightly longer than 1.0, 0.9%); sum of both 5-I with 9–19 branches (100%) (10–19 branches, 98.4%); sum of both 6-I usually with 8–16 branches (94.6%), range 6–16; sum of both 6-III usually with 9–21 branches (98.5%), range 8–21; sum of both 7-I usually with 11–22 branches (94.4%), range 8–22; sum of both 7-I plus both 6-III usually with 20–37 branches (97.3%), range 18–37; 9-III-V spiniform, usually broad, flattened, straight (tapered in 2.7% of IV, 12.3% of V), 9-VI,VII spiniform, often tapered (56.2% of VI, 53.4% of VII) (curved in 1.4% of VI, 41.4% of VII); ratio of length of 9-III/9-IV 0.42–0.73 (mean 0.53), 9-IV/9-V 0.62–0.99 (mean 0.85), 9-V/9-VI 0.75–1.04 (mean 0.88), 9-VI/9-VII 0.76–1.04 (mean 0.92), 9-III/9-VII 0.26–0.46 (mean 0.36); sum of both 9-VIII usually with 24–41 branches (89.2%); range 14–41; 1-IX usually with 2,3 branches, range 1–4; male genital lobe index 1.04–1.22, mean 1.13. *Paddle:* Seta 1-Pa usually single (99.7%) (with 2 branches, 0.3%), range 1–2; sum of both 1-Pa usually with 2 branches (98.7%) (with 3 branches, 1.3%), range 2–3; index 1.27–1.49, mean 1.40; length 0.74–0.98 mm, mean 0.88 mm; width 0.53–0.70 mm, mean 0.63 mm; refractile border on basal 0.66–0.83 of outer margin, submarginal row of aciculae as in *An. diluvialis*; nonrefractile border on apical 0.17–0.34 of outer and apical 0.05–0.16 of inner margins, with sparse short thin aciculae.

4th-instar larva (Fig. 14). Chaetotaxy as figured and recorded in Tables 19 and 23–31. *Head:* Medium brown with extensive more darkly pigmented areas on dorsal and ventral surfaces (100%), arrangement of darker areas somewhat variable, frontal ecdysial line with broad darkly pigmented area on both sides along posterior *ca.* 0.3–0.4 of length, dorsal surface without spicules or occasionally with minute spicules; seta 2-C usually 1.30 or greater length of 3-C (95.6%), range 1.23–1.83; 3-C dendritic, with 22–31 somewhat thicker, apical, branches that are more or less bunched together distally, few stout basal branches; sum of both 3-C often with 53–57 branches (73.3%), range 48–59. *Antenna:* Flagellum length 0.24–0.30 mm (100%); seta 1-A usually shorter than apex of flagellum (90.1%) (equal to apex, 8.8%; slightly longer than apex, 1.1%), inserted usually 0.21–0.30 from base of flagellum (95.2%), range 0.21–0.34; sum of both 1-A with 6–17 branches (100%); 3-A darkly pigmented, usually with 4–6 serrations on mesal margin at about midlength (99.0%) (margin smooth, 1.0%); 4-A with 4–9 branches. *Mouthparts:* Dorsomentum darkly pigmented with 9 teeth. *Thorax:* Seta 1-P often longer than 3-P (70.6%) (equal in length, 8.8%; slightly shorter in length, 20.6%); 1-P often with 2,3 branches (79.0%) (single, 10.5%), range 1–5, branching often on apical 0.40–0.81 (74.8%) (branching on apical 0.18–0.39, 17.4%); sum of both 4-P usually with 22–30 branches (81.5%), range 22–36; 9-P usually both single (84.8%) (single on 1 side and other with 2 branches, 12.8%; both sides with 2 branches, 2.4%); sum of both 14-P usually with 8–16 branches (90.5%) (8–15 branches, 75.8%), range 8–22. *Abdomen:* Tergum II occasionally with accessory tergal plate absent; seta 1-VII palmate, leaflets usually moderately broad or broad (95.2%) (narrow, 4.8%); sum of both 2-I usually with 11–18 branches (97.2%) (12–18 branches, 87.7%), range 10–18; sum of both 2-I plus both 2-III usually with 19–34 branches (99.6%), range 18–34; sum of both 2-I plus both 9-I usually with 25–35 branches (86.0%), range 20–35; sum of both 2-III usually with 9–18 branches (95.8%) (10–18 branches, 90.5%), range 7–18; sum of both 8-II with 8–17 branches (100%); sum of both 8-II plus both 8-III usually with 18–27 branches (92.9%) (19–27 branches, 85.5%), range 15–27; sum of both 8-II plus both 9-II usually with 26–40 branches (96.6%), range 23–40; sum of both 8-III usually with 6–10 branches (94.8%), range 6–14; sum of both 8-III plus both 8-VI usually with 12–18 branches (97.6%), range 12–20; sum of both 8-V usually with 4–6 branches (89.7%), range 4–8; sum of both 8-VI often with 5–7 branches (79.2%), range 5–11; sum of both 9-II usually with 13–19 branches (80.5%), range 13–24; sum of both 10-VI with 4–8 branches (100%); sum of both 13-I usually with 10–14 branches (82.4%), range 8–14; segment VIII often with small sternal plate anteromesally (64.2%) (absent, 35.8%), pecten plate with 7–11 long spines with well-developed spicules laterally, usually both with 9–11 long spines (96.1%) (both sides with 8, 3.3%; 1 side with 8 and other with 7, 0.6%); seta 7-S both with 2 branches (50.0%) (with 2 branches on 1 side and 3 on other, 3.8%; single on 1 side and other with 2 branches, 7.7%; both sides single, 38.5%); segment X with saddle having small, more darkly pigmented area posterodorsally, small spicules forming large patch on posterior *ca.* 0.6 of saddle, numerous other spicules covering much of segment X on area ventral and posterior to saddle.

Egg (Figs. 18B, 19–1D, and 19-2D). The following information is taken from Linley et al. (1993). *Linear dimensions:* Mean length 512.9 ± 4.4 μm; mean width 181.9 ± 1.5 μm. *Deck:* Plastron large, separating deck into anterior and posterior areas; number of lobed tubercles, anterior 6.6 ± 0.3, posterior 5.7 ± 0.2; mean anterior deck tubercle area 1.42 ± 0.05 μm, density 89.8 ± 2.9 μm. *Float:* Mean length of both floats 267.9 ± 3.6

μm; mean float length as percent of egg length 52.2 ± 0.4; mean float length/mean number of ribs 13.2 ± 0.2. *Dorsal plastron:* Mean individual pore area 0.94 ± 0.05 μm, small.

Type data. The type series of *An. inundatus* was reared from a single F_1 progeny brood of a female collected in nature from a large rot cavity (extending from ground level to a height of *ca.* 1 m) in a tree located in a heavily shaded, shallow, partly dried-up, freshwater, flood plain pool on May 31, 1995, by John F. Reinert and Paul E. Kaiser. This site was situated *ca.* 60 m from the western margin of the Choctawhatchee River immediately south of Rook's Bluff Fish Camp, which is located from Florida State Highway 20 at Bruce south 3.5 mi. on Florida State Highway 81 then east *ca.* 0.1 mi on Rook's Bluff Road, Walton Co., FL. The holotype (FL95.25-67) consists of a female glued to a small triangular paper point on an insect pin and its associated pupal and 4th-instar larval exuviae mounted in Canada balsam on a microscope slide. The remainder of the type series includes the following specimens: paratypes, FL95.25–1 ♂pl, -2 ♂pl, -3 ♂pl, -4 ♂pl, -5 ♂pl, -6 ♂pl, -7 ♀pl, -8 ♂pl, -9 ♂plg, -10 ♂pl, -11 ♂pl, -12 ♂plg, -13 ♂pl, -14 ♂pl, -15 ♀pl, -16 ♂pl, -17 ♂pl, -18 ♂pl, -19 ♂pl, -20 ♂pl, -21 ♂pl, -22 ♂pl, -23 ♀pl, -24 ♂plg, -25 ♂pl, -26 ♀pl, -27 ♀pl, -28 ♀pl, -29 pl, -30 ♀pl, -31 ♀pl, -32 ♀pl, -33 ♀pl, -34 ♀pl, -35 ♂pl, -36 ♀pl, -37 pl, -38 ♂pl, -39 ♀pl, -40 ♂pl, -41 ♂plg, -42 ♂pl, -43 Pl, -44 ♂pl, -45 ♂pl, -46 ♂pl, -47 pl, -48 ♀pl, -49 ♂ and g, -49A ♀pl, -50 ♀pl, -51 ♂pl, -52 ♂pl, -53 ♀pl, -54 ♂pl, -55 ♀pl, -56 ♀pl, -57 ♀pl, -58 Pl, -59 ♂pl, -60 ♀pl, -61 ♀pl, -62 ♀pl, -63 ♂plg, -64 ♀pl, -65 ♂pl, -66 ♀pl, -68 ♂pl, -69 ♀pl, -70 ♂pl, -71 ♀pl, -72 pl, -73 ♀pl, -74 ♂pl, -75 Pl, -76 Pl, -77 l, -78 ♀pl, -79 pl, -80 ♂plg, -81 ♀pl, -82 Pl, -83 l, -84 l, -85 ♀pl, -86 ♀pl, -87 ♀pl, -88 ♀pl, -89 ♀pl, -90 ♀pl, -91 ♀pl, -92 ♂pl, -93 ♀pl, -94 ♂pl, -95 ♀pl, -96 Pl, -98 ♀pl, -100 Pl, -101 Pl, and 35 L.

The holotype and some paratypes with their associated immature exuviae are deposited in the NMNH, Washington, DC. Most of the paratypes are deposited in the FSCA, Gainesville, FL. Additional paratypes are deposited in the NHM, London, United Kingdom, and the ORSTOM, Montpellier, France.

Bionomics. Habitats of immatures appear to be similar to those of *An. diluvialis.* Larvae were collected in temporary pools located in heavily shaded swamps adjacent to or near rivers (e.g., Choctawhatchee River) that experienced seasonal flooding and inundated the surrounding flood plain. Immatures were found in floating debris (e.g., recently fallen leaves and small twigs) at the water's interface with the shore, logs or bases of trees. Hatching of eggs obtained from feral females was usually low; however, the eggs hatched normally when they were deoxygenated or placed in water taken from the swamp habitat. Kaiser (1994) recorded larvae (as *An. quadrimaculatus* sp. C) from leaf litter and floatage in swamps with temporary water and a dense overhead canopy that allowed little sunlight to penetrate.

Daytime-resting adults were usually collected from large rot cavities in trees or from under bridges, but also from under eaves of buildings and from a plastic garbage can. Adults tended to be gregarious in these sites. The most common resting sites were in rot cavities of bald cypress and tupelo trees that were found within riverine swamps subject to intermittent flooding (e.g., panhandle of Florida and near Savannah, GA). Sweet gum, red maple, and bay trees also were typically found in these swamps. In Louisiana and western Florida, *An. inundatus* adult resting sites were usually shared by *An. quadrimaculatus* and, less frequently, by *An. maverlius* and *An. smaragdinus*; however, adult collections from Georgia often included all 4 species. Rutledge and Meek (1994) and Rutledge et al. (1996) collected adults from a cypress swamp in Vermilion Parish, LA.

Discussion. In addition to the characters used in the keys, the following features, used in combination, can be used for the identification of most specimens and for their separation from the other members of the complex: females by the combination of (1) tibiae I,II dark-scaled (100%), (2) femora I dark-scaled (100%) and II dark-scaled (86.2%), (3) dorsocentral area with golden piliform scales on anterior margin (97.5%), (4) sum of setae on both scutal fossal areas 38–59 (94.4%), (5) interocular area with 6–9 setae (90.1%), (6) prescutellar area with setae separated by moderately wide area on posterior margin (88.0%), (7) sum of setae on both scutal fossal areas 41–59 (86.5%), (8) scutal fossal area with 21–32 setae (82.7%), and (9) prealar area with 2–5 setae (82.1%); males by (10) maxillary palpus length longer than proboscis (90.0%); male genitalia by the combination of (11) tergum IX with heavily pigmented area of laterobasal band narrow and ventrolateral margin relatively smooth (100%), (12) claspette with seta 4 noticeably shorter than seta 5 (100%), (13) gonostylus with 21–34 tiny setae (100%), and (14) claspette with seta 5 not borne on short elevated process (97.1%); pupae by the combination of (15) sum of both seta 1-Pa with 2,3 branches (100%), (16) sum of both seta 6-III with 9–21 branches (98.5%), (17) sum of both seta 5-I with 10–19 branches (98.4%), (18) sum of both seta 7-I plus both seta 6-III with 20–37 branches (97.3%), (19) sum of both seta 6-I with 8–16 branches (94.6%), (20) sum of both seta 7-I with 11–22 branches (94.4%), (21) seta 1-VII 0.75 or less length of tergum VIII (93.3%), and (22) sum of both seta 9-VIII with 24–41 branches (89.2%); and 4th-instar larvae by the combination of (23) sum of both seta 8-II with 8–17 branches (100%), (24) sum of both seta 2-I plus both seta 9-I with 20–35 branches (100%), (25) sum of both seta 10-VI with 4–8 branches (100%), (26) sum of both

seta 2-I plus both seta 2-III with 19–34 branches (99.6%), (27) seta 3-A with serrations mesally (99.0%), (28) sum of both seta 2-I with 11–18 branches (97.2%), (29) sum of both seta 2-III with 9–18 branches (95.8%), (30) sum of both seta 8-III with 6–10 branches (94.8%), (31) sum of both seta 8-II plus both seta 8-III with 18–27 branches (92.9%), (32) sum of both seta 2-III with 10–18 branches (90.5%), (33) seta 1-A shorter than apex of flagellum (90.1%), (34) sum of both seta 2-I with 12–18 branches (87.7%), (35) sum of both seta 8-II plus both seta 8-III with 19–27 branches (85.5%), (36) sum of both seta 13-I with 10–14 branches (82.4%), (37) sum of both seta 9-II with 13–19 branches (80.5%), (38) seta 1-P longer than or equal to seta 3-P length (79.4%), and (39) sum of both seta 14-P with 8–15 branches (75.8%).

The following primary features, given above by numbers in parentheses and listed here in decreasing order of separation, best distinguish specimens of *An. inundatus* from *An. diluvialis*: in females, 3 (97.1%), 4 (93.3%), 7 (91.9%), and 8 (89.7%); and in 4th-instar larvae, 35 (87.6%) and 34 (80.6%); from *An. maverlius*: in females, 2 (95.7%), 5 (86.4%), 7 (84.0%), and 8 (82.4%); in male genitalia, 11 (100%) and 14 (92.7%); in pupae, 17 (98.8%) and 22 (89.0%); and in 4th-instar larvae, 26 (99.0%), 29 (97.7%), 28 (96.3%), 30 (95.9%), 25 (95.8%), 37 (86.9%), 36 (84.1%), and 33 (81.1%); from *An. quadrimaculatus*: in females, 1 (100%), 2 (96.6%), 6 (94.4%), and 9 (84.6%); in males, 10 (95.0%); in male genitalia, 11 (100%), 14 (98.6%), 12 (90.8%), and 13 (90.0%); in pupae, 15 (98.9%), 21 (95.7%), and 19 (93.6%); and in 4th-instar larvae, 27 (99.5%), 26 (97.4%), 32 (93.1%), 38 (89.7%), and 39 (83.7%); from *An. smaragdinus*: in females, 1 (100%), 2 (96.6%), 6 (94.4%), 7 (90.1%), and 8 (88.3%); in males, 10 (95.0%); in male genitalia, 11 (100%) and 13 (95.8%); in pupae, 17 (98.4%), 18 (98.3%), 16 (98.2%), 19 (95.5%), 21 (93.7%), 20 (93.4%), and 22 (93.2%); and in 4th-instar larvae, 26 (99.7%), 28 (98.6%), 27 (98.4%), 23 (96.9%), 31 (96.5%), 29 (95.1%), 24 (94.1%), 36 (91.2%), 39 (87.0%), and 38 (85.1%).

Male genitalia of *An. inundatus* often had the lateral margin of sternum VIII without a subbasal depression (71.4%), while this depression was present in the other species of the complex.

Pupal exuviae of *An. inundatus* were most similar to those of *An. diluvialis* (see discussion section of the latter species).

The species name *inundatus* is of Latin origin and refers to the habitat of this species, i.e., the overflowed or inundated flood plains along rivers.

Anopheles (Anopheles) maverlius Reinert, new species

Literature references

Anopheles quadrimaculatus species D of Narang et al. 1989a:317; Cockburn 1990:191; Apperson and Lanzaro 1991:507; Narang and Seawright 1991:59; Seawright et al. 1992:289; Mitchell et al. 1992:939; Mallet and Fritzius 1993:25; Linley et al. 1993:24 (E*); Johnson et al. 1993:939; Kaiser 1994:8; Rutledge and Meek 1994:585; Cornel et al. 1996:109; Reinert et al. 1996:310; Rutledge et al. 1996:952.

Female (Figs. 1G and 2C; Tables 1–5). *Head:* Antennal flagellum 0.64–0.80 length of proboscis, mean 0.73; maxillary palpus often less than 1.00 length of proboscis (70.0%), range 0.92–1.06, mean 0.98, with few semirecumbent scales basally; proboscis 0.91–1.09 length of femur I, mean 1.02; vertex, occiput, and dorsal area of postgena with erect forked scales long and narrow, pale-scaled patch of erect forked scales moderate- to small-sized; interocular area usually with 3–6 setae (96.6%) (3–5 setae, 82.6%), range 3–8, usually dark but occasionally 1–3 pale. *Thorax:* Anterior promontory with several to numerous golden piliform scales, usually 6 golden or golden-brown setae, range 4–10; acrostichal area with somewhat reduced number of golden-brown scales, more or less directed posteriorly; dorsocentral area usually with few to several golden piliform scales on anterior margin (99.1%), usually 2–6, range 2–10, remainder of dorsocentral area usually with sparse (occasionally with moderate number), somewhat longer brown or golden piliform scales, directed mainly posteriorly; prescutellar area usually with few brown piliform scales on anterior area mesally (some specimens without scales), moderate number of dark brown setae on anterior and lateral areas, posterolateral area usually without or with only 1,2 dark setae leaving wide median bare space (85.2%); scutal fossal area usually with 9–20 setae (82.1%), range 9–26, most long, sum of setae on both scutal fossal areas usually 22–40 (81.5%), range 22–49; supraalar setae dark; parascutellar area frequently with 2 setae (2 on both sides, 38.7%; 1 on both sides, 26.2%; 1 on 1 side and 2 on other, 27.0%; 1 on 1 side and 3 on other, 1.1%; 2 on 1 side and 3 on other, 6.8%; 3 on both sides, 0.3%), range 1–3, second seta usually 0.85–1.00 (very rarely 0.7) length of other seta; scutellum with few brown piliform scales on median 0.5–0.7 (occasional specimen also with 1–4 golden scales mesally); proepisternum usually with 7–14 upper setae (94.4%), range 4–14; prespiracular area with 1–6 setae, usually longer than other species of complex; mesokatepisternum usually with 1–3 upper (91.7%) setae, range 1–6, and with 2–7 lower setae, sum of upper and lower setae usually 3–8 (90.8%), range 3–11; prealar area often with 2–5 setae (55.6%), range 2–11; mesepimeron with 5–15 upper setae. *Legs:* Coxa I without scales anterodorsally; femora I often with fringe of pale scales apically (63.7%) (with very narrow pale-scaled band apically, 26.5%; with narrow pale-scaled band apically, 7.1%; dark-scaled, 2.7%), femur II usually with pale scales apically

(with fringe of pale scales, 36.3%; with very narrow pale-scaled band, 32.7%; with narrow pale-scaled band, 30.1%; dark-scaled, 0.9%), femur III often with moderate-sized pale-scaled band apically (67.0%) (with narrow pale-scaled band apically, 33.0%; tibiae I,II dark-scaled, III usually dark-scaled, 98.2%; with few pale scales apically, 1.8%).

Male. Essentially similar to female except for sexual features. *Head:* Antennal flagellum 0.75–0.89 length of proboscis, mean 0.80; maxillary palpus 1.01–1.13 length of proboscis, mean 1.05; proboscis 1.27–1.42 length of femur I, mean 1.33.

Male genitalia (Figs. 4A, 5A, and 6C). *Tergum VIII:* Apical margin with moderately deep median depression separating broadly rounded lobes (100%); length 0.41–0.49 mm, mean 0.45 mm; width 0.56–0.65 mm, mean 0.60 mm; VIII-Te index 0.71–0.79, mean 0.75. *Sternum VIII:* Lateral margin with subbasal depression. *Tergum IX:* Median band relatively long, length usually 0.04–0.05 mm, range 0.03–0.05 mm, mean 0.04 mm, width 0.16–0.21 mm, mean 0.18 mm; Te-IX index 0.18–0.27, mean 0.22; posterolateral lobe with apex truncate and smooth, length usually 0.07–0.09 mm, range 0.05–0.09 mm, mean 0.07 mm; PlL/Te-IX index 1.32–2.58, mean 1.96; laterobasal band with heavily pigmented area moderately wide (100%), ventrolateral margin relatively smooth (100%). *Phallosome:* Aedeagus length 0.20–0.23 mm, mean 0.22 mm, apex bearing 2–4 (2 on 1 side and 3 on other, 2.9%; 3 on both sides, 50.0%; 3 on 1 side and 4 on other, 26.5%; 4 on both sides, 20.6%) dark leaflets on each side of midline, apical leaflet broader, long and 0.36–0.53 length of aedeagus, other leaflets narrow, shorter, basal 1–3 leaflets usually each with 1 stout spicule anterobasally. *Gonocoxite:* Length 0.32–0.38 mm, mean 0.35 mm; ventral surface with internal seta situated slightly more distal than *An. quadrimaculatus,* apical portion usually recurved (occasionally curved). *Gonostylus:* Moderately pigmented; length 0.39–0.44 mm, mean 0.42 mm; often with 20–27 tiny setae (72.1%), range 15–27, most usually in more or less irregular line on distal 0.67–0.70 (85.0%); Gs/Gc index 1.13–1.27, mean 1.20. *Claspette:* Seta 2 usually absent (94.1%) (present 5.9%); seta 4 usually noticeably shorter than seta 5 (97.1%) (4 slightly longer than 5, 2.9%), situated dorsally at same level (53.1%) or slightly mesal (43.8%) to seta 3 (4 slightly dorsolateral to 3, 3.1%); seta 5 formed of 2–4 (2 on both sides, 17.6%; 2 on 1 side and 3 on other, 11.8%; 3 on both sides, 55.9%; 3 on 1 side and 4 on other, 11.8%; 4 on both sides, 2.9%) apically fused stems (occasionally not fused apically) and usually borne on short elevated process (88.2%).

Pupa (Fig. 10). Chaetotaxy as figured and recorded in Tables 8 and 11–17. *Cephalothorax:* Integument light tan, occasionally with some moderately pigmented areas; dorsal apotome with median apical projection, well defined, flaplike (100%); lateralia with cuticular ocular facets of compound eye poorly developed; scutum with anterior angle broad, *ca.* 90° (99.6%) (rarely 82°, 0.4%); median keel with lateral line moderately long, extending posteriorly to near seta 8-CT (99.6%) (extending slightly posterior to 8-CT, 0.4%); mesothoracic wing usually light tan, occasionally with moderately pigmented indistinct longitudinal lines; postscutal area completely split to metanotum by dorsal ecdysal opening (100%). *Trumpet:* Light tan; index 3.35–4.00, mean 3.67; length 0.45–0.52 mm, mean 0.51 mm; width 0.12–0.14 mm, mean 0.13 mm; pinna 0.77–0.89 of trumpet length, mean 0.83. *Metanotum:* Metanotal wing usually with most of basal area moderately pigmented, occasionally entirely pale; area between pair of seta 10-CT with several well-defined deep grooves; sum of both 10-CT usually with 7–18 branches (98.2%), (8–18 branches, 92.5%), range 6–18. *Abdomen:* Tergum I pigmentation similar to *An. diluvialis* and *An. inundatus*; tergum III with many spicules arranged in short rows of 2,3 spicules; terga III–VII with well-developed curved transverse ridge posterior to seta 1; tergum VIII with posterolateral corner moderately pigmented; seta 1-VII usually 0.85 or less length of tergum VIII (92.5%) (length 1.0, 5.8%; slightly longer than 1.0, 1.7%); sum of both 5-I usually with 4–9 branches (99.2%) (4–8 branches, 95.1%), range 4–10; sum of both 6-I with 3–12 branches; sum of both 6-III with 5–14 branches; sum of both 7-I with 6–16 branches; sum of both 7-I plus both 6-III with 13–27 branches; 9-III-VII spiniform, usually broad, flattened, straight (tapered in 26.9% of VI, 15.4% of VII) (curved in 7.7% of VII); ratio of length of 9-III/9-IV 0.39–0.79 (mean 0.57), 9-IV/9-V 0.67–1.07 (mean 0.92), 9-V/9-VI 0.67–0.99 (mean 0.84), 9-VI/9-VII 0.68–0.96 (mean 0.81), 9-III/9-VII 0.28–0.43 (mean 0.36); sum of both 9-VIII usually with 9–24 branches (93.0%) (9–23 branches, 88.7%), range 9–30; 1-IX single (rarely 2-branched); sternal transverse ridges as in *An. quadrimaculatus*; male genital lobe usually longer, with stout pointed spicule at about midlength, index 1.25–1.33, mean 1.27. *Paddle:* Seta 1-Pa usually single (88.6%) (with 2 branches, 10.5%; with 3 branches, 0.9%), range 1–3; sum of both 1-Pa usually with 2 branches (82.1%) (with 3 branches, 11.4%; with 4 branches, 4.2%; with 5 branches, 2.2%; with 6 branches, 0.2%), range 2–6; 2-Pa extends beyond apex of paddle; index 1.32–1.52, mean 1.43; length 0.77–0.98 mm, mean 0.87 mm; width 0.53–0.71 mm, mean 0.62 mm; refractile border on basal 0.68–0.79 of outer margin; nonrefractile border on apical 0.21–0.32 of outer and apical 0.03–0.11 of inner margins with sparse short thin aciculae.

4th-instar larva (Fig. 15). Chaetotaxy as figured and recorded in Tables 20 and 23–31. *Head:* Light brown, usually with sparse, more darkly pigmented small areas on dorsal and ventral surfaces, arrange-

ment of darker areas somewhat variable, frontal ecdysial line without or with narrow moderately pigmented area on both sides along posterior *ca.* 0.4 of length; dorsal surface usually with well-developed spicules, most in short rows of 3–5 spicules, occasionally with spicules smaller; seta 2-C shorter, usually 1.29 or less length of 3-C (86.2%), range 1.05–1.53, distance between alveoli usually slightly wider; 3-C lightly dendritic, usually with 31–49 apical branches (96.7%) that are bunched together, range 28–49, very few stout basal branches; 5,6-C and often 7-C usually with branches more widely spaced along shaft. *Antenna:* Flagellum length often 0.31–0.32 mm (55.6%), range 0.28–0.32 mm; seta 1-A often extending noticeably beyond apex of flagellum (56.5%) (equal to apex, 15.5%; shorter than apex, 28.0%), inserted usually 0.31–0.53 from base (97.6%), range 0.29–0.53, often plumose; sum of both 1-A usually with 18–29 branches (98.9%), range 17–29; 3-A darkly pigmented, usually with 2–5 small serrations on mesal margin about midlength (94.5%) (margin smooth, 5.5%); 4-A with 4–8 branches. *Mouthparts:* Dorsomentum darkly pigmented with 9 teeth. *Thorax:* Most plumose setae usually with branches somewhat more widely spaced along shaft; seta 1-P usually noticeably longer than 3-P (93.3%) (slightly shorter, 6.7%); 1-P usually with 2–6 branches (98.8%) (single, 1.2%), branches longer and more basal, usually on apical 0.53–0.81 (98.8%), range 0.47–0.81; sum of both 4-P with 22–30 branches (100%); 9-P usually both single (97.4%) (single on 1 side and other with 2 branches, 2.2%; both sides with 2 branches, 0.4%); sum of both 14-P usually with 8–15 branches (97.0%), range 8–18. *Abdomen:* Seta 1-VII palmate, leaflets usually broad (88.5%) (moderately broad, 11.5%); sum of both 2-I usually with 6–10 branches (95.4%), range 6–14; sum of both 2-I plus both 2-III usually with 10–18 branches (98.4%), range 10–21; sum of both 2-I plus both 9-I with 21–32 branches (100%); sum of both 2-III usually with 2–8 branches (99.6%), range 2–10; 6-III with branches more widely spaced along shaft; 8-II longer, sum of both 8-II usually with 11–21 branches (99.3%), range 10–21; sum of both 8-II plus both 8-III with 21–34 branches (100%); sum of both 8-II plus both 9-II usually with 29–49 branches (95.9%), range 27–49; 8-III usually on pigmented setal support plate; sum of both 8-III usually with 11–17 branches (96.9%), range 9–17; sum of both 8-III plus both 8-VI usually with 19–28 branches (99.5%), range 18–28; sum of both 8-VI usually with 8–14 branches (97.6%), range 7–14; sum of both 8-V usually with 7–9 branches (82.8%), range 6–9; sum of both 8-VI usually with 8–14 branches (97.5%), range 7–14; sum of both 9-II usually with 20–32 branches (93.2%), range 18–32; sum of both 10-VI usually with 2,3 branches (91.5%), range 2–5; sum of both 13-I usually with 6–9 branches (85.7%), range 6–11; segment VIII usually with small sternal plate anteromesally (98.5%) (absent 1.5%), somewhat larger; pecten plate with 6–10 long spines with well-developed spicules laterally, long spines somewhat shorter than in *An. quadrimaculatus* and *An. smaragdinus,* most anterior long spine strongly bent near base, specimens from Florida often with both plates with 8 or fewer long spines (78.6%) (1 or both plates with 9,10 long spines, 21.4%), specimens from Georgia usually with 1 or both plates with 9,10 long spines (94.3%) (both plates with 8 or fewer long spines, 5.7%), specimens from Mississippi usually with 1 or both plates with 9,10 long spines (87.5%) (both plates with 8 or fewer long spines, 12.5%); seta 7-S both often with 2 branches (76.6%) (single on 1 side and other with 2 branches, 19.9%; both sides single, 3.5%), sternal plate usually present (98.5%), somewhat larger; segment X with spicules forming small patch located more or less posterior to point of insertion of seta 1-X, also segment X with spicules on posterior *ca.* 0.2 and few on ventral margin.

Egg (Figs. 18A, 19–1E, and 19-2E). The following information is taken from Linley et al. (1993). *Linear dimensions:* Mean length 547.9 ± 4.4 μm; width 193.4 ± 2.9 μm. *Deck:* Plastron large, separating deck into anterior and posterior areas; number of lobed tubercles, anterior 6.8 ± 0.3, posterior 5.5 ± 0.3; mean anterior deck tubercle area 1.68 ± 0.05 μm, density 94.7 ± 2.2. *Float:* Mean length of both floats 273.4 ± 4.9 μm; mean float length as percentage of egg length 50.3 ± 0.6; mean float length/mean number of ribs 13.3 ± 0.3. *Dorsal plastron:* Mean individual pore area 2.38 ± 0.13 μm, small.

Type data. The type series of *An. maverlius* was reared from a single F_1 progeny brood of a female collected in nature from a very large rot cavity (extending from ground level to a height of *ca.* 1.1 m and about 0.4 m wide) in a large live oak tree located at the margin of a heavily shaded, shallow, partly dried up, freshwater, flood plain pool on May 31, 1995, by John F. Reinert and Paul E. Kaiser. This site was situated *ca.* 30 m from the western margin of the Choctawhatchee River and was located *ca.* 0.1 mi. north of the Florida State Highway 20 bridge at the eastern end of Whitten Road; the nearest town was Bruce, Walton County, FL. The holotype (FL95.14–16) consists of a female glued to a small triangular paper point on an insect pin and its associated pupal and 4th-instar larval exuviae mounted in Canada balsam on a microscope slide. The remainder of the type series includes the following specimens: paratypes, FL95.14–1 ♂pl, -2 ♂p, -3 ♂pl, -4 ♂pl, -5 ♂pl, -6 ♂pl, -7 ♀pl, -8 ♂plg, -9 ♂pl, -10 ♂pl, -11 ♂pl, -12 ♂pl, -13 ♂pl, -14 ♀pl, -15 ♂pl, -17 ♂pl, -18 ♀pl, -19 ♂pl, -20 ♂plg, -21 ♂pl, -22 ♀pl, -23 ♂pl, -24 ♀pl, -25 ♂pl, -26 ♀pl, -27 ♂plg, -28 ♂pl, -30 ♀pl, -31 ♂pl, -32 ♂pl, -33 ♀l, -34 ♂pl, -35 ♂pl, -36 ♂ and ♂g, -37 ♀pl, -38 ♀pl, -39 ♀pl, -40 ♂pl, -41 ♂pl, -42 ♂plg, -43 ♂pl, -44 ♀pl, -45 ♀, -46 ♀pl, -47 ♀pl, -48 ♀pl, -49 ♂pl, -50 ♀, -51 ♀pl, -52 ♂, -53 ♂, -54 ♀pl,

-55 ♀pl, -56 ♂pl, -57 ♀pl, -58 ♀, -59 ♂pl, -60 ♂plg, -61 ♀pl, -62 ♂pl, -63 ♂pl, -64 ♀pl, -65 ♂pl, -66 ♀pl, -67 ♂plg, -68 ♀, -69 ♂pl, -70 ♂pl, -71 ♀pl, -72 ♂pl, -73 ♀pl, -74 ♂pl, -75 ♀pl, -76 ♀pl, -77 ♀pl, -78 ♀pl, -79 ♀pl, -80 ♂pl, -81 ♀pl, -82 ♀pl, -83 ♀pl, -84 ♀pl, -85 ♀pl, -86 ♀pl, -87 Pl, 3 p, and 19 L.

The holotype and some paratypes with their associated immature exuviae are deposited in the NMNH, Washington, DC. Most of the paratypes are deposited in the FSCA, Gainesville, FL. Additional paratypes are deposited in the NHM, London, United Kingdom, and the ORSTOM, Montpellier, France.

Bionomics. In Tishomingo Co., MS, larvae were collected near the margin of a wooded drainage ditch that flowed into the Yellow Creek, which extended between the Pickwick Reservoir and the Tombigbee Waterway. The ditch usually contained water, but on occasion it was dry because of fluctuations in water levels of the reservoir. Shore grasses and parrot feather (*Myriophyllum* spp.) were found in the ditch, and the wooded area was composed of bald cypress, sweet gum, and birch trees. Kaiser (1994) reported that the typical habitat for *An. maverlius* (as *An. quadrimaculatus* sp. D) was similar to that of *An. diluvialis* and/or *An. inundatus* (as *An. quadrimaculatus* sp. C) but was found further inland.

Daytime-resting adults were collected primarily from large cavities in trees but also from under bridges and under the eaves of buildings. Adults tended to be gregarious in these sites. Adult resting sites were near rivers or tributaries of reservoirs that experienced either temporary flooding or fluctuations in water level that resulted in a transitory inundation of adjacent wetlands. Adults collected from swamps in the southernmost portion of the species range contained bald cypress, sweet gum, red maple, and tupelo trees, while those farther north also contained ash and birch trees. Adult collections of *An. maverlius* from riverine environments often also included *An. inundatus, An. quadrimaculatus,* and *An. smaragdinus,* whereas collections from reservoir sites always included *An. quadrimaculatus.* Frequency of *An. maverlius* in adult collections was usually low (Seawright et al. 1992), but 74% of populations at Yellow Creek, Tishomingo Co., MS, were this species. Only 2 of 353 adults of the Quadrimaculatus Complex collected by Apperson and Lanzaro (1991) from 2 small utility sheds at Noxubee Wildlife Refuge, Noxubee Co., MS, were *An. maverlius* (as *An. quadrimaculatus* sp. D). These 2 females had fed on white-tailed deer. Mallet and Fritzius (1993) reported that in Mississippi this species was restricted to relatively undisturbed forest habitats near major rivers to the east and south of the Delta, and that it was susceptible to malathion and permethrin. Rutledge et al. (1996) aspirated adults from under a bridge in Mississippi.

Discussion. In addition to the characters used in the keys, the following features, used in combination, can be used for the identification of most specimens and for separation from the other members of the complex: females by the combination of (1) tibiae I,II dark-scaled (100%), (2) dorsocentral area with golden piliform scales on anterior margin (99.1%), (3) femora I (97.3%) and II (99.1%) with pale scales apically, (4) prescutellar area with setae widely separated on posterior margin (85.2%), (5) sum of setae on both scutal fossal areas 22–40 (81.5%), and (6) maxillary palpus less than 1.0 length of proboscis (70.0%); males by (7) maxillary palpus length longer than proboscis (100%); male genitalia by the combination of (8) tergum IX with heavily pigmented area of laterobasal band moderately wide and ventrolateral margin relatively smooth (100%), (9) gonostylus length 0.39–0.44 mm (100%), (10) claspette with seta 4 noticeably shorter than seta 5 (97.1%), (11) claspette with seta 4 at same level or slightly mesal to seta 3 (96.9%), (12) claspette with seta 5 borne on short elevated process (88.2%), and (13) gonostylus with 20–27 tiny setae (72.1%); pupae by the combination of (14) exuviae with postscutal area of cephalothorax completely split to metanotum by dorsal ecdysal opening (100%), (15) cephalothorax with lateral line of median keel moderately long, extending posteriorly to near seta 8-CT (99.6%), (16) sum of both seta 5-I with 4–9 branches (99.2%), (17) sum of both seta 10-CT with 7–18 branches (98.2%), (18) sum of both seta 5-I with 4–8 branches (95.1%), (19) sum of both seta 1-Pa with 2,3 branches (93.5%), (20) sum of both seta 9-VIII with 9–24 branches (93.0%), (21) seta 1-VII 0.85 or less length of tergum VIII (92.5%), (22) sum of both seta 10-CT with 8–18 branches (92.5%), and (23) sum of both seta 9-VIII with 9–23 branches (88.7%); and 4th-instar larvae by the combination of (24) sum of both seta 8-II plus both seta 8-III with 21–34 branches (100%), (25) sum of both seta 2-I plus both seta 9-I with 21–32 branches (100%), (26) sum of both seta 4-P with 22–30 branches (100%), (27) sum of both seta 2-III with 2–8 branches (99.6%), (28) sum of both seta 8-III with 10–17 branches (99.6%), (29) sum of both seta 8-II plus both seta 9-II with 29–49 branches (99.5%), (30) sum of both seta 8-II with 11–21 branches (99.3%), (31) sum of both seta 2-I plus both seta 2-III with 10–18 branches (98.4%), (32) sum of both seta 8-VI with 8–14 branches (97.5%), (33) sum of both seta 14-P with 8–15 branches (97.0%), (34) sum of both seta 8-III with 11–17 branches (96.9%), (35) sum of both seta 2-I with 6–10 branches (95.4%), (36) seta 3-A with serrations mesally (94.5%), (37) seta 1-P noticeably longer than seta 3-P length (93.3%), (38) sum of both seta 9-II with 20–32 branches (93.2%), (39) sum of both seta 10-VI with 2,3 branches (91.5%), (40) sum of both seta 13-I with 6–9 branches (85.7%), (41) seta

7-S both with 2 branches (76.6%), and (42) seta 1-A longer than apex of flagellum (72.0%).

The following primary features, given above by numbers in parentheses and listed here in decreasing order of separation, best distinguish specimens of *An. maverlius* from *An. diluvialis*: in females, 2 (97.9%), 3 (97.1%), and 6 (85.0%); in male genitalia, 8 (100%), 11 (98.5%), and 12 (90.5%); in pupae, 14 (100%), 15 (99.8%), 17 (97.6%), 18 (95.1%), and 23 (90.5%); and in 4th-instar larvae, 27 (98.7%), 24 (97.9%), 28 (96.6%), 38 (96.0%), 31 (95.9%), 39 (95.8%), 32 (92.3%), 33 (90.6%), 40 (88.9%), 42 (84.5%), and 41 (83.0%); from *An. inundatus*: in females, 3 (96.1%), 6 (85.0%), and 5 (84.0%); in male genitalia, 8 (100%), 11 (98.5%), and 12 (92.7%); in pupae, 14 (100%), 15 (99.8%), 16 (98.8%), and 23 (89.0%); and in 4th-instar larvae, 31 (99.0%), 27 (97.7%), 35 (96.3%), 34 (95.9%), 39 (95.8%), 38 (86.9%), 40 (84.1%), and 42 (81.1%); from *An. quadrimaculatus*: in females, 1 (100%), 4 (92.6%), 5 (89.3%), and 6 (80.0%); in males, 7 (100%); in male genitalia, 8 (100%), 9 (99.0%), 11 (90.5%), 10 (89.4%), and 13 (76.1%); in pupae, 19 (95.7%), 21 (95.3%), and 20 (93.4%); and in 4th-instar larvae, 24 (100%), 28 (99.2%), 30 (99.1%), 29 (98.1%), 36 (97.3%), 26 (97.0%), 37 (96.7%), 33 (94.3%), 38 (93.7%), and 39 (91.2%); and from *An. smaragdinus*: in females, 1 (100%), 4 (92.6%), and 6 (82.5%); in males, 7 (100%); in male genitalia, 9 (100%), 8 (100%), 11 (98.5%), 13 (81.8%), and 12 (73.4%); in pupae, 21 (93.3%) and 22 (90.4%); and in 4th-instar larvae, 24 (100%), 28 (99.8%), 29 (99.8%), 30 (99.7%), 32 (98.0%), 33 (97.6%), 25 (96.3%), 36 (96.2%), 38 (94.3%), 37 (92.1%), 42 (85.1%), 39 (84.8%), and 41 (80.1%).

Male genitalia of *An. maverlius* usually had the gonostylus with an irregular line of tiny setae on the distal 0.67–0.70 (85.0%), leaving a larger proximal bare space that distinguished them from those of other species of the complex in which the setae occur on the distal 0.71–0.86.

Other features of pupal exuviae, in addition to those mentioned above, useful in distinguishing *An. maverlius* were: tergum VIII with posterolateral corner moderately pigmented; metanotum with several well-defined deep grooves on the area between the pair of seta 10-CT; and seta 9-VII usually broad and straight (tapered in 15% and curved in 8% of specimens). The male genital lobe was usually longer (index 1.25–1.33) and with a stout spicule at about midlength (*An. quadrimaculatus* very rarely with a minute spicule, other species without a spicule).

The species name *maverlius* was derived from the acronym for the Medical and Veterinary Entomology Research Laboratory (MAVERL) of the United States Department of Agriculture (USDA), where most of the investigations on the new species of the Quadrimaculatus Complex were conducted. The MAVERL was combined with the USDA's Insect Attractants, Behavior and Basic Biology Research Laboratory in 1996 to form the Center for Medical, Agricultural, and Veterinary Entomology. The name is to be taken as masculine in gender.

Anopheles (Anopheles) quadrimaculatus Say

Synonymy

Anopheles 4-maculatus Say, 1824:356 (♀).
Anopheles annulimanus Van der Wulp, 1867:129 (♂).

Literature references

Anopheles annulimanus of Giles 1900:170 (♂), 1902:325 (♂); Theobald 1901:212 (♂), 1907:26; Blanchard 1905:202; Howard et al. 1917:1029 (♂); Edwards 1925:260, 1932:39; Dyar 1928: 453; Carpenter and LaCasse 1955:50; Stone et al. 1959:27; Knight and Stone 1977:29; Belkin 1968:9.

Anopheles quadrimaculatus of Theobald 1910:5; Howard et al. 1917:1028 (in part); Daggy et al. 1941:883; French et al. 1962:377; Klassen et al. 1965:328; Belkin et al. 1966:3 (restricted type locality); Seawright and Anthony 1972:47; Kitzmiller 1982:445; Mitchell and Seawright 1984a: 341, 1984b:421; Wing et al. 1985:511; Benedict and Seawright 1987:55; Shetty et al. 1987:s9.

Anopheles quadrimaculatus species A of Lanzaro 1987:0955; Kim et al. 1987b:50, 1987a:187; Kaiser and Seawright 1987:222; Willis et al. 1987: s45; Kaiser et al. 1988b:34, 1988a:138; Lanzaro et al. 1988:248; Cockburn and Seawright 1988: 261; Cockburn et al. 1988:299; Narang and Seawright 1988:303; Kaiser 1988:311; Kaiser et al. 1988c:494; Mitchell and Seawright 1989a:58; Cockburn and Mitchell 1989:105; Narang et al. 1989b:508, 1989a:317, 1990b:3.194; Mitchell and Seawright 1989b:496; Cockburn et al. 1990: 31; Cockburn 1990:191; Lanzaro et al. 1990:578; Collins et al. 1990:417; Narang and Seawright 1990:533; Seawright et al. 1991:221; Lanzaro et al. 1991:349; Apperson and Lanzaro 1991:507; Narang and Seawright 1991:59; Nayar et al. 1992:61; Johnson et al. 1992:231; Seawright et al. 1992:289; Mitchell et al. 1992:939; Mallet and Fritzius 1993:25; Linley et al. 1993:124 (E*); Mitchell et al. 1993b:3.273; Johnson et al. 1993:939; Jensen et al. 1993:1038; Narang et al. 1993:463; Mitchell et al. 1993a:1058; Kaiser 1994:8; Rutledge and Meek 1994:585; Jensen et al. 1995:141; Perera et al. 1995:836; Cornel et al. 1996:109; Jensen et al. 1996:523; Reinert et al. 1996:310; Rutledge et al. 1996:952.

Female (Figs. 1A-C, 2A, and 2F; Tables 1–5). *Head:* Antennal flagellum 0.67–0.78 length of proboscis, mean 0.73; pedicel dark, with patch of small spatulate dark scales mesally and dorsally, 2,3 very short thin curved dark setae dorsolaterally; antennal flagellum dark, flagellomere 1 with few small spatulate dark scales mesally; maxillary palpus usually

1.00–1.09 length of proboscis (90.0%), range 0.94–1.09, mean 1.04, dark-scaled, several semirecumbent longer scales dorsally on basal area, palpomere 2 usually with 2 (range 1–3) of basal setae dark, moderately long, semierect and positioned dorsolaterally; proboscis 0.99–1.13 length of femur I, mean 1.06, dark-scaled, few to several semirecumbent longer scales ventrally on basal area, labellum pale brown; clypeus dark, bare; entire dorsal and dorsolateral portions of head posterior to ocular line covered with dense patch of dark long narrow erect forked scales except often scales on dorsolateral area moderately long and often more or less moderately broad, and moderate-sized patch of pale erect forked scales on median anterior area; vertex with small, more or less diamond-shaped patch of narrow curved recumbent white scales anteromesally and extending anteriorly onto posterior portion of ocular line; frontal tuft with similar but much longer scales extended over interocular space; several long and few moderately long dark curved ocular setae; interocular area usually with 7–12 long setae (98.0%), range 6–12, few dark and 2–5 pale, rarely all dark; postgenal area with several moderately long, dark setae. *Thorax:* Anterior promontory with long linear, erect or semierect, usually white or golden-white scales forming dense patch and extending slightly onto anterior margin of acrostichal area, 6–8 pale setae; acrostichal area with entire length having numerous golden semirecumbent piliform scales directed more or less laterally and presenting shaggy appearance, numerous brown semierect short setae in 2 rows intermingled with scales; dorsocentral area always with several white or golden-white piliform scales on anterior margin, remainder of entire length of dorsocentral area with numerous scales similar to those on acrostichal area but more or less directed posterolaterally, irregular row of slightly longer brown setae, scales of acrostichal and dorsocentral areas merge together on area posterior to level of juncture of prescutal suture with dorsocentral area and form uniformly scaled area with prescutellar area; prescutellar area with golden piliform scales, numerous setae on lateral margins of which lateral longer ones are brown and mesal shorter ones are often golden, several golden or golden-brown (occasional specimens also with few brown) setae on anterior area intermixed with scales, other golden setae nearly forming line on posterior margin leaving only narrow median bare space; scutal fossal area usually with 21–45 dark brown setae (93.1%), range 15–45, many moderately long and often thin, remainder long (usually small bare areas separating scutal fossal and dorsocentral setae), also usually posteromesal 1–3 setae dark brown, shorter, foliform, strongly curved, usually apex truncate, sum of setae on both scutal fossal areas usually 41–75 (97.1%), range 35–75; antealar and supraalar areas covered with continuous patch of numerous dark brown setae except 2–6 supraalar pale short setae anterior to base of wing, setae moderately long on antealar area, long on supraalar area except for small patch of shorter ones anterior to base of wing; parascutellar area often with 2,3 long dark brown setae (2 on both sides, 55.1%; 2 on 1 side and 1 on other, 22.6%; 1 on both sides, 11.3%; 3 on 1 side and 1 on other, 0.3%; 3 on 1 side and 2 on other, 10.0%; 3 on both sides, 0.8%), range 1–3, second seta usually 0.85–1.00 (very rarely 0.7) length of other seta; scutellum with numerous golden (occasional specimen also with very few golden-brown to brown) piliform scales (scales short relative to setae) and slightly anterior to numerous, evenly spaced, moderately long and long dark brown setae on posterior margin; paratergite and mesopostnotum bare; anteprononotum with patch of several brown setae, moderately long anteriorly and dorsally, short and fine posteriorly and ventrally; prespiracular area with 1–10 setae, usually golden; proepisternum usually with 2–6 dark upper setae (98.6%), range 2–8; mesokatepisternum usually with 4–9 upper (93.8%), range 2–9, and usually with 3–10 lower dark setae, sum of upper and lower setae usually 9–18 (92.1%) (10–18 setae, 85.5%), range 6–18; prealar area usually with 6–12 dark setae (87.0%), range 3–12; mesepimeron with 5–17 upper golden setae; other pleural areas bare. *Legs:* Coxae I–III with several setae, I usually with 1,2 (range 1–3) moderately broad brown scales anterodorsally; trochanters I–III with few broad brown scales, numerous curved pale setae; femora I–III each dark-brown-scaled with well-defined pale-scaled apical band, width of band very narrow to narrow on I, moderate-sized on II, well developed on III; tibiae I–III each dark-brown-scaled, I,II with very narrow to narrow pale-scaled apical band (I with only several pale scales apically, 17.2%), III with distinct narrow pale-scaled band apically, apex of tibiae with 1 seta longer, straight on I, slightly curved on II, strongly curved on III; tarsi I–III each dark-brown-scaled; posttarsi I–III each with 2 ungues, equal in size, simple. *Wing:* All veins and wing fringe dark-scaled but with 4 darker areas presenting appearance of spots, i.e., first spot (most proximal one) formed by scales on radius-one (R_1) for short distance both directions at juncture with radial sector (R_S), plus scales on anterior and posterior margins of R_S from base *ca.* 0.6 distance to juncture of radius-four-plus-five (R_{4+5}), second spot (large median one) formed by scales on anterior and posterior margins of following veins, R_{2+3} for short distance both directions at juncture of short basal crossveinlike segment of R_{4+5}, plus short distal area of media and proximal area of media-one-plus-two (M_{1+2}) on both sides at juncture with radiomedial crossvein (rm), plus distal portion of mediocubital crossvein (mcu) at juncture with and for short distance on proximal portion of media-three-plus-four (M_{3+4}), third spot (anterodistal one) formed by scales on anterior and posterior margins of proximal portions of veins radius-two (R_2) and

radius-three (R_3), and fourth spot (posterodistal one) formed by scales on anterior and posterior margins of proximal portions of veins media-one (M_1) and media-two (M_2), plus distal portion of M_{3+4}; remigium with ventral surface having well-developed patch of scales (20 or more); alula without scales; upper calypter with numerous long, pale setae on margin. *Halter:* Capitellum dark-brown-scaled; pedicel pale. *Abdomen:* Terga and sterna with integument somewhat darker on posterior portion, without scales, covered with numerous golden-brown to brown setae, those on lateral areas of terga longer.

Male. Essentially similar to female except for sexual features. *Head:* Antennal flagellum 0.74–0.81 length of proboscis, mean 0.78; maxillary palpus 0.89–0.99 length of proboscis, mean 0.96; proboscis 1.33–1.41 length of femur I, mean 1.37. *Legs:* Posttarsus I with 1 large unguis, with 1 median long narrow tooth and 1 basal short curved tooth, posttarsi II,III each with 2 ungues, equal in size, simple.

Male genitalia (Figs. 3, 4C, 5C, and 6A). *Tergum VIII:* Moderately pigmented and covered with minute spicules, except basal *ca.* 0.35 heavily pigmented and nonspiculate; scales absent; numerous moderately long and long setae; basolateral seta absent; apical margin very gently convex (38.8%) or straight (59.2%) (gently concave, 2.0%); basal margin straight; wider than long; length 0.38–0.46 mm, mean 0.43 mm; width 0.47–0.64 mm, mean 0.53 mm; tergum VIII index 0.69–0.88, mean 0.80. *Sternum VIII:* Moderately pigmented; covered with minute spicules; scales absent; numerous short and moderately long setae, several long setae apically and laterally; 2 tiny basomedian setae near midline; apical margin straight; basal margin slightly concave mesally; lateral margin with subbasal depression; wider than long. *Tergum IX:* Heavily pigmented; consists of relatively wide and narrow median band, covered with short spicules, length 0.02–0.03 mm, mean 0.02 mm, width 0.16–0.22 mm, mean 0.19 mm; PIL elongate, narrow, apex bluntly rounded and usually smooth (96.0%) (slightly ridged, 4.0%), length 0.03–0.10 mm, mean 0.07 mm; Te-IX index 0.10–0.15, mean 0.13; PIL/Te–IX index 1.33–4.43, mean 2.92; laterobasal band with heavily pigmented area wide (100%), ventrolateral margin noticeably irregular in shape (100%), covered with minute spicules, very narrowly connected with sternum IX. *Proctiger:* Paraproct lightly pigmented; cercus long, conical, membranous, covered with numerous short spicules. *Tergum X:* Consists of narrow, lightly pigmented band at base of cercus. *Phallosome:* Aedeagus length 0.16–0.20 mm, mean 0.18 mm, consists of elongate, dorsally curved, narrow, tubelike structure bearing 2–4 (2 on 1 side and 3 on other, 20.4%; 3 on both sides, 71.4%; 3 on 1 side and 4 on other, 6.1%; 4 on both sides, 2.0%) pale narrow leaflets apically on each side of midline, apical leaflet moderately long and 0.27–0.41 length of aedeagus, other leaflets shorter, basal 1,2 leaflets usually with 1–3 short pointed spicules anterobasally, base of aedeagus split and directed slightly laterally; paramere heavy pigmented, short, expanded posteriorly; basal piece heavily pigmented, short, curved. *Gonocoxite:* Heavily pigmented; covered with short spicules; scales absent; length 0.27–0.34 mm, mean 0.30 mm; mesal surface scleritized and contiguous with dorsal and ventral surfaces, with few short fine setae; dorsal surface with several short setae, 2 heavily pigmented long stout parabasal setae, mesal seta shorter, both attached to common elevated process (parabasal lobe) which is heavily pigmented, large, situated dorsobasally; lateral surface with numerous long stout setae from near base to apex, proximal 3–5 setae moderately long; ventral surface narrowly connected with other gonocoxite by median basal triangular projection, with short scattered setae, few moderately long setae mesally and apically, internal seta long, stout, apically curved, situated mesally about midway between base and apex. *Gonostylus:* Longer than gonocoxite, length 0.31–0.38 mm, mean 0.35 mm; heavily pigmented, tubular, long, mesally curved, usually with 11–19 tiny setae (80.0%), range 11–24, most in more or less irregular line from near base to apex (usually on distal 0.79–0.86, 91.8%); Gs/Gc index 1.02–1.30, mean 1.17; gonostylar claw heavily pigmented, short, bluntly pointed, attached subapically. *Claspette:* Heavily pigmented; ventral lobe connected mesally with its mate, covered with moderately long stout spicules, seta 1 moderately long, narrow, curved, lanceolate, situated on sternomesal margin, seta 2 present (100%), short, thin, simple, situated lateral to seta 1, seta 3 long, distally curved, lanceolate, situated lateral to seta 1, seta 4 moderately long, usually slightly longer than seta 5 (81.6%) (4 equal to 5, 14.3%; 4 slightly shorter than 5, 4.1%), lanceolate, situated dorsolateral to seta 3 (100%) and ventromesal to seta 5; dorsal lobe fused with ventral lobe, nonspiculate, seta 5 composed of 1–3 (1 on 1 side and 2 on other, 14.3%; 1 on 1 side and 3 on other, 2.0%; 2 on both sides, 51.0%; 2 on 1 side and 3 on other, 26.5%; 3 on both sides, 6.1%) moderately long, flattened, apically fused and expanded setae, borne on short elevated process (100%). *Sternum IX:* Heavily pigmented; covered with short spicules; apex broadly convex; base broadly concave.

Pupa (Fig. 11). Chaetotaxy as figured and recorded in Tables 9 and 11–17. *Cephalothorax:* Integument light tan, often with some darker pigmented areas; dorsal apotome with apex usually slightly convex or flat (98.7%) (broadly rounded, 1.3%), without median apical flaplike projection (100%); lateralia with cuticular ocular facets of compound eye well developed; scutum with anterior angle acute, produced into short blunt lobe (100%); median keel with transverse striations poorly developed, lateral line long and extending

from seta 4-CT posteriorly to at least base of seta 8-CT (100%); mesothoracic wing usually with poorly defined pale pigmented longitudinal lines (occasionally absent); postscutal area completely split to metanotum by dorsal ecdysial line (100%). *Trumpet:* Angusticorn type; simple with deep meatal cleft; integument median tan; index 2.53–3.73, mean 3.18; length 0.39–0.56 mm, mean 0.48 mm; width 0.12–0.20 mm, mean 0.15 mm; pinna 0.76–0.91 of trumpet length, mean 0.83. *Metanotum:* Metanotal wing with most of basal area darkly pigmented (rarely restricted to small spot); area between pair of seta 10-CT without or with few indistinct shallow transverse grooves; 1 short stout pointed spicule slightly posterolateral to 12-CT and 2nd one near middle of metanotal wing lateral to 12-CT; sum of both 10-CT with 4–13 branches. *Abdomen:* Tergum I with darkly pigmented U- or O-shaped area extending from posterior margin anteriorly between pair of seta 1-I (rarely restricted to dark bar on posterior margin); terga and sterna II–VIII with numerous spicules, absent or fewer on lateral areas, tergum III usually with most spicules arranged singularly; terga III–VII with curved transverse ridge posterior to seta 1 poorly developed; tergum VIII with posterolateral corner not pigmented; 1-VII usually slightly longer than length of tergum VIII (98.1%) (length 0.95–1.00, 1.9%); ratio of length of 2-II/1-II 0.42–0.63, mean 0.54; sum of both 5-I with 6–13 branches; 5-III often with branches arising from proximal 0.24–0.42; sum of both 6-I usually with 4–7 branches (92.5%), range 4–10; sum of both 6-III with 5–12 branches; sum of both 7-I with 6–16 branches; sum of both 7-I plus both 6-III with 12–27 branches; 9-III–VII spiniform, usually broad, flattened, straight (tapered in 7.0% of III, 29.1% of IV, 25.6% of V, 38.4% of VI, 27.9% of VII) (curved in 1.2% of IV,V, 5.4% of VI, 32.6% of VII); ratio of length of 9-III/9-IV 0.44–0.87 (mean 0.63), 9-IV/9-V 0.70–1.00 (mean 0.88), 9-V/9-VI 0.75–1.05 (mean 0.91), 9-VI/9-VII 0.73–1.05 (mean 0.93), 9-III/9-VII 0.32–0.66 (mean 0.46); sum of both 9-VIII usually with 24–43 branches (97.4%) (25–43 branches, 93.7%), range 17–43; 1-IX usually with 2,3 branches, range 1–3; sterna III–VII with well-developed transverse ridge posterior to seta 14; male genital lobe index 1.01–1.13, mean 1.06. *Paddle:* Seta 1-Pa usually with 2–6 branches (98.3%), range 1–6; sum of both 1-Pa usually with 4–12 branches (97.8%) (with 2 branches, 0.2%; with 3 branches, 2.0%), range 2–12; 2-Pa often not reaching apex of paddle; index 1.27–1.50, mean 1.43; length 0.75–1.00 mm, mean 0.89 mm; width 0.59–0.75 mm, mean 0.64 mm; refractile border on basal 0.66–0.83 of outer margin, with continuous submarginal row (occasionally 2 rows) of short stout pointed aciculae extending from near base to about middle of well-defined marginal serrations; nonrefractile border on apical 0.17–0.33 of outer and apical 0.03–0.20 of inner margins, with sparse short thin aciculae.

4th-instar larva (Fig. 16). Chaetotaxy as figured and recorded in Tables 21 and 23–31. *Head:* Moderately brown with darker pigmented pattern on dorsal and ventral surfaces, pattern shape somewhat variable (pattern absent dorsally, 1.8%), frontal ecdysial line with broad darkly pigmented area on both sides along posterior *ca.* 0.5 of length; dorsal surface usually without spicules, occasionally with minute spicules; collar very dark; seta 2-C simple, single (rare specimen with 1 of pair 2-forked), usually 1.3 or greater length of 3-C (97.8%), range 1.29–1.81; 2-C with distance between pair of alveoli *ca.* 1.50–1.85 times diameter of alveolus; 3-C dendritic, usually with 11–30 thin apical branches (98.8%), range 11–35, branches usually more widely separated distally, few stout basal branches; sum of both 3-C with 25–63 branches (100%). *Antenna:* Flagellum pale with dark stout spicules apically or very narrow, moderately dark, pigmented band apically in addition to spicules, numerous pale spicules over entire length except on lateral surface, length usually 0.31–0.35 mm (91.9%), range 0.28–0.36 mm; seta 1-A usually noticeably shorter than apex of flagellum (99.1%) (equal to apex, 0.6%; slightly longer than apex, 0.3%), inserted usually 0.31–0.56 from base of flagellum (98.5%), range 0.27–0.56; sum of both 1-A with 5–16 branches (100%); 2,3-A darkly pigmented, bladelike; 3-A with mesal margin smooth and not serrated (100%); 4-A with 4–8 branches. *Mouthparts:* Dorsomentum darkly pigmented with 9 teeth. *Thorax:* Setae 5,6-P, 9–12-P,M,T on common setal support plates; 4,7,8-P, 1,8-M, 5,7,8-T each singly on setal support plates; seta 1-P shorter than 3-P (100%), usually 0.50–0.74 length of 3-P, range 0.40–0.97; 1-P single (44.0%) or with 2–4 branches (56.0%), branched usually on distal 0.39 (on distal 0.08–0.39, 41.6%; on distal 0.40–0.64, 14.4%); 4-P plumose, stout; sum of both 4-P usually with 31–46 branches (94.0%), range 24–46; 9-P often with 1 or both setae of pair 2,3-forked (81.6%) (both sides with 2 branches, 25.9%; 2-branched on 1 side and other with 3 branches, 8.6%; both sides with 3 branches, 2.4%; 4-branched on 1 side and other with 2 branches, 0.6%; single on 1 side and other with 2 branches, 17.6%; both sides single, 44.9%); sum of both 14-P usually with 16–26 branches (91.5%), range 12–26. *Abdomen:* Segments I–VII each with moderately large heavily pigmented tergal plate anteromesally, II–VII each also with small pigmented accessory tergal plate posteromesally to larger plate (occasionally absent on II), VIII with single large pigmented tergal plate anteromesally; III–VIII with patch of small spicules mesally on ventral surface; setae 6,7-I,II on common setal support plates; 6-III on setal support plate; 1-II palmate, leaflets pale or moderately pigmented, usually with weakly developed shoulders and poorly demarcated terminal filament, marginal serrations

poorly developed or absent, *ca.* 0.75–0.80 size of 1-III; 1-III–VI palmate, with leaflets well developed, broad, darkly pigmented but blotched with pale spots, with lateral serrations and short broad terminal filament; 1-VII palmate, with leaflets often broad (69.4%) or moderately broad (30.6%); sum of both 2-I usually with 6–10 branches (94.4%), range 6–12; sum of both 2-I plus both 2-III usually with 12–18 branches (95.1%), range 12–21; sum of both 2-III usually with 3–9 branches (95.7%), range 3–11; sum of both 6-IV usually with 10–14 branches (91.3%), range 7–14; sum of both 8-II usually with 4–10 branches (98.9%), range 4–11; sum of both 8-II plus both 8-III with 11–20 branches (100%); sum of both 8-II plus both 9-II usually with 17–28 branches (96.6%), range 17–30; sum of both 8-III usually with 5–9 branches (98.8%), range 5–10; sum of both 8-III plus both 8-VI usually with 11–18 branches (99.7%), range 11–19; sum of both 8-V usually with 4,5 branches, range 3–6; sum of both 8-VI with 5–10 branches (100%); sum of both 9-II usually with 13–19 branches (94.2%), range 13–22; sum of both 10-VI usually with 4–8 branches (90.9%), range 3–8; sum of both 13-I with 6–12 branches (100%); segment VIII usually with small moderately pigmented sternal plate on median area anterior to setae 14-VIII (94.9%) (absent, 5.1%), plate often somewhat constricted medially on anterior and posterior margins, occasionally split into 2 ovoid plates lying side by side (sternal plate best seen in exuviae); usually with both pecten plates each with 6–8 long spines (97.4%) (both sides with 8, 15.7%; 1 side with 8 and other with 7, 33.8%; 1 side with 8 and other with 6, 3.6%; both sides with 7, 27.5%; 1 side with 7 and other with 6, 13.5%; both sides with 6, 3.3%; 1 side with 10 and other with 8, 0.5%; 1 side with 9 and other with 8, 1.4%; 1 side with 9 and other with 7, 0.8%) and 13–19 short spines, minute lateral spicules often on posterior 2,3 long spines, rarely on other long spines, pecten plate connected with its mate posteriorly by darkly pigmented, U-shaped band; 3-S represented by an alveolus; 4,5-S minute, single; 7-S both usually with 2 branches (84.6%) (with 2 branches on 1 side and other with 3 branches, 0.4%; single on 1 side and other with 2 branches, 12.3%; both sides single, 2.7%); segment X with saddle incomplete, small spicules forming large triangular patch on posterior *ca.* 0.7, other spicules ventral to and longer ones posterior to saddle, median dorsal caudal process well developed and darkly pigmented, 1-X single, nearly always ventral to saddle or in small ventral notch of saddle, 2,3-X more pectinate than plumose, 4-X composed of 18 long, unevenly branched plumose setae attached evenly along convex ventral margin of large caudally produced boss, long narrow pigmented plate extending from boss anteriorly along midventral area of segment X, grid poorly developed with very short transverse bars, 4 moderately long fingerlike anal papillae.

Egg (Figs. 18E, 19-1A, 19-2A, 19-3, and 19-4). The following information is taken from Linley et al. (1993). *Linear dimensions:* Mean length 551.4 ± 8.1 μm; mean width 192.9 ± 2.5 μm. *Deck:* Plastron confined to 2 narrow strips adjacent to floats and not dividing deck; number of lobed tubercles, anterior 6.6 ± 0.2, posterior 7.3 ± 0.3; mean anterior deck tubercle area 1.86 ± 0.05 μm, density 68.4 ± 2.9. *Float:* Mean length of both floats 285.6 ± 4.5 μm; mean float length as percentage of egg length 51.8 ± 0.4; mean float length/mean number of ribs 12.4 ± 0.2. *Dorsal plastron:* Mean individual pore area 3.72 ± 0.48 μm, large.

Type data. Say's (1824) original description of this species indicated that it "inhabits North-west Territory" and he did not indicate a holotype repository. Belkin et al. (1966) and Knight and Stone (1977) indicated that no type material of *An. quadrimaculatus* was extant. Belkin et al. (1966) stated the following concerning the female upon which Say's description was based: "The material was collected on Long's Second Expedition (Keating 1824) and it is most likely that it was obtained between 25 June and 2 July 1823 between Prairie du Chien (Wisconsin) and Fort Anthony [Fort Snelling] (Minnesota) while Say was in command of the party which ascended the Mississippi by barge. This is also the only area in the Northwest Territory of that period where quadrimaculatus, as currently interpreted, is common. Accordingly, we are restricting the type locality of this species to the vicinity of Wabasha, Minnesota where quadrimaculatus has been reported to be very abundant (Daggy, Muegge and Riley 1940)." The correct date for Daggy et al. is 1941.

A neotype (MN95.10–125) for *An. quadrimaculatus* is hereby designated (by J.F.R.) and consists of a female glued to a small triangular paper point on a pin and its associated pupal and 4th-instar larval exuviae mounted in Canada balsam on a microscope slide. The neotype was reared from a single F_1 progeny brood of a female collected, as part of a group of adult mosquitoes, in nature from several large rot cavities in trees adjacent to a partly shaded, shallow, freshwater, small marsh next to the Mississippi River on August 19, 1995, by John F. Reinert and Paul E. Kaiser. This site was located in a municipal park, northeastern part of Lake City, Goodhue Co., MN, and was less than 0.5 mi. north of Wabasha Co. and about 14 mi. from the town of Wabasha. The neotype is deposited in the NMNH, Washington, DC. The IPB from which the neotype was selected consists of the following specimens: 67 ♀pl, 59 ♂pl, 9 ♂g, 2 pl, 2 Pl, 13 l, and 31 L.

The holotype male of *An. annulimanus* Van der Wulp (1867), deposited in the National Museum of Natural History, Leiden, the Netherlands, was examined (by J.F.R.) and, even though the specimen was in poor condition, it was found to be conspecific with the neotype of *An. quadrimaculatus*. The following characters of the type support this con-

clusion, i.e., femora I,II and tibiae I,II with narrow pale-scaled bands apically, 23 scutal fossal setae on one side (other side damaged), anterior margin of dorsocentral area with several golden piliform scales, prealar area with 7–8(?) setae, median anterior promontory area with moderate-sized tuft of whitish scales, interocular area with 10(?) setae, parascutellar area with 3 setae on each side, male genitalia with claspette having seta 4 longer than seta 5, aedeagus with apical leaflet moderately long and pale, and tergum IX with laterobasal band with heavily pigmented area wide and ventrolateral margin irregular in shape. Four labels were affixed to the pin of *An. annulimanus* and were as follow: HOLOTYPE (red rectangular label); Kumlien, Wisconsin (white rectangular label); *Anopheles annulimanus,* type, v.d.W. (white rectangular label); and T96.191 Term. (genitalia preparation number; small white rectangular label). The genitalia were dissected and mounted in Canada balsam on a microscope slide.

Bionomics. Immatures of *An. quadrimaculatus* were found in a wide variety of habitats and appeared to be opportunistic in habitat selection. Larvae have been taken in association with other members of the complex (i.e., *An. diluvialis, An. inundatus,* and *An. smaragdinus*). Typically, larvae were collected from vegetation at margins of lakes, ponds, streams, canals, and roadside ditches. Immatures also were collected from blue-green algal mats that had developed on the water surface, usually in association with emergent or floating aquatic vegetation. Extensive immature populations occurred in rice fields in Arkansas, Louisiana, and Mississippi, where the abundance of emergent aquatic vegetation was related to the water management of individual fields. In Florida, larvae were routinely found throughout the year among vegetation in spring-fed streams where the water temperature was 20–23°C; predominant vegetation was eel grass, red *Ludwigia,* and blue-green algal mats. Immatures were plentiful in stream-fed beaver ponds that contained coon-tail, blue-green algae, and pondweeds at the Hartwell and Clark Hill Reservoirs on the South Carolina–Georgia border. Reinert et al. (1997) reported atypical habitats for this species as a highly polluted sewage retention pond and a plastic 5-gallon bucket; in the first site larvae were associated with *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, and *Uranotaenia lowii* Theobald. Kaiser et al. (1988b) collected immatures from a borrow pit near Montgomery, AL, and a shallow lake in the Kanapaha Botanical Gardens, Gainesville, FL; both sites contained large amounts of aquatic vegetation. Kaiser (1994) recorded *An. quadrimaculatus* immatures associated with *An. punctipennis* during cooler months of the year or in moving streams but associated with *Psorophora columbiae* (Dyar and Knab) in rice fields. In 1995, Jensen et al. collected *An. quadrimaculatus* larvae from temporary pools in an intermittently flooded swamp near Coleman, Sumter Co., FL, and in association with *An. diluvialis, An. crucians s.l., An. perplexens, Ae. infirmatus, Ae. vexans* (Meigen), *Culex territans* Walker, *Ps. ferox, Ps. howardii* Coquillett, and *Uranotaenia sapphirina* (Osten Sacken).

Daytime-resting adults were collected from large rot cavities in trees, from livestock barns, from under bridges and culverts, from under eaves of buildings, and from wooden resting boxes. Adults tended to be gregarious in these sites. A collection from a single site frequently included one or more other members of the complex (i.e., *An. diluvialis, An. inundatus, An. maverlius,* and *An. smaragdinus*). Adults appeared to be most active at dusk and dawn. Females were collected once in large numbers from a stable 1.5 mi. from the nearest breeding site (by P.E.K.). Females have been observed feeding on large domesticated animals and on numerous occasions engorged females have been collected from horse stables and cattle barns, and they were collected twice from pig farm shelters. In St. Tammany Parish, LA, engorged females were collected from a pheasant-breeding farm (by P.E.K.). Apperson and Lanzaro (1991) reported that of 353 engorged females of *An. quadrimaculatus* collected from 2 small utility sheds at Noxubee Wildlife Refuge, Noxubee Co., MS, 1.1% had fed on opossum, 2.2% had fed on cottontail rabbit, and 96.7% had fed on white-tailed deer. Jensen et al. (1993), using updraft CDC traps baited with dry ice, monitored changes in the abundance and parity rate of this species in an intermittently flooded swamp at Coleman, Sumter Co., FL. They found that of 1,178 *An. quadrimaculatus s.l.* collected in an 18-day period, only 5.3% were *An. quadrimaculatus s.s.* (94.7% were *An. diluvialis*), 36 of 37 females had primary follicles at stage II, none appeared to have blood-fed, and 19% of the 36 were parous. Jensen et al. (1996) collected blood-engorged females of this species from daytime resting sites at Manatee Springs State Park, Levy Co., FL, and found that 10.7% (19 of 177) from a campground area, but none of 19 from a wooded area, had fed on humans. Wing et al. (1985) compared the susceptibilities to *Plasmodium yoelii* (Landau and Killick-Kendrick) of the ORLANDO strain and 4 homozygous mutant stocks of *An. quadrimaculatus.* Nayar et al. (1992) found that the ORLANDO strain, in laboratory studies, had infection rates of 29.7% with *Brugia malayi* and 66.2% with *Brugia pahangi.* Adults were collected from tree holes (Kaiser et al. 1988b); tree holes, outdoor latrines, and eaves of buildings (Kaiser et al. 1988a); tree holes, farm buildings, and boxes (Lanzaro et al. 1988); tree holes and barns (Lanzaro et al. 1990); tree holes, crevices of trees and logs, wooden boxes, culverts, bridges, and barns (Seawright et al. 1991, 1992); and inside a garage and a resting station in a rice field (Rutledge et al. 1996). Seawright et al. (1992) reported that for 11 of 13 locations sampled around Kentucky

Lake, TN, the populations consisted of over 94% *An. quadrimaculatus* (as sp. A). *Anopheles quadrimaculatus* (as *An. quadrimaculatus* sp. A) was found to be widespread and common throughout Mississippi and was the main mosquito pest in the Mississippi Delta (Mallet and Fritzius 1993), also Delta and Noxubee populations were found to be highly resistant to malathion (*ca.* 10×–1,000×) and Delta populations were *ca.* 10 times more tolerant of permethrin than were Noxubee populations.

Discussion. In addition to the characters used in the keys, the following features, used in combination, can be used for the identification of most specimens and for separation from the other members of the complex: females by the combination of (1) dorsocentral area with several white or golden-white piliform scales on anterior margin (100%), (2) femora I,II pale-scaled apically (100%), (3) tibiae I,II pale-scaled apically (100%), (4) interocular area with 7–12 setae (98.0%), (5) sum of setae on both scutal fossal areas 41–75 (97.1%), (6) scutal fossal area with 21–45 setae (93.1%), (7) maxillary palpus 1.0 or greater than length of proboscis (90.0%), and (8) prealar area with 6–12 setae (87.0%), 2–5 setae pale; males by (9) maxillary palpus length shorter than proboscis (100%); male genitalia by the combination of (10) tergum IX with heavily pigmented area of laterobasal band wide and ventrolateral margin noticeably irregular in shape (100%), (11) claspette with seta 5 borne on short elevated process (100%), (12) gonostylus length 0.32–0.37 mm (98.0 %), (13) claspette with seta 4 usually longer than seta 5 (81.6%), and (14) gonostylus with 11–19 tiny setae (80.0%); pupae by the combination of (15) seta 1-VII slightly longer than length of tergum VIII (98.1%), (16) sum of both seta 1-Pa with 4–12 branches (97.8%), (17) sum of both seta 9-VIII with 25–43 branches (93.7%), and (18) sum of both seta 6-I with 4–7 branches (92.5%); and 4th-instar larvae by the combination of (19) sum of both seta 8-II plus both seta 8-III with 11–20 branches (100%), (20) seta 3-A without serrations (100%), (21) seta 1-P shorter than seta 3-P length (100%), (22) seta 1-A noticeably shorter than apex of flagellum (99.1%), (23) sum of both seta 8-II with 4–10 branches (98.9%), (24) sum of both seta 8-III with 5–9 branches (98.8%), (25) sum of both seta 8-II plus both seta 9-II with 17–28 branches (96.6%), (26) sum of both seta 2-III with 3–9 branches (95.7%), (27) sum of both seta 2-I plus both seta 2-III with 12–18 branches (95.1%), (28) sum of both seta 2-I with 6–10 branches (94.4%), (29) sum of both seta 9-II with 13–19 branches (94.2%), (30) sum of both seta 4-P with 31–46 branches (94.0%), (31) sum of both seta 14-P with 16–26 branches (91.5%), (32) sum of both seta 10-VI with 4–8 branches (90.9%), (33) sum of both seta 2-I plus both seta 9-I with 20–27 branches (88.5%), and (34) seta 7-S both with 2,3 branches (85.0%).

The following primary features, given above by numbers in parentheses and listed here in decreasing order of separation, best distinguish specimens of *An. quadrimaculatus* from *An. diluvialis*: in females, 3 (100%), 1 (98.4%), 2 (97.6%), 5 (97.2%), 4 (95.9%), 6 (94.9%), and 8 (90.8%); in males, 9 (100%); in male genitalia, 10 (100%), 11 (96.4%), and 14 (87.5%); in pupae, 16 (98.9%), 15 (95.1%), and 18 (91.3%); and in 4th-instar larvae, 27 (94.3%), 26 (93.1%), 20 (92.1%), 34 (87.2%), and 21 (86.3%); from *An. inundatus*: in females, 3 (100%), 2 (96.6%), and 8 (84.6%); in males, 9 (95.0%); in male genitalia, 10 (100%), 11 (98.6%), and 14 (90.0%); in pupae, 16 (98.9%), 15 (95.7%), and 18 (93.6%); and in 4th-instar larvae, 20 (99.5%), 27 (97.4%), 28 (95.8%), 26 (93.1%), and 21 (89.7%); from *An. maverlius*: in females, 3 (100%), 4 (97.4%), 5 (89.3%), 6 (87.6%), and 7 (80.0%); in males, 9 (100%); in male genitalia, 10 (100%), 12 (99.0%), and 14 (76.1%); in pupae, 16 (95.7%), 15 (95.3%), and 17 (93.4%); and in 4th-instar larvae, 19 (100%), 24 (99.2%), 23 (99.1%), 25 (98.1%), 20 (97.3%), 30 (97.0%), 21 (96.7%), 31 (94.3%), 29 (93.7%), 32 (91.2%), and 22 (85.6%); and from *An. smaragdinus*: in females, 5 (95.4%), 6 (93.5%), 4 (89.2%), and 8 (87.3%); in male genitalia, 13 (79.8%) and 11 (79.3%); in pupae, 16 (98.0%); and in 4th-instar larvae, 33 (88.4%) and 34 (84.3%).

Other pupal features, in addition to those mentioned above, useful in distinguishing *An. quadrimaculatus* from *An. smaragdinus* were: ratio of length of seta 2-II/1-II; absence or reduced pigmentation on the mesothoracic wing; and tergum I almost always with a heavily pigmented U- or O-shaped area between the pair of seta 1-I. The latter feature was usually with a somewhat wider, moderately pigmented, U-shaped area in *An. smaragdinus* and was usually pale in *An. inundatus* and/or strongly reduced in *An. diluvialis* and *An. maverlius.*

Anopheles (Anopheles) smaragdinus Reinert, new species

Literature references

Anopheles quadrimaculatus species B of Lanzaro 1987:0955; Kim et al. 1987a:187; Willis et al. 1987:s45; Kaiser et al. 1988b:34, 1988a:138; Lanzaro et al. 1988:248; Cockburn and Seawright 1988:261; Cockburn et al. 1988:299; Narang and Seawright 1988:303; Kaiser 1988:311; Kaiser et al. 1988c:494; Cockburn and Mitchell 1989:105; Narang et al. 1989b:508, 1989a:317; Cockburn 1990:191; Lanzaro et al. 1990:578; Seawright et al. 1991:221; Apperson and Lanzaro 1991:507; Narang and Seawright 1991:59; Nayar et al. 1992:61; Seawright et al. 1992:289; Mitchell et al. 1992:939; Mallet and Fritzius 1993:25; Linley et al. 1993:124 (E*); Johnson et al. 1993:939; Kaiser 1994:8; Rutledge and Meek 1994:585; Cornel et al. 1996:109; Jensen et al.

1996:523; Reinert et al. 1996:310; Rutledge et al. 1996:952.

Female (Figs. 1D and 2B; Tables 1–5). *Head:* Antennal flagellum 0.67–0.80 length of proboscis, mean 0.75; maxillary palpus usually 1.00–1.12 length of proboscis (95.0%), range 0.99–1.12, mean 1.04; usually with (occasionally without) few semirecumbent scales basally; proboscis 0.82–1.07 length of femur I, mean 0.98; vertex, occiput and dorsal area of postgena with erect forked scales long and narrow; interocular area usually with 4–6 setae (80.4%), range 4–8. *Thorax:* Anterior promontory scales usually golden, occasionally golden-white, and similarly shaped as scales on acrostichal area, 4–6 golden setae; prescutellar area without golden piliform scales or with only few scales on anterior portion, setae number and arrangement similar to *An. quadrimaculatus* but all dark brown (occasional specimen also with very few golden setae); scutal fossal area normally with fewer setae, usually with 8–20 setae (93.9%), range 8–28, sum of setae on both scutal fossal areas usually with 17–40 setae (93.7%), range 17–51; supraalar setae dark; parascutellar area with 1–4 setae (1 on both sides, 36.3%; 2 on both sides, 35.3%; 2 on 1 side and 1 on other, 24.7%; 3,4 on 1 or both sides, 3.2%), 2nd to 4th setae usually 0.85–1.00 length of other seta; scutellum with several golden and golden-brown or brown piliform scales; proepisternum usually with 2–6 upper setae (98.5%), range 2–9; prespiracular area with 1–9 setae; mesokatepisternum usually with 4–9 upper setae (81.0%), range 2–8, and with 2–7 lower setae, sum of upper and lower setae often 4–9 (76.6%), range 6–18; prealar area usually with 1–5 setae (87.6%), range 1–8; mesepimeron with 3–17 upper setae. *Legs:* Coxa I usually with 3,4 (range 2–6) moderately long and moderately broad brown scales anterodorsally; femur I often with very narrow pale-scaled apical band (68.3%) or with only a few pale scales apically (31.7%); tibia I very rarely without pale scales apically, II with pale scales apically (with few pale scales, 55.0%; with very narrow pale-scaled band, 45.0%), III usually with pale-scaled, very narrow band or occasionally few pale scales apically (100%).

Male. Essentially similar to female except for sexual features. *Head:* Antennal flagellum 0.69–0.80 length of proboscis, mean 0.76; maxillary palpus 0.87–0.99 length of proboscis, mean 0.95; proboscis 1.23–1.53 length of femur I, mean 1.34.

Male genitalia (Figs. 4B, 5B, and 6B). *Tergum VIII:* Apical margin often straight (78.0%) (very gently convex, 19.5%; very gently concave, 2.5%); length 0.37–0.47 mm, mean 0.41 mm; width 0.49–0.59 mm, mean 0.54 mm; VIII-Te index 0.71–0.83, mean 0.76. *Tergum IX:* Length usually 0.02–0.03 mm, range 0.02–0.04 mm, mean 0.03 mm; width 0.17–0.22 mm, mean 0.19 mm; posterolateral lobe usually with apex bluntly rounded (rarely sharply pointed) and often with small ridges (51.2%); length 0.07–0.10 mm, mean 0.08 mm; Te-IX index 0.09–0.23, mean 0.16; PlL/Te-IX index 2.00–4.43, mean 3.03; laterobasal band with heavily pigmented area wide (100%), ventrolateral margin noticeably irregular in shape (100%). *Phallosome:* Aedeagus length 0.17–0.18 mm, mean 0.17 mm; apex with 2–4 (2 on both sides, 12.2%; 2 on 1 side and 3 on other, 17.1%; 3 on both sides, 26.8%; 3 on 1 side and 4 on other, 36.6%; 4 on both sides, 7.3%) pale narrow leaflets on each side of midline, apical leaflet moderately long and 0.31–0.41 length of aedeagus. *Gonocoxite:* Length 0.30–0.34 mm, mean 0.32 mm; ventral surface with internal seta situated slightly more distal than in *An. quadrimaculatus*. *Gonostylus:* Length 0.32–0.38 mm, mean 0.35 mm; usually with 12–19 tiny setae (91.5%), range 12–22, most in more or less irregular line from near base to apex; Gs/Gc index 1.05–1.15, mean 1.11. *Claspette:* Seta 2 present (100%), usually slightly longer (approximately 1.2 length of *An. quadrimaculatus*); seta 4 often slightly shorter than seta 5 (78.0%) (4 equal to 5, 14.6%; 4 longer than 5, 7.3%), situated dorsolateral to seta 3 (100%); seta 5 formed of 1–3 (1 on both sides, 2.4%; 2 on both sides, 26.8%; 2 on 1 side and 3 on other, 26.8%; 3 on both sides, 43.9%) apically fused stems, often not borne on short elevated process (58.5%).

Pupa (Fig. 12). Chaetotaxy as figured and recorded in Tables 10–17. Similar to *An. quadrimaculatus* in development of integument (except metanotal wing and tergum I), dorsal apotome (slightly convex or flat, 95.0%; broadly rounded, 5.0%), lateralia, anterior angle of scutum, lateral line of median keel (99.6%), complete splitting of postscutal area of cephalothorax by dorsal ecdysial opening, trumpet, metanotum, and sternal transverse ridges. *Cephalothorax:* Mesothoracic wing usually with well-defined, moderately dark to darkly pigmented longitudinal lines. *Trumpet:* Index 2.84–3.88, mean 3.20; length 0.43–0.57 mm, mean 0.49 mm; width 0.13–0.20 mm, mean 0.16 mm; pinna 0.84–0.93 of trumpet length, mean 0.88. *Metanotum:* Sum of both seta 10-CT usually with 2–7 branches (88.2%), range 2–14. *Abdomen:* Tergum I with moderately pigmented area between pair of seta 1-I usually U-shaped, usually somewhat wider than in *An. quadrimaculatus*; seta 1-VII usually slightly longer than length of tergum VIII (94.0%) (length 1.0, 5.2%); ratio of length of 2-II/1-II 0.66–0.89 (mean 0.81); sum of both 5-I usually with 4–9 branches (98.3%) (4–8 branches, 92.2%), range 4–12; 5-III often with branches arising from proximal 0.04–0.15; sum of both 6-I usually with 2–7 branches (96.3%), range 2–9; sum of both 6-III usually with 4–8 branches (97.8%), range 4–10; sum of both 7-I usually with 4–10 branches (92.4%), range 4–12; sum of both 7-I plus both 6-III usually with 8–19 branches (99.3%), range 8–20; 9-III–VII spiniform, usually broad, flattened, straight (tapered in 5.9% of III, 11.8% of IV, 25.0%

of V, 69.1% of VI, 36.8% of VII) (curved in 16.2% of VII); ratio of length of 9-III/9-IV 0.30–0.73 (mean 0.56), 9-IV/9-V 0.73–1.07 (mean 0.91), 9-V/9-VI 0.67–0.99 (mean 0.84), 9-VI/9-VII 0.73–1.13 (mean 0.93), 9-III/9-VII 0.21–0.52 (mean 0.37); sum of both 9-VIII usually with 12–23 branches (97.2%) (12–22 branches, 91.8%), range 12–26; 1-IX usually with 2 branches, range 1–3; male genital lobe index 1.01–1.17, mean 1.10. *Paddle:* Seta 1-Pa usually single (92.3%) (with 2 branches, 7.6%; with 3 branches, 0.1%), range 1–3; sum of both 1-Pa usually with 2 branches (85.3%) (with 3 branches, 12.9%; with 4 branches, 1.9%), range 2–4; index 1.28–1.45, mean 1.38; length 0.79–0.94 mm, mean 0.88 mm; width 0.59–0.70 mm, mean 0.63 mm; refractile border on basal 0.68–0.83 of outer margin; nonrefractile border on apical 0.17–0.33 of outer and apical 0.02–0.12 of inner margins with fewer short, thin aciculae than in *An. quadrimaculatus.*

4th-instar larva (Fig. 17). Chaetotaxy as figured and recorded in Tables 22–31. *Head:* Usually pale brown, occasionally moderately brown, usually without more darkly pigmented pattern on dorsal and ventral surfaces (pattern present in 16.9% of specimens, but paler than *An. quadrimaculatus*); frontal ecdysial line with area on both sides pale, rarely with narrow, more darkly pigmented area along posterior 0.5 of length; dorsal surface usually without spicules, occasionally with sparse minute spicules; seta 2-C 1.32–1.92 length of 3-C (100%); 3-C dendritic, with 28–53 (100%) apical, somewhat thicker, darker branches, somewhat bunched together distally, several stout basal branches; sum of both 3-C usually with 64–101 branches (99.6%), range 60–101. *Antenna:* Flagellum length usually 0.31–0.38 mm (92.3%), range 0.29–0.38 mm; seta 1-A usually shorter than apex of flagellum (98.2%) (equal to apex, 1.6%; slightly longer than apex, 0.3%), inserted usually 0.31–0.42 from base of flagellum (89.7%), range 0.28–0.42; sum of both 1-A usually with 6–17 branches (98.0%), range 6–22; 3-A pale to moderately pigmented, margins usually not serrated (97.8%) (rarely with few small serrations on mesal margin at about midlength, 2.2%); 4-A with 4–7 branches. *Mouthparts:* Dorsomentum darkly pigmented, usually with 9 teeth, rarely with 11. *Thorax:* Seta 1-P usually shorter than 3-P (90.8%), length often 0.75–0.95 of 3-P (78.3%) (0.50–0.74 length, 12.5%; equal in length, 5.3%; very slightly longer, 3.9%), range 0.60–1.08; 1-P usually with 2–5 branches (94.2%) (single, 5.8%), branched usually on distal 0.13–0.80 (94.2%) (on distal 0.13–0.39, 65.8%; on distal 0.40–0.80, 28.4%); sum of both 4-P often with 31–41 branches (60.5%), range 21–41; 9-P usually single (89.6%) (single on 1 side and other side with 2 branches, 7.6%; both sides with 2 branches, 2.8%); sum of both 14-P usually with 16–28 branches (98.2%), range 15–28. *Abdomen:* Seta 1-VII palmate, with leaflets usually broad (95.7%) (moderately broad, 4.3%); sum of both 2-I with 4–10 branches (100%); sum of both 2-I plus both 2-III usually with 8–18 branches (99.7%), range 8–19; sum of both 2-I plus both 9-I usually with 14–20 branches (92.6%) (14–19 branches, 88.2%), range 14–22; sum of both 2-III usually with 4–8 branches (94.3%), range 4–10; sum of both 6-IV usually with 6–9 branches (87.1%), range 6–11; sum of both 8-II usually with 4–7 branches (93.8%), range 4–9; sum of both 8-II plus both 8-III with 8–17 branches (100%) (8–14 branches, 95.7%); sum of both 8-II plus both 9-II with 16–28 branches (100%); sum of both 8-III with 4–8 branches (100%); sum of both 8-III plus both 8-VI with 8–16 branches (100%); sum of both 8-V usually with 4–6 branches (92.6%), range 4–8; sum of both 8-VI usually with 4–7 branches (98.5%), range 4–8; sum of both 9-II usually with 10–19 branches (95.4%), range 10–20; sum of both 10-VI often with 4–6 branches (78.0%), range 2–6; sum of both 13-I with 6–9 branches (100%); segment VIII usually without small sternal plate anteromesally (92.9%) (present, 7.1%); both pecten plates usually each with 6–8 long spines (both sides with 8, 26.9%; 8 on 1 side and 7 on other, 35.2%; 8 on 1 side and 6 on other, 1.0%; both sides with 7, 18.6%; 7 on 1 side and 6 on other, 5.3%; both sides with 6, 0.8%; both sides with 9, 2.0%; 9 on 1 side and 8 on other, 8.8%; 9 on 1 side and 7 on other, 1.5%) and 13–18 short spines, long spines smooth or with minute lateral spicules primarily on posterior 4 or 5; 7-S both usually single (83.6%) (single on 1 side and other side with 2 branches, 12.9%; both sides with 2 branches, 3.4%); segment X with saddle having small spicules forming large triangular patch on posterior *ca.* 0.7, other spicules ventral to insertion of seta 1-S and posterior to saddle.

Egg (Figs. 18D, 19-1B, and 19-2B). The following information is taken from Linley et al. (1993). *Linear dimensions:* Mean length 529.7 ± 8.1 μm; mean width 192.6 ± 5.6 μm. *Deck:* Plastron confined to 2 narrow strips adjacent to floats and not dividing deck; number of lobed tubercles, anterior 5.2 ± 0.3, posterior 5.1 ± 0.3; mean anterior deck tubercle area 1.79 ± 0.05 μm, density 82.8 ± 3.0. *Float:* Mean length of both floats 253.1 ± 5.2 μm; mean float length as percent of egg length 47.8 ± 0.4; mean float length/mean number of ribs 12.3 ± 0.2. *Dorsal plastron:* Mean individual pore area 2.83 ± 0.20 μm, large.

Type data. The type series of *An. smaragdinus* was reared from a single F_1 progeny brood of a female collected in nature from a large rot cavity (extending from ground level to a height of *ca.* 0.75 m) in a tree situated at the margin of a partly shaded, shallow freshwater swamp on August 31, 1995, by John F. Reinert and Paul E. Kaiser. This site was located about midway on the North End Trail, Manatee Springs State Park, and the nearest town was Chiefland, Levy Co., FL. The holotype (FL95.57–35) consists of a female glued to a small triangular

paper point on an insect pin and its associated pupal and 4th-instar larval exuviae mounted in Canada balsam on a microscope slide. The remainder of the type series includes the following specimens: paratypes, FL95.57–1 ♂pl, -2 ♂pl, -3 ♂plg, -4 ♂pl, -5 ♂pl, -6 ♂pl, -7 ♂pl, -8 ♀pl, -9 ♂pl, -10 ♂pl, -11 ♂pl, -12 ♀pl, -13 ♂pl, -14 ♀pl, -15 ♀pl, -16 ♀pl, -17 ♀pl, -18 ♀pl, -19 ♀pl, -20 ♀pl, -21 ♀pl, -22 ♂plg, -23 ♂pl, -24 ♂plg, -25 ♂pl, -26 ♀pl, -27 ♀pl, -28 ♀pl, -29 ♀pl, -30 ♂pl, -31 ♂pl, -32 ♀pl, -33 Pl, -34 ♀pl, -36 ♀pl, -37 ♂plg, -38 ♀pl, -39 ♂pl, -40 ♂pl, -41 ♂plg, -42 ♀pl, -43 ♀pl, -44 ♀pl, -45 ♂pl, -46 ♀pl, -47 ♂plg, -48 ♀pl, -49 ♀pl, -50 ♀pl, -51 ♀pl, -52 ♂pl, -53 ♀pl, -54 ♀pl, -55 ♂plg, -56 ♀pl, -57 ♀pl, -58 ♀pl, -59 ♀pl, -60 Pl, 23 p, and 41 L.

The holotype and some paratypes with their associated immature exuviae are deposited in the NMNH, Washington, DC. Most of the paratypes are deposited in the FSCA, Gainesville, FL. Additional paratypes are deposited in the NHM, London, United Kingdom, and the ORSTOM, Montpellier, France.

Bionomics. Immatures appear to prefer habitats in permanent-water swamps with moderate amounts of emergent vegetation in which the tree canopy allows filtered sunlight to reach the water surface. Larvae were collected from emergent grass at the habitat margins, in floating algal mats, and in floating debris at the water's interface with bases of trees and fallen limbs. Larvae were taken from a swamp predominated by sweet gum trees, but also with red maple and buttonbush, in Hamilton Co., FL; this site had a tree canopy allowing only filtered sunlight onto the water. In Levy Co., FL, immatures were taken from a moderately shaded, shallow swamp with bald cypress trees, emergent grasses at the margins and floating algal mats. Kaiser (1994) reported that *An. smaragdinus* (as *An. quadrimaculatus* sp. B) breed in permanent-water swamps that have filtered sunlight and limited aquatic vegetation.

Daytime-resting adults were collected from large rot cavities in trees, from under bridges and culverts, from under eaves of buildings, and from wooden boxes. Adults tended to be gregarious in these sites. Adults of this species frequently shared resting sites with *An. quadrimaculatus* and occasionally with *An. diluvialis, An. inundatus,* and *An. maverlius.* Specimens were often collected from sites associated with cypress ponds and sweet gum swamps; however, some collections of adults were taken adjacent to beaver-dammed ponds. In Montgomery Co., AL, feral adults of the complex (87% *An. smaragdinus*) were taken from resting sites adjacent to a sweet gum swamp and at Hart Co., GA, near a beaver-dammed pond (77% *An. smaragdinus*). Seawright et al. (1992) reported that there were indications that the species frequencies of some mixed populations of adults of the complex were stable for several years (e.g., at Lake Octahatchee, Hamilton Co., FL, for *An. smaragdinus,* mean frequencies were 91.9% in 1986, 91.8% in 1987, and 95.5% in 1990, and for *An. quadrimaculatus,* mean frequencies were 8.1% in 1986, 8.2% in 1987, and 4.5% in 1990). However, for other populations, the frequencies of the sibling species varied considerably for sites located within close proximity and from year to year. Large numbers of adults were collected from wooden boxes at Wheeler Reservoir, AL, and at Benton Co., TN. Resting adults have been collected from: tree holes (Kaiser et al. 1988b); tree holes, outdoor latrines, and eaves of buildings (Kaiser et al. 1988a); tree holes, farm buildings, and boxes (Lanzaro et al. 1988); tree holes and barns (Lanzaro et al. 1990); tree holes, crevices of trees and logs, wooden boxes, culverts, bridges, and barns (Seawright et al. 1991, 1992); and under a bridge and in chicken coops (Rutledge et al. 1996). Mallet and Fritzius (1993) reported that this species (as *An. quadrimaculatus* sp. B) was restricted to relatively undisturbed forest habitats near major rivers to the east and south of the Delta in Mississippi, and that it was susceptible to malathion and permethrin. Apperson and Lanzaro (1991) found that 201 engorged females of *An. smaragdinus* (as *An. quadrimaculatus* sp. B), collected from 2 small utility sheds at Noxubee Wildlife Refuge, Noxubee Co., MS, had fed on the following animals: 0.5% on dog/fox, 0.5% on pig, 3.0% on raccoon, 6.5% on cottontail rabbit, and 89.5% on white-tailed deer. Jensen et al. (1996) collected blood-engorged females of this species from daytime resting sites at Manatee Springs State Park, Levy Co., FL, and found that 1.6% (2 of 129) from a wooded area, but none of 62 from a campground area, had fed on humans. Nayar et al. (1992) reported that feral females of *An. smaragdinus* in laboratory studies had infection rates of 13.3% with *Brugia malayi* and 21.3% with *Brugia pahangi.*

Discussion. In addition to the characters used in the keys, the following features, used in combination, can be used for the identification of most specimens and for their separation from the other members of the complex: females by the combination of (1) dorsocentral area with several golden or golden-white piliform scales on anterior margin (100%), (2) femora I,II with pale scales apically (100%), (3) tibiae II (and nearly always I) with pale scales apically (100%), (4) maxillary palpus 1.0 or greater than length of proboscis (95.0%), and (5) sum of setae on both scutal fossal areas 17–40 (93.7%); males by (6) maxillary palpus length shorter than proboscis (100%); male genitalia by the combination of (7) tergum IX with heavily pigmented area of laterobasal band wide and with ventrolateral margin irregular in shape (100%), (8) gonostylus shorter, 0.32–0.38 mm (100%), (9) gonostylus with 12–19 tiny setae (91.5%), (10) claspette with seta 4 slightly shorter than seta 5 (78.0%), and (11) claspette with seta 5 not borne on short elevated process (58.5%); pupae by the

combination of (12) sum of both seta 7-I plus both seta 6-III with 8–19 branches (99.3%), (13) sum of both seta 5-I with 4–9 branches (98.3%), (14) sum of both seta 6-III with 4–8 branches (97.8%), (15) sum of both seta 9-VIII with 12–23 branches (97.2%), (16) sum of both seta 6-I with 2–7 branches (96.3%), (17) seta 1-VII slightly longer than tergum VIII (94.0%), (18) sum of both seta 7-I with 4–10 branches (92.4%), (19) sum of both seta 5-I with 4–8 branches (92.2%), (20) sum of both seta 9-VIII with 12–22 branches (91.8%), and (21) sum of both seta 10-CT with 2–7 branches (88.2%); and 4th-instar larvae by the combination of (22) sum of both seta 8-II with 4–9 branches (100%), (23) sum of both seta 8-II plus both seta 8-III with 8–17 branches (100%), (24) sum of both seta 8-III with 4–8 branches (100%), (25) sum of both seta 2-I with 4–10 branches (100%), (26) sum of both seta 8-II plus both seta 9-II with 16–28 branches (100%), (27) sum of both seta 13-I with 6–9 branches (100%), (28) sum of both seta 2-I plus both seta 2-III with 8–18 branches (99.7%), (29) sum of both seta 8-VI with 4–7 branches (98.5%), (30) sum of both seta 14-P with 16–28 branches (98.2%), (31) seta 1-A shorter than apex of flagellum (98.2%), (32) seta 3-A without serrations (97.8%), (33) sum of both seta 8-II plus both seta 8-III with 8–14 branches (95.7%), (34) sum of both seta 9-II with 10–19 branches (95.4%), (35) sum of both seta 2-III with 4–8 branches (94.3%), (36) sum of both seta 8-II with 4–7 branches (93.8%), (37) seta 1-P shorter than seta 3-P length (90.8%), (38) sum of both seta 2-I plus both seta 9-I with 14–19 branches (88.2%), (39) seta 7-S both single (83.6%), and (40) sum of both seta 10-VI with 4–6 branches (78.0%).

The following primary features, given above by numbers in parentheses and listed here in decreasing order of separation, best distinguish specimens of *An. smaragdinus* from *An. diluvialis*: in females, 3 (100%), 1 (98.4%), and 2 (97.6%); in males, 6 (100%); in male genitalia, 7 (100%) and 9 (93.3%); in pupae, 12 (98.4%), 14 (97.1%), 20 (95.1%), 19 (93.7%), 16 (93.2%), 17 (93.1%), and 18 (92.5%); and in 4th-instar larvae, 27 (96.6%), 35 (96.1%), 27 (96.0%), 36 (96.0%), 38 (94.1%), 32 (91.0%), and 37 (90.8%); from *An. inundatus*: in females, 3 (100%), 2 (96.6%), and 5 (90.1%); in males, 6 (95.0%); in male genitalia, 7 (100%) and 9 (95.8%); in pupae, 13 (98.4%), 12 (98.3%), 14 (98.2%), 16 (95.5%), 17 (93.7%), and 18 (93.4%); and in the 4th-instar larvae, 28 (99.7%), 25 (98.6%), 32 (98.4%), 36 (96.9%), 23 (96.5%), 35 (95.1%), 38 (94.1%), 27 (91.2%), and 37 (85.1%); from *An. maverlius*: in females, 3 (100%) and 4 (82.5%); in males, 6 (100%); in male genitalia, 8 (100%), 7 (100%), 9 (81.8%), and 11 (73.4%); in pupae, 21 (90.4%); and in 4th-instar larvae, 24 (99.8%), 26 (99.8%), 22 (99.7%), 33 (97.9%), 30 (97.6%), 29 (97.0%), 32 (96.2%), 34 (94.3%), 38 (94.1%), 37 (92.1%), 31 (85.1%), and 40 (84.8%); and from *An. quadrimaculatus*: in females, 5 (95.4%); in male genitalia, 10 (79.8%) and 11 (79.3%); in pupae, 15 (97.3%); and in 4th-instar larvae, 38 (88.4%) and 39 (84.3%).

Male genitalia of *An. smaragdinus* often had the posterolateral lobe of tergum IX with the apex ridged (51.2%), while the apex was smooth in the other species of the complex (*An. quadrimaculatus* usually smooth, ridged in 4.0% of specimens).

In pupal exuviae of *An. smaragdinus,* the mesothoracic wing usually possessed well-defined, darkly pigmented longitudinal lines that distinguished it from the usually pale integument of many specimens of *An. quadrimaculatus* and most specimens of *An. diluvialis, An. inundatus,* and *An. maverlius.* Additionally, all living 4th-instar larvae and early pupae examined during this study were emerald green (or a shade close to emerald green); larvae of *some* specimens of species *An. diluvialis, An. inundatus, An. maverlius,* and *An. quadrimaculatus* were pale green but not emerald green.

The sternal plate located anteromesally on segment VIII was usually absent (92.9%) in the 4th-instar larvae of *An. smaragdinus* but was present in *An. diluvialis* (93.5%), *An. inundatus* (64.2%), *An. maverlius* (98.5%), and *An. quadrimaculatus* (94.9%).

The species name *smaragdinus* is of Latin origin and refers to the emerald green color of the living 4th-instar larvae and early pupae.

DISTRIBUTION

The format used in the distribution of species for the Quadrimaculatus Complex follows. Entries for specimens used in the morphological studies list the state in capital letters; county or parish italicized; nearest town or city; locality (e.g., river, creek); date of collection (day, month, year); collectors' initials listed alphabetically (full names provided in Appendix 2); collection number (e.g., FL95.57); indication of isofemale progeny brood (IPB is enclosed in parentheses, followed by an asterisk if at least the mother or one adult from the brood also had been identified by starch gel electrophoresis); and specimens examined (e.g., 27 ♀pl, 10 L; indicates 27 females individually reared with associated pupal and 4th-instar larval exuviae and 10 4th-instar larvae). Also, some adults from each IPB were frozen for use in molecular studies. The format used for distribution of specimens examined in biochemical, molecular, and genetic studies is similar to the above, but with some data excluded. The format used for records in the published literature includes state, county or parish, and citation. Distributions for the 5 species of the complex are listed below and are displayed on maps (Figs. 23–27). Seawright et al. (1992) provided a summary of the distribution for the species of the complex from collections made by IAMARL and MAVERL personnel to that date.

Anopheles diluvialis

Specimens examined in morphological studies: 26 IPBs, 527 ♀pl, 26 ♀, 310 ♂pl, 26 ♂, 31 ♂g, 9 pl, 37 Pl, and 309 L. FLORIDA, *Dixie Co.*, Cross City, Bear Bay Swamp, 12 Oct 1994, JFR, PEK, FL94.11 (IPB*), 46 ♀pl, 54 ♂pl, 3 ♂g, 17 L; FL94.13 (IPB*), 31 ♀pl, 30 ♂pl, 13 L; FL94.14 (IPB*), 24 ♀pl, 25 ♂pl, 1 ♂g, 24 L; FL94.15 (IPB*), 78 ♀pl, 7 ♂pl, 1 ♂g, 1 pl, 10 L; FL94.16 (IPB*), 25 ♀pl, 14 ♂pl, 1 ♂g, 14 L; FL94.17 (IPB*), 10 ♀pl, 7 ♂pl, 1 ♂g, 13 L; FL94.18 (IPB*), 35 ♀pl, 15 ♂pl, 1 ♂g, 16 L; FL94.19 (IPB*), 1 ♀pl, 14 ♂pl, 1 ♂g, 1 Pl, 11 L; FL94.20 (IPB*), 3 ♀pl, 6 ♂pl, 1 ♂g, 1 Pl, 11 L; FL94.21 (IPB*), 9 ♀pl, 8 ♂pl, 1 ♂g, 25 L; FL94.24 (IPB*), 10 ♀pl, 2 ♂pl, 5 L; FL94.26 (IPB*), 19 ♀pl, 12 ♂pl, 1 ♂g, 6 L; 14 Mar 1996, PEK, FL96.1 (IPB), 21 ♀pl, 8 ♂pl, 3 ♂g, 2 Pl, 2 L; FL96.2 (IPB), 13 ♀pl, 5 Pl; FL96.3 (IPB), 17 ♀pl, 6 ♂pl, 2 ♂g, 4 Pl; 26 Apr 1996, KRK, PEK, FL96.9 (IPB), 4 ♀pl; FL96.10 (IPB), 12 ♀pl; 6 Jun 1996, SEW, FL96.15 (IPB), 12 ♀pl, 2 ♀, 10 ♂pl, 2 ♂, 2 pl, 3 Pl; 10 Jul 1996, SEW, FL96.18, 18 ♀pl, 26 ♂pl; 29 Sep 1987, SEM, TJZ-28 (IPB*), 12 ♀pl, 21 ♀, 8 ♂pl, 20 ♂, 1 ♂g, 10 L; TJZ-29 (IPB*), 13 ♀pl, 13 ♀, 6 ♂pl, 4 ♂, 1 pl, 10 L; *Levy Co.*, Chiefland, Manatee Springs State Park, Magnolia Camp, 31 Aug 1995, JFR, PEK, FL95.51 (IPB*), 12 ♀pl, 30 ♂pl, 7 ♂g, 1 pl, 1 Pl, 13 L; FL95.64 (IPB*), 36 ♀pl, 1 ♂pl, 1 pl, 1 Pl, 37 L; FL95.66 (IPB*), 21 ♀pl, 14 ♂pl, 2 ♂g, 3 pl, 1 Pl, 17 L; North End Trail, 31 Aug 1995, JFR, PEK, FL95.55 (IPB*), 10 ♀pl, 12 ♂pl, 2 ♂g, 3 Pl, 10 L; FL95.56 (IPB*), 40 ♀pl, 10 Pl, 27 L; Hickory Camp, 31 Aug 1995, JFR, PEK, FL95.63 (IPB*), 13 ♀pl, 21 ♂pl, 2 ♂g, 6 Pl, 18 L; Chiefland, Usher boat ramp on Suwannee River, 21 May 1996, SEW, 10 ♀pl, 20 ♂pl, 2 ♂g.

Specimens examined in biochemical, molecular, and genetic studies: FLORIDA, *Alachua Co.*, High Springs, Santa Fe River, 26 May 1988, 28 May 1991, 3 Jan, 31 Jul 1992, PEK, TJ, 71 adults; *Citrus Co.*, Chassahowitzka, 30 Jun 1987, BJS, PEK, 24 adults; Homosassa, Homosassa River, 30 Jun 1987, BJS, PEK, 2 adults; *Dixie Co.*, Cross City, Bear Bay Swamp, 2 Jun 1987, 2 Apr 1990, 15 May 1991, 12 Oct 1994, BJS, BKB, JFR, PEK, SEM, 338 adults; Horseshoe Beach, 2 Jun 1987, 26 Mar 1990, BJS, PEK, 106 adults; Jena, 2 Jun 1987, BJS, PEK, 75 adults; Old Town, Guaranto Springs, 8 Jun 1988, 10 May 1991, PEK, 14 adults; *Gilchrist Co.*, Bell, Ginnie Springs, 17 May 1988, GCL, PEK, SEM, 37 adults; *Hernando Co.*, Weeki Wachee, 30 Jun 1987, BJS, PEK, 11 adults; *Jefferson Co.*, Wacissa, Wacissa Springs, 28 Sep 1989, MQB, PEK, 5 adults; *Lafayette Co.*, Branford, Sawannee River, 8,21 Jun 1988, PEK, 4 adults; *Levy Co.*, Chiefland, Manatee Springs State Park, 8 Jun 1988, 10 May 1989, 24 May, 16 Sep, 3 Oct, 20 Dec 1991, 26 Jun, 22 Jul, 18 Aug, 24 Sep, 13 Oct, 23 Nov 1992, 10 May 1993, 31 Aug 1995, BJS, JFR, PEK, SEM, SGS, TJ, 262 adults; Fowler Bluff, Lower Sawannee National Wildlife Refuge, 4 Oct 1986, 14 Jan, 25 Jun, 21 Jul 1992, 13 Jul 1993, BJS, PEK, TJ, 430 adults; Gulf Hammock, Waccasassa Bay State Preserve, 27 May 1987, BJS, PEK, 51 adults; *Marion Co.*, Eureka, Ocklawaha River, 22 May 1985, 14 Jun 1990, 29 May 1992, MQB, PEK, TJ, 21 adults; Ocala, Ocklawaha River, 29 May 1992, TJ, 46 adults; *Putnam Co.*, Welaka, Ocklawaha River, 5 Jun 1992, PEK, TJ, 11 adults; *Sumter Co.*, Coleman, Lake Panasoffkee, 22 Mar, 23 Aug 1990, 23 Apr, 4 Jun, 2 Jul, 8 Sep 1991, 24 Feb, 26 Mar 1992, 6 Jan, 16 Mar 1993, PEK, SEM, SGS, TJ, 528 adults; Sumterville, Lake Panasoffkee, 23 Aug 1990, BJS, PEK, 3 adults; Rutland, Withlacoochee River, 12 Feb 1990, BJS, PEK, 1 adult; Tarrytown, Withlacoochee State Forest, 11 Jun 1991, TJ, 14 adults; *Suwannee Co.*, Falmouth, Suwannee River State Park, 21 Jun 1988, PEK, 1 adult; Hildreth, Ichetucknee Springs State Park, 3 Jun 1988, BJS, PEK, 10 adults; *Taylor Co.*, Smith-McCullan Creek, 9 Jun 1987, BJS, PEK, 45 adults; *Wakulla Co.*, St. Marks, St. Marks National Wildlife Refuge, 9 Jun 1987, BJS, PEK, 15 adults, Wakulla, Wakulla Springs, 28 Sep 1989, MQB, PEK, 33 adults.

Records in literature: FLORIDA, *Levy Co.* (Cockburn and Seawright 1988, Kaiser et al. 1988c, Narang et al. 1989b, Jensen et al. 1996), *Dixie Co., Levy Co.* (Narang and Seawright 1988), *Calhoun Co., Levy Co.* (Kaiser 1988), *Dixie Co.* (Narang et al. 1989a, Cockburn 1990, Mitchell et al. 1992, Linley et al. 1993, Cornel et al. 1996), *Alachua Co., Citrus Co., Dixie Co., Gilchrist Co., Levy Co., Taylor Co.* (Narang et al. 1990a), *Dixie Co., Sumter Co.* (Nayar et al. 1992, Jensen et al. 1994), *Alachua Co., Citrus Co., Dixie Co., Gilchrist Co., Hernando Co., Jefferson Co., LaFayette Co., Leon Co., Levy Co., Marion Co., Sumter Co., Suwannee Co., Taylor Co., Wakulla Co.* (Seawright et al. 1992), *Sumter Co.* (Jensen et al. 1993, 1995).

Anopheles inundatus

Specimens examined in morphological studies: 32 IPBs, 837 ♀pl, 1 ♀p, 503 ♂pl, 2 ♂p, 35 ♂g, 20 pl, 139 Pl, 1 P, 38 l, and 781 L. FLORIDA, *Walton Co.*, Bruce, Choctawhatchee River at FL-20 bridge, 11 Nov 1994, PEK, FL94.61 (IPB*), 50 ♀pl, 24 ♂pl, 1 ♂p, 2 ♂g, 3 Pl, 32 L; FL94.64 (IPB*), 28 ♀pl, 19 ♂pl, 1 ♂g, 1 pl, 5 Pl, 2 l, 1 P, 31 L; FL94.65 (IPB*), 54 ♀pl, 29 ♂pl, 2 ♂g, 4 Pl, 2 l, 22 L; 31 May 1995, JFR, PEK, FL95.29 (IPB*), 16 ♀pl, 10 ♂pl, 1 ♂g, 10 Pl, 9 L; FL95.30 (IPB*), 5 ♀pl, 4 ♂pl, 12 Pl, 11 L; FL95.31 (IPB*), 21 ♀pl, 12 ♂pl, 1 ♂g, 2 Pl, 24 L; Choctawhatchee River at Rook's Bluff Fish Camp, 11 Nov 1994, PEK, FL94.62 (IPB*), 17 ♀pl, 1 Pl, 1 l, 19 L; FL94.66 (IPB*), 7 ♀pl, 1 l, 2 L; FL94.67 (IPB*), 75 ♀pl, 36 ♂pl, 1 ♂p, 2 ♂g, 2 Pl, 6 l, 31 L; FL94.69 (IPB*), 31 ♀pl, 27 ♂pl, 1 ♂g, 6 Pl, 2 l, 42 L; FL94.70 (IPB*), 29 ♀pl, 14 ♂pl, 1 ♂g, 2 pl,

4 Pl, 2 l, 20 L; FL94. 71 (IPB*), 24 ♀pl, 16 ♂pl, 2 ♂g, 1 l, 7 L; 31 May 1995, JFR, PEK, FL95.16 (IPB*), 16 ♀pl, 8 L; FL95.17 (IPB*), 45 ♀pl, 35 ♂pl, 1 ♂g, 9 Pl, 53 L; FL95.18 (IPB*), 19 ♀pl, 22 ♂pl, 1 ♂g, 7 Pl, 55 L; FL95.19 (IPB*), 4 ♀pl, 13 ♂pl, 1 ♂g, 12 Pl, 57 L; FL95.20 (IPB*), 23 ♀pl, 22 ♂pl, 1 ♂g, 11 Pl, 58 L; FL95.21 (IPB*), 2 ♀pl, 6 ♂pl, 4 Pl, 10 L; FL95.22 (IPB*), 5 ♀pl, 6 ♂pl, 5 Pl, 14 L; FL95.23 (IPB*), 58 ♀pl, 1 ♀p, 35 ♂pl, 3 ♂g, 1 pl, 1 Pl, 18 L; FL95.24 (IPB*), 10 ♀pl, 6 ♂pl, 2 ♂g, 5 pl, 10 Pl, 33 L; FL95.25 (IPB*), 41 ♀pl, 42 ♂pl, 1 ♂, 7 ♂g, 5 pl, 8 Pl, 3 l, 35 L; FL95.26 (IPB*), 1 ♀pl, 3 ♂pl, 2 Pl, 5 L; FL95.27 (IPB*), 1 ♀pl, 2 ♂pl, 2 Pl, 12 L; FL95.28 (IPB*), 1 ♀pl, 6 ♂pl, 3 Pl, 6 L. GEORGIA, *Bullock Co.*, Dover, Ogeechee River at US-301 bridge, 18 Sep 1995, PEK, GA95.31 (IPB*), 3 ♀pl, 2 ♂pl, 2 L; GA95.34 (IPB*), 26 ♀pl, 28 ♂pl, 1 ♂g, 1 pl, 1 Pl, 39 L; GA95.37 (IPB*), 57 ♀pl, 18 L; GA95.38 (IPB*), 45 ♀pl, 28 ♂pl, 1 ♂g, 28 L; GA95.40 (IPB*), 59 ♀pl, 1 pl, 2 Pl, 12 L; GA95.42 (IPB*), 41 ♀pl, 29 ♂pl, 2 ♂g, 2 Pl, 11 l, 34 L. LOUISIANA, *Iberia Parish,* Dauterive Landing on Dauterive Lake, 7 Nov 1994, PEK, LA94.9 (IPB*), 23 ♀pl, 27 ♂pl, 2 ♂g, 4 pl, 11 Pl, 7 l, 34 L.

Specimens examined in biochemical, molecular and genetic studies: FLORIDA, *Calhoun Co.,* Blountstown, Apalachicola River, 26 Sep 1989, PEK, 8 adults; Chippola Park, Dead Lake, 28 Sep 1987, PEK, 6 adults; *Gulf Co.,* Wewahitchka, Dead Lake, 14 Jun 1988, PEK, 1 adult; Willis Landing, Apalachicola River, 26 Sep 1989, 1 Jun 1990, PEK, 82 adults; *Holmes Co.,* Westville, Choctawhatchee River, 28 Sep 1988, PEK, 10 adults; *Jackson Co.,* Chattahoochee, Apalachicola River, 26 Sep 1989, MQB, PEK, 8 adults; *Liberty Co.,* Hosford, Ochlockonee River, 27 Sep 1989, MQB, PEK, 9 adults; *Walton Co.,* Bruce, Choctawhatchee River, 14 Jun, 28 Sep 1988, 10,31 Jul 1991, 26 May 1992, 11 Nov 1994, 31 May, 1 Jun 1995, BKB, JFR, MQB, PEK, SEM, SGS, TJ, 407 adults; Red Bay, Morrison Springs, 28 Sep 1988, PEK, SEM, 53 adults; *Washington Co.,* New Hope, Holmes Creek, 28 Sep 1988, 26 May 1992, PEK, SEM, SGS, 74 adults. GEORGIA, *Bullock Co.*, Dover, Ogeechee River, 18 Sep 1995, PEK, 17 adults; *Chatham Co.,* Bloomingdale, Ogeechee River, 14 Jul, 10 Oct 1988, 9 Jun 1992, PEK, SEM, 16 adults; *Effingham Co.,* Guyton, Ogeechee River, 14 Jul, 11 Oct 1988, 3 Aug 1989, 9 Jun 1992, PEK, SEM, SGS, TJ, 49 adults; *Long Co.,* Ludowici, Altamaha River, 10 Jun 1992, PEK, TJ, 1 adult; *Screven Co.,* Dover, Ogeechee River, 11 Oct 1988, 9 Jun 1992, 18 Sep 1995, PEK, SEM, SGS, TJ, 23 adults; Oliver, Ogeechee River, 11 Oct 1988, 9 Jun 1992, PEK, SEM, TJ, 10 adults. LOUISIANA, *Iberia Parish,* Loreauville, Dauterive Lake, 7 Nov 1994, PEK, 3 adults; *Vermilion Parish,* Ester, 16 Jun, 7 Jul, 4 Aug 1993, 5,12,26 May, 9 Jun, 12 Sep, 6 Nov 1994, 26 Jul 1995, BBB, CRR, PEK, 110 adults.

Records in literature: FLORIDA, *Walton Co., Washington Co.* (Narang et al. 1990a), *Calhoun Co., Gulf Co., Holmes Co., Jackson Co., Liberty Co., Walton Co., Washington Co.* (Seawright et al. 1992), *Walton Co.* (Linley et al. 1993, Cornel et al. 1996). GEORGIA, *Calhoun Co., Effingham Co., Screven Co.* (Narang et al. 1990a), *Bullock Co., Chatham Co., Effingham Co., Screven Co.* (Seawright et al. 1992). LOUISIANA, *Vermilian Parish* (Rutledge and Meek 1994, Rutledge et al. 1996).

Anopheles maverlius

Specimens examined in morphological studies: 13 IPBs, 412 ♀pl, 1 ♀p, 4 ♀, 428 ♂pl, 7 ♂, 33 ♂g, 1 pl, 21 Pl, 8 p, 13 l, and 366 L. FLORIDA, *Walton Co.*, Bruce, Choctawhatcee River at FL-20 bridge, 31 May 1995, JFR, PEK, FL95.14 (IPB*), 34 ♀pl, 1 ♀p, 4 ♀, 43 ♂pl, 4 ♂, 8 ♂g, 2 Pl, 3 p, 19 L. GEORGIA, *Bullock Co.*, Dover, Ogeechee River at US-301 bridge, 18 Sep 1995, PEK, GA95.30 (IPB*), 19 ♀pl, 20 ♂pl, 2 ♂g, 1 Pl, 1 l, 6 L. MISSISSIPPI, *Tishomingo Co.*, Pickwick Reservoir, Sep 1987, BJS, PEK, 5 ♀pl, 1 pl, 8 L; 2 Sep 1987, TJZ-25 (IPB*), 11 ♀pl, 9 ♀, 10 ♂pl, 3 ♂, 1 ♂g, 7 L; High Point, Chambers Creek at Highway 350 bridge, 27 Sep 1995, SPR, MS95.2 (IPB*), 25 ♀pl, 13 ♂pl, 1 ♂g, 41 L; MS95.6 (IPB*), 64 ♀pl, 60 ♂pl, 3 ♂g, 1 Pl, 16 L; MS95.7 (IPB*), 31 ♀pl, 22 ♂pl, 3 ♂g, 3 Pl, 44 L; MS95.8 (IPB*), 62 ♀pl, 57 ♂pl, 3 ♂g, 1 Pl, 36 L; 10 Oct 1995, SPR, MS95.10 (IPB*), 20 ♀pl, 31 ♂pl, 2 ♂g, 2 Pl, 4 l, 28 L; MS95.11 (IPB*), 35 ♀pl, 31 ♂pl, 2 ♂g, 7 Pl, 39 L; MS95.12 (IPB*), 27 ♀pl, 54 ♂pl, 2 ♂g, 30 L; MS95.13 (IPB*), 22 ♀pl, 22 ♂pl, 2 ♂g, 1 Pl, 8 l, 35 L; MS95.14 (IPB*), 30 ♀pl, 32 ♂pl, 2 ♂g, 2 Pl, 22 L; MS95.15 (IPB*), 27 ♀pl, 33 ♂pl, 2 ♂g, 1 Pl, 13 L; Yellow Creek at Highway 25, 22 Sep 1987, BRM, KJT, 5 p, 22 L.

Specimens examined in biochemical, molecular and genetic studies: FLORIDA, *Calhoun Co.,* Blountstown, Apalachicola River, 26 Sep 1989, PEK, 1 adult; *Holmes Co.,* Westville, Choctawhatchee River, 28 Sep 1988, PEK, 7 adults; *Jackson Co.,* Chattahoochee, Apalachicola River, 26 Sep 1989, MQB, PEK, 2 adults; *Leon Co.,* Bradfordville, Lake Iamonia, 28 Sep 1989, MQB, PEK, 1 adult; *Liberty Co.,* Hosford, Ochlockonee River, 27 Sep 1989, MQB, PEK, 1 adult; *Walton Co.,* Bruce, Choctawhatchee River, 14 Jun, 28 Sep 1988, 10,31 Jul 1991, 26 May 1992, 11 Nov 1994, 31 May 1995, BKB, JFR, MQB, PEK, SEM, SGS, TJ, 163 adults; Red Bay, Morrison Springs, 28 Sep 1988, PEK, SEM, 9 adults. GEORGIA, *Bullock Co.,* Dover, Ogeechee River, 18 Sep 1995, PEK, 1 adult; *Camden Co.,* Jerusalem, Satilla River, 30 Aug 1989, PEK, 1 adult; *Chatham Co.,* Bloomingdale, Ogeechee River, 14 Jul, 10 Oct 1988, 9 Jun 1992, PEK, SEM, 6 adults; *Effingham Co.,* Eden, Ogeechee River, 10 Oct 1988, PEK, 1 adult; Guyton, Ogeechee River, 14 Jul, 11 Oct 1988, 3 Aug 1989, 9 Jun 1992, PEK, SEM, SGS, TJ, 19 adults;

McDuffie Co., Thompson, Clark Hill Reservoir, 27 Jun 1988, PEK, 1 adult; *Screven Co.,* Dover, Ogeechee River, 11 Oct 1988, 9 Jun 1992, 18 Sep 1995, PEK, SEM, SGS, TJ, 4 adults; Oliver, Ogeechee River, 11 Oct 1988, 9 Jun 1992, PEK, SEM, TJ, 2 adults. LOUISIANA, *Calcasieu Parish,* Lake Charles, 8 Aug 1993, CRR, 3 adults; *Iberia Parish,* Delcambre, 19 Aug 1993, CRR, 2 adults; *Jefferson Davis Parish,* Pine Island, 16 Jun, 4,11,18 Aug 1993, 26 May, 9 Jun 1994, CRR, 13 adults; Mermentau, Lake Mermentau, 4 Aug 1993, 9 Jun 1994, 3 adults; *Lafourche Parish,* Thibodaux, 11 Aug 1994, CRR, 1 adult; *Natchitoches Parish,* Robeline, 15 Aug 1994, CRR, 1 adult; *Ouachita Parish,* Swartz, Russell Sage Wildlife Management Area, Bayou Lafourche, 25 Jun, 20 Aug, 14 Sep 1993, 19 Jul 1994, CRR, 13 adults; Richwood, Ouachita Wildlife Management Area, Bayou Lafourche, 20 Aug, 28 Sep 1993, CRR, 4 adults; *St. James Parish,* Union, 17 Aug 1994, CRR, 2 adults; *St. Tammany Parish,* Pearl River, Pearl River Wildlife Management Area, 20 Jul 1994, CRR, 2 adults; Slidell, West Pearl River, 1 Nov 1994, PEK, 1 adult; *Vermilion Parish,* Ester, 27 Jul, 12 Sep 1994, CRR, 3 adults; Lake Arthur, Lake Arthur, 4 Aug 1993, 9 Jun 1994, CRR, 5 adults. MISSISSIPPI, *Hancock Co.,* Bay St. Louis, Pearl River, 19 Jul 1990, JKN, PEK, 1 adult; *Itawamba Co.,* Fairview, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 9 adults; *Noxubee Co.,* Brooksville, Noxubee National Wildlife Refuge, 7 Aug, 28 Sep 1986, BKB, GCL, PEK, 4 adults; *Tishomingo Co.,* High Point, Chambers Creek, 27 Sep, 10 Oct 1995, SR, 11 adults; North Crossroads, Yellow Creek, 1 Sep 1987, 26 Aug 1988, BRM, PEK, SEM, 134 adults. SOUTH CAROLINA, *McCormick Co.,* Clarks Hill, Savannah River, 27 Jun 1988, PEK, 6 adults. TENNESSEE, *Benton Co.,* Eagle Creek, Tennessee River, 12 Aug 1988, BKB, PEK, 1 adult; *Decatur Co.,* Bath Springs, Tennessee River, 13 Aug 1988, BKB, PEK, 1 adult.

Records in literature: ALABAMA, *Colbert Co., Montgomery Co.* (Seawright et al. 1992). FLORIDA (Cockburn 1990), *Calhoun Co., Holmes Co., Jackson Co., Liberty Co., Walton Co.* (Seawright et al. 1992), *Walton Co.* (Linley et al. 1993, Cornel et al. 1996). GEORGIA, *Camden Co., Chatham Co., Effingham Co., McDuffie Co., Screven Co.* (Seawright et al. 1992). LOUISIANA (Rutledge and Meek 1994). MISSISSIPPI, *Tishomingo Co.* (Narang et al. 1989a, Mitchell et al. 1992, Rutledge et al. 1996), *Noxubee Co.* (Apperson and Lanzaro 1991), *Itawamba Co., Tishomingo Co.* (Seawright et al. 1992), *Noxubee Co.*; restricted to relatively undisturbed forest habitats near major rivers to east and south of Mississippi Delta (Mallet and Fritzius 1993). SOUTH CAROLINA, *McCormick Co.* (Seawright et al. 1992). TENNESSEE, *Benton Co., Decatur Co.* (Seawright et al. 1992).

Anopheles quadrimaculatus

Specimens examined in morphological studies: 38 IPBs, 961 ♀pl, 1 ♀p, 121 ♀, 722 ♂ pl, 95 ♂, 34 ♂g, 36 pl, 73 Pl, 21 p, 6 P, 53 l, and 877 L. FLORIDA, *Alachua Co.*, Gainesville, USDA-IA-MARL, ORLANDO Laboratory Strain, 2 Sep 1991, JFR, FL91.105, 11 ♀pl, 6♀, 11 ♂, 10 p, 36 L; 4 Aug 1995, JFR, FL95.33, 38 ♀pl, 24 ♀, 13 ♂pl, 24 ♂, 38 L; 28 Nov 1995, JFR, FL95.115, 5 ♀pl, 31 ♀, 7 ♂, 26 L; Gainesville, Kanapaha Botanical Gardens, 26 Oct 1987, SEM, TJZ-30 (IPB*), 9 ♀pl, 32 ♀, 14 ♂pl, 26 ♂, 1 ♂g, 7 L; TJZ-31 (IPB*), 5 ♀pl, 14 ♀, 18 ♂pl, 21 ♂, 10 L; Gainesville, Lake Alice, 25 Oct 1994, JFR, PEK, FL94.52 (IPB*), 43 ♀pl, 23 ♂pl, 1 pl, 9 L; FL94.53 (IPB*), 20 ♀pl, 14 ♂pl, 1 ♂g, 2 pl, 11 L; FL94.54 (IPB*), 1 ♀pl, 1 ♂pl, 1 ♂g, 1 pl; FL94.55 (IPB*), 34 ♀pl, 26 ♂pl, 1 ♂g, 24 L; FL94.56 (IPB*), 4 ♀pl, 11 ♂pl, 2 Pl, 10 L; FL94.57 (IPB), 1 ♂pl, 1 Pl; Gainesville, Paynes Prairie State Preserve, 24 Aug 1995, JFR, PEK, FL95.42 (IPB*), 38 ♀pl, 13 ♂pl, 2 ♂g, 11 l, 3 P, 30 L; FL95.43 (IPB*), 26 ♀pl, 11 ♂pl, 4 l, 16 L; Gainesville, 3 Nov 1995, ORW, FL95.71, 16 ♀pl, 22 ♂pl, 1 pl, 1 Pl, 1 l, 13 L; Gainesville, 4,17,21,28 Oct 1996, 24 Mar 1997, HMS, JFR, SEW, FL96.32, 3 ♀pl, 1 ♀p, 2 ♂pl, 3 L; FL97.10, 2 ♀pl, 1 ♂pl, 1 Pl, 31 L; Gainesville, 21 Jan 1997, JFR, ORW, FL97.1, 1 ♀pl, 5 ♂pl; *Dixie Co.*, Cross City, Bear Bay Swamp, 6 Jun 1996, SEW, FL96.14 (IPB), 43 ♀pl, 2 ♀, 36 ♂pl, 1 ♂, 20 L; *Levy Co.*, Chiefland, Manatee Springs State Park, JFR, PEK, FL95.46, 4 ♀pl, 1 ♂pl; FL95.54 (IPB*), 32 ♀pl, 12 ♂pl, 2 ♂g, 6 pl, 48 L; FL95.60 (IPB*), 26 ♀pl, 10 ♂pl, 10 pl, 1 Pl, 24 L; Chiefland, Usher boat ramp on Suwannee River, 26 Apr 1996, KRK, PEK, FL96.8 (IPB), 30 ♀pl, 25 ♂pl, 15 L; *Palm Beach Co.*, West Palm Beach, Jul 1996, JN, JKN96.1, 4 ♀pl, 3 ♂pl; JKN96.2, 6 ♀pl, 2 ♂pl; *Sumter Co.*, Coleman, Coleman's Landing on Lake Panasoffkee, 21 Aug 1996, HMS, SEW, FL96.25 (IPB), 19 ♀pl, 12 ♂pl, 6 p, 2 P, 5 l; *Walton Co.*, Bruce, Choctawhatchee River at FL-20 bridge, 11 Nov 1994, PEK, FL94.60 (IPB*), 10 ♀pl, 12 ♂pl, 2 Pl; FL94.72 (IPB*), 25 ♀pl, 12 ♂pl, 1 ♂g, 1 Pl, 12 L; 31 May 1995, JFR, PEK, FL95.15 (IPB*), 13 ♀pl, 14 ♂pl, 1 ♂g, 9 Pl, 1 l, 1 P. GEORGIA, *Bullock Co.*, Dover, Ogeechee River at US-301 bridge, 18 Sep 1995, PEK, GA95.35 (IPB*), 18 ♀pl, 12 ♂pl, 1 ♂g, 1 Pl, 20 L; GA95.36 (IPB*), 23 ♀pl, 5 ♂pl, 1 ♂g, 1 Pl, 9 L; GA95.43 (IPB*), 20 ♀pl, 12 ♂pl, 1 Pl, 42 L; GA95.44 (IPB*), 42 ♀pl, 19 ♂pl, 2 ♂g, 1 Pl, 28 L. LOUISIANA, *Iberia Parish,* Dauterive Landing on Dauterive Lake, 7 Nov 1994, PEK, LA94.1 (IPB*), 24 ♀pl, 23 ♂pl, 1 ♂g, 4 l, 24 L; LA94.2 (IPB*), 21 ♀pl, 22 ♂pl, 2 ♂g, 1 pl, 7 l, 20 L; *Vermilion Parish,* Ester, 7 Nov 1994, BBB, PEK, LA94.11 (IPB*), 19 ♀pl, 3 ♂pl, 1 ♂g, 1 Pl, 1 l, 18 L; LA94.12 (IPB*), 11 ♀pl, 14 ♂pl, 3 pl, 4 Pl, 1 l, 12 L; LA94.13 (IPB*), 1 ♀pl, 5 ♂pl, 2 pl, 4 Pl, 5 p, 3 L; LA94.15 (IPB*),

12 ♀pl, 17 ♂pl, 1 ♂g, 3 pl, 3 l, 23 L; LA94.17 (IPB*), 27 ♀pl, 20 ♂pl, 1 ♂g, 1 l, 29 L; Palmetto Island, 26 Jun 1995, PEK, LA95.2 (IPB), 5 ♀pl, 20 ♂pl, 12 Pl, 38 L; LA95.3 (IPB), 6 ♀pl, 26 ♂pl, 1 ♂g, 4 pl, 10 Pl, 37 L; LA95.4 (IPB), 52 ♀pl, 35 ♂pl, 1 ♂g, 7 Pl, 38 L; LA95.5 (IPB), 14 ♀pl, 1 ♂pl, 4 Pl, 10 L. MINNESOTA, *Goodhue Co.*, Lake City, 19 Aug 1995, JFR, PEK, MN95.10 (IPB*), 67 ♀pl, 59 ♂pl, 9 ♂g, 2 pl, 2 Pl, 13 l, 31 L; *Wabasha Co.*, 6 Sep 1939, RHD, 1 ♀; East Indian Creek at US-61 bridge, 18 Aug 1995, JFR, PEK, 1 L. MISSISSIPPI, *Tishomingo Co.*, High Point, Chambers Creek at Highway 350 bridge, 27 Sep 1995, SPR, MS95.3 (IPB*), 34 ♀pl, 28 ♂pl, 1 ♂g, 1 Pl, 34 L; MS95.4 (IPB*), 28 ♀pl, 27 ♂pl, 1 ♂g, 8 L; MS95.5 (IPB*), 22 ♀pl, 23 ♂pl, 1 ♂g, 1 Pl, 47 L; MS95.9 (IPB*), 36 ♀pl, 33 ♂pl, 1 ♂g, 4 Pl, 22 L. WISCONSIN, Kumlien (holotype *An. annulimanus*), 1♂; *La Crosse Co.*, La Crosse, 19 Aug 1987, BJS, PEK, 11 ♀pl, 11 ♀, 4 ♂pl, 4 ♂.

Specimens examined in biochemical, molecular, and genetic studies: ALABAMA, *Baldwin Co.*, Stockton, Tensaw River, 18 Jul 1990, JKN, PEK, 51 adults; *Colbert Co.*, Riverton, Pickwick Lake, 26 Aug 1987, BRM, PEK, SEM, 150 adults; *Greene Co.*, Forkland, Lake Demopolis, 1 Sep 1988, PEK, SEM, 40 adults; *Houston Co.*, Pansey, Chattahoochee State Park, 27 Jun, 10 Sep, 12 Nov 1985, BKB, PEK, 88 adults; *Jackson Co.*, Fackler, Guntersville Lake, 9 Sep 1986, MQB, PEK, 50 adults; *Lauderdale Co.*, Florence, Pickwick Lake, 26 Aug 1988, BRM, PEK, SEM, 95 adults; Killen, Wilson Lake, 11 Aug 1988, BRM, PEK, SEM, 46 adults; *Lawrence Co.*, Town Creek, Wilson Lake, 11 Aug 1988, BRM, PEK, SEM, 22 adults; *Limestone Co.*, Mooresville, Wheeler Lake, 8 Sep 1986, 30 Aug 1987, 9 Aug 1988, BRM, KJT, MQB, PEK, SEM, 348 adults; *Madison Co.*, Triana, Wheeler Lake, 30 Aug 1987, 9 Aug 1988, BRM, KJT, MQB, PEK, SEM, 41 adults; *Marshall Co.*, Guntersville, Browns Creek, 9 Sep 1986, MQB, PEK, 51 adults; *Montgomery Co.*, Waugh, 23 Jul, 27 Aug, 17 Oct 1984, MQB, PEK, SEM, 30 adults; *Morgan Co.*, Decatur, Wheeler Lake, 31 Aug 1987, 10 Aug 1988, BRM, KJT, MQB, PEK, SEM, 268 adults; *Pickens Co.*, Pickensville, Aliceville Lake, 31 Aug 1988, PEK, SEM, 46 adults; *Russell Co.*, Cottonton, Chattahoochee River, 30 Aug 1990, PEK, 24 adults; *Sumter Co.*, Gainesville, Tennessee Tombigbee, 1 Sep 1988, PEK, SEM, 36 adults. ARKANSAS, *Arkansas Co.*, Stuttgart, 19 Jul 1985, GCL, MQB, PEK, 100 adults. CONNECTICUT, *Litchfield Co.*, Harwinton, 31 Aug 1992, PEK, 20 adults. FLORIDA, *Alachua Co.*, Cross Creek, 23 Jul 1986, PEK, 19 adults; Gainesville, Kanapaha Botanical Gardens, 22 May, 5 Jun, 15 Aug, 10 Oct, 2 Dec 1985, 19 Mar, 22 May 1986, GCL, MQB, PEK, SEM, 840 adults; Lake Alice, 7 Nov 1990, 25 Oct 1994, JFR, PEK, 32 adults; Paynes Prairie State Preserve, 10 Mar 1992, 24 Aug 1995, JFR, PEK, SGS, 37 adults; High Springs, Santa Fe River, 26 May 1988, 28 May 1991, 3 Jan, 31 Jul 1992, PEK, TJ, 66 adults; *Calhoun Co.*, Blountstown, Apalachicola River, 26 Sep 1989, PEK, 38 adults; Chippola Park, Dead Lake, 28 Sep 1987, PEK, 72 adults; *Citrus Co.*, Chassahowitzka, 30 Jun 1987, BJS, PEK, 28 adults; Citrus Springs, Lake Rousseau, 27 May 1987, 2 Apr 1990, PEK, BJS, 54 adults; Homosassa, Homosassa River, 30 Jun 1987, BJS, PEK, 39 adults; *Collier Co.*, Copeland, 14 Nov 1988, PEK, 103 adults; Immokalee, Corkscrew Swamp Sanctuary, 14 Nov 1988, PEK, 22 adults; *Dixie Co.*, Cross City, Bear Bay Swamp, 2 Jun 1987, 12 May 1992, PEK, 3 adults; Horseshoe Beach, 2 Jun 1987, 26 May 1990, BJS, PEK, 51 adults; Jena, 2 Jun 1987, BJS, PEK, 23 adults; Old Town, Guaranto Springs, 8 Jun 1988, 10 May 1991, PEK, 32 adults; *Escambia Co.*, McDavid, Escambia River, 19 Sep 1989, PEK, SEM, 93 adults; *Flagler Co.*, Palm Coast, 6 Jul 1987, BJS, PEK, 22 adults; *Gilchrist Co.*, Bell, Ginnie Springs, 17 May 1988, GCL, PEK, SEM, 107 adults; *Glades Co.*, Moore Haven, 15 Nov 1988, PEK, 94 adults; *Gulf Co.*, Wewahitchka, Dead Lake, 29 May 1987, 14 Jun 1988, PEK, 57 adults; Willis Landing, Apalachicola River, 26 Sep 1989, 1 Jun 1990, PEK, 38 adults; *Hamilton Co.*, Jennings, Lake Octahatchee, 12 Jun, 14 Jul, 18 Aug, 16 Sep, 27 Oct, 17 Nov 1986, 19 May, 15 Jun, 13 Jul, 4 Aug, 8 Sep, 20 Oct, 16 Nov 1987, 22 May 1990, 27 Jul, 7 Oct 1991, 18 Oct 1994, BJS, BKB, JFR, MQB, PEK, SEM, 118 adults; White Springs, 12 Jun 1986, PEK, 39 adults; *Hernando Co.*, Weeki Wachee, 30 Jun 1987, BJS, PEK, 24 adults; *Holmes Co.*, Westville, Choctawhatchee River, 28 Sep 1988, PEK, 17 adults; *Indian River Co.*, Vero Beach, Blue Cypress Lake, 27 Oct 1988, PEK, 25 adults; *Jackson Co.*, Chattahoochee, Apalachicola River, 26 Sep 1989, MQB, PEK, 32 adults; *Jefferson Co.*, Wacissa, Wacissa Springs, 28 Sep 1989, MQB, PEK, 24 adults; *Lafayette Co.*, Branford, Sawannee River, 8,21 Jun 1988, PEK, 58 adults; *Leon Co.*, Tallahassee, Lake Carr, 28 Sep 1989, MQB, PEK, 54 adults; Lake Talquin, 27 Sep 1989, MQB, PEK, 23 adults; Bradfordville, Lake Iamonia, 28 Sep 1989, PEK, MQB, 4 adults; Miccosukee, Lake Miccosukee, 28 Sep 1989, MQB, PEK, 25 adults; *Levy Co.*, Chiefland, Manatee Springs State Park, 8 Jun 1988, 10 May 1989, 24 May, 16 Sep, 3 Oct, 20 Dec 1991, 26 Jun, 22 Jul, 18 Aug, 24 Sep, 13 Oct, 23 Nov 1992, 10 May 1993, 31 Aug 1995, BJS, JFR, PEK, SEM, SGS, TJ, 476 adults; Fowler Bluff, Lower Sawannee National Wildlife Refuge, 4 Oct 1986, 14 Jan, 25 Jun, 21 Jul 1992, 13 Jul 1993, BJS, PEK, TJ, 65 adults; Gulf Hammock, Waccasassa Bay State Preserve, 27 May 1987, BJS, PEK, 21 adults; *Liberty Co.*, Hosford, Ochlockonee River, 27 Sep 1989, MQB, PEK, 24 adults; *Marion Co.*, Eureka, Ocklawaha River, 22 May 1985, 14 Jun 1990, 29 May 1992, MQB, PEK, TJ, 150 adults; Ocala, Ocklawaha River, 29 May 1992, TJ, 2 adults; *Okaloosa Co.*, Milligan, Yellow River, 20 Sep 1989, PEK,

SEM, 24 adults; *Okeechobee Co.,* Okeechobee, 15 Nov 1988, PEK, 22 adults; *Orange Co.,* Christmas, St. Johns River, 1 May 1990, PEK, 26 adults; *Palm Beach Co.,* Belle Glade, 15 Nov 1988, PEK, 59 adults; *Putnam Co.* Welaka, Ocklawaha River, 5 Jun 1992, PEK, TJ, 2 adults; *Santa Rosa Co.,* Milton, Blackwater River, 20 Sep 1989, PEK, SEM, 21 adults; *Sarasota Co.,* Bee Ridge, Myakka River State Park, 5 Nov 1985, MQB, PEK, 115 adults; *Sumter Co.,* Coleman, Lake Panasoffkee, 22 Mar, 23 Aug 1990, 23 Apr, 4 Jun, 2 Jul, 8 Sep 1991, 24 Feb, 26 Mar 1992, 6 Jan, 16 Mar 1993, PEK, SEM, SGS, TJ, 127 adults; Sumterville, Lake Panasoffkee, 19 Aug 1985, 23 Aug 1990, 16 Aug, 27 Dec 1991, BJS, PEK, 192 adults; Rutland, Withlacoochee River, 12 Feb 1990, BJS, PEK, 23 adults; Tarrytown, Withlacoochee State Forest, 11 Jun 1991, TJ, 6 adults; *Suwannee Co.,* Falmouth, Suwannee River State Park, 21 Jun 1988, PEK, 44 adults; Hildreth, Ichetucknee Springs State Park, 3 Jun 1988, BJS, PEK, 40 adults; *Taylor Co.,* Dekle Beach, 23 Aug 1989, MQB, PEK, 23 adults; Hampton Springs, Econfina River, 23 Aug 1989, MQB, PEK, 15 adults; Smith-McCullan Creek, 9 Jun 1987, BJS, PEK, 22 adults; *Wakulla Co.,* St. Marks, St. Marks National Wildlife Refuge, 9 Jun 1987, BJS, PEK, 57 adults; Wakulla, Wakulla Springs, 28 Sep 1989, MQB, PEK, 38 adults; *Walton Co.,* Bruce, Choctawhatchee River, 14 Jun, 28 Sep 1988, 10,31 Jul 1991, 26 May 1992, 11 Nov 1994, 31 May 1995, BKB, JFR, MQB, PEK, SEM, SGS, TJ, 43 adults; Red Bay, Morrison Springs, 28 Sep 1988, PEK, SEM, 2 adults; *Washington Co.,* New Hope, Holmes Creek, 28 Sep 1988, PEK, SEM, SGS, 1 adult. GEORGIA, *Brooks Co.,* Clyattville, Withlacoochee River, 31 Aug 1989, PEK, 16 adults; *Bryan Co.,* Richmond Hill, Ogeechee River, 10 Oct 1988, PEK, 31 adults; *Bullock Co.,* Dover, 18 Sep 1995, PEK, 22 adults; Stilson, Ogeechee River, 11 Oct 1988, PEK, 15 adults; *Chatham Co.,* Bloomingdale, Ogeechee River, 14 Jul, 10 Oct 1988, 9 Jun 1992, PEK, SEM, 36 adults; *Effingham Co.,* Eden, Ogeechee River, 10 Oct 1988, PEK, 9 adults; Guyton, Ogeechee River, 14 Jul, 11 Oct 1988, 3 Aug 1989, 9 Jun 1992, 18 Sep 1995, PEK, SEM, SGS, TJ, 100 adults; *Glynn Co.,* Darien, Altamaha River, 27 Sep 1990, PEK, 23 adults; *Hart Co.,* Hartwell, Hartwell Lake, 28 Jun 1988, PEK, 16 adults; *Lanier Co.,* Lakeland, Banks Lake, 14 Sep 1989, PEK, 10 adults; *Long Co.,* Ludowici, Altamaha River, 10 Jun 1992, PEK, TJ, 23 adults; *McDuffie Co.,* Thompson, Clark Hill Reservoir, 27 Jun 1988, PEK, 11 adults; *Screven Co.,* Dover, Ogeechee River, 11 Oct 1988, 9 Jun 1992, 18 Sep 1995, PEK, SEM, SGS, TJ, 48 adults; Oliver, Ogeechee River, 11 Oct 1988, 9 Jun 1992, PEK, SEM, TJ, 56 adults; *Seminole Co.,* Reynoldsville, Lake Seminole, 27 Jun, 10 Sep, 12 Nov 1985, BKB, MQB, PEK, 103 adults. KENTUCKY, *Calloway Co.,* New Concord, Blood River, 15 Aug 1988, BKB, PEK, 50 adults; *Marshall Co.,* Fairdealing, Kentucky Lake, 15 Aug 1988, BKB, PEK, 114 adults; *Trigg Co.,* Land Between the Lakes, Lake Barkley, Energy Lake, 16 Aug 1988, BKB, PEK, 44 adults; Fords Creek, 16 Aug 1988, BKB, PEK, 44 adults; Honker Lake, 16 Aug 1988, BKB, PEK, 40 adults. LOUISIANA, *Allen Parish,* Oakdale, Calcasieu River, 17 Jun 1994, CRR, 21 adults; *Assumption Parish,* Napoleonville, Lake Verret, 18 Jul 1990, JKN, PEK, 13 adults; *Calcasieu Parish,* Lake Charles, 8 Jul, 18 Aug 1993, CRR, 60 adults; *Cameron Parish,* Grand Chenier, Superior Canal, 19 Jul 1990, JKN, PEK, 28 adults; Johnsons Bayou, 10 Aug 1993, CRR, 56 adults; Lake Arthur, 8 Jul 1993, CRR, 4 adults; Sweet Lake, 8 Jul 1993, CRR, 57 adults; *Concordia Parish,* Lettsworth, Three Rivers Wildlife Management Area, 16 Aug 1993, 15 Jun 1994, CRR, 30 adults; *East Baton Rouge Parish,* Port Hudson, 17 Aug 1993, CRR, 11 adults; Alsen, 17 Aug 1993, CRR, 10 adults; *Iberia Parish,* Delcambre, 19 Aug 1993, 27 Jul 1994, CRR, 101 adults; Loreauville, Dauterive Lake, 7 Nov 1994, PEK, 27 adults; *Iberville Parish,* Baton Rouge, 13 May 1994, JT, VW, 20 adults; Ramah, Atchafalaya River, 9 Jun 1994, CRR, 26 adults; St. Gabriel, 17 Aug 1993, CRR, 14 adults; White Castle, 10 Jun 1994, CRR, 20 adults; *Jefferson Davis Parish,* Jennings, 17 Jun 1986, JSB, 116 adults; Mermentau, Lake Mermentau, 16 Jun, 13 Jul, 11 Aug, 7 Sep, 20 Oct 1993, 26 May, 9 Jun, 6,27 Jul, 12 Sep 1994, CRR, 167 adults; Pine Island, 23 Jun, 29 Jul, 18 Aug, 20 Oct 1993, 26 May, 9 Jun, 6 Jul 1994, CRR, 166 adults; *Lafourche Parish,* Thibodaux, 11 Aug 1994, CRR, 7 adults; *Natchitoches Parish,* Natchitoches, 15 Aug 1994, CRR, 7 adults; Robeline, 15 Aug 1994, CRR, 6 adults; *Ouachita Parish,* Fondale, Ouachita Wildlife Management Area, Ouachita River, 16 Jul, 29 Aug, 2 Sep 1993, 21 Jun, 19 Jul 1994, CRR, 131 adults; Richwood, Ouachita Wildlife Management Area, Bayou Lafourche, 20 Aug, 28 Sep 1993, CRR, 83 adults; Swartz, Russell Sage Wildlife Management Area, Bayou Lafourche, 18 Jun, 20 Aug, 14 Sep 1993, 21 Jun, 19 Jul 1994, CRR, 128 adults; *Pointe Coupee Parish,* Batchelor, 19 Aug 1994, CRR, 5 adults; New Roads, 19 Aug 1994, CRR, 5 adults; *Rapides Parish,* Alexandria, 4 Aug 1993, CRR, 8 adults; *Red River Parish,* Coushatta, 15 Aug 1994, CRR, 6 adults; *St. Bernard Parish,* Violet, 18 Aug 1994, CRR, 23 adults; *St. Helena Parish,* Montpelier, 16 Aug 1994, CRR, 3 adults; *St. James Parish,* Union, 17 Aug 1994, CRR, 39 adults; *St. Landry Parish,* Eunice, 20 Jul 1993, CRR, 24 adults; Melville, Atchafalaya River, 17 Jun 1994, CRR, 5 adults; *St. Tammany Parish,* Mandeville, 4 Nov 1994, KS, 9 adults; Slidell, West Pearl River, 1 Nov 1994, 28 Jun 1995, KS, PEK, 8 adults; *Tangipahoa Parish,* Ponchatoula, Joyce Wildlife Management Area, 13 Jul 1994, CRR, 5 adults; Tangipahoa River, 3 Jun 1994, CRR, 3 adults; *Terrebonne Parish,* Chacahoula, 3 Oct 1993, EO, 3 adults; *Union Parish,* Sterlington, Ouachita River, 9 Aug 1994, CRR, CLM, 9 adults;

Vermilion Parish, Ester, Palmetto Island, 16 Jun, 7 Jul, 4 Aug 1993, 5 May, 12 May, 26 May, 9 Jun, 5,27 Jul, 12 Sep, 7 Nov 1994, 26 Jun, 26 Jul 1995, BBB, CRR, PEK, 128 adults; Gueydan, Bayou Queue de Tortue, 21 Apr 1994, 26 Jun 1995, CRR, PEK, 48 adults; Kaplan, 12 Jun 1988, BBB, 46 adults; Lake Arthur, 4 Aug 1993, 9 Jun, 27 Jul 1994, CRR, 101 adults; Wright, 4 Aug 1993, 21 Apr 1994, CRR, 33 adults. MASSACHUSETTS, *Worcester Co.,* Leicester, 30 Aug 1992, PEK, 12 adults; Upton, Upton State Forest, 30 Aug 1992, PEK, 28 adults. MICHIGAN, *Ingham Co.,* East Lansing, 6 Sep 1987, EDW, 52 adults. MINNESOTA, *Goodhue Co.,* Lake City, 19 Aug 1995, JFR, PEK, 4 adults; *Wabasha Co.,* Wabasha, 7 Oct 1994, PEK, SGS, 1 adult. MISSISSIPPI, *Bolivar Co.,* Skene, 5 Aug 1986, GCL, MQB, PEK, 46 adults; *Hancock Co.,* Bay St. Louis, Pearl River, 19 Jul 1990, JKN, PEK, 39 adults; *Itawamba Co.,* Fairview, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 25 adults; Fulton, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 47 adults; New Salem, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 44 adults; *Lowndes Co.,* Columbus, Aliceville Lake, 31 Aug 1988, PEK, SEM, 54 adults; Columbus Lake, 31 Aug 1988, PEK, SEM, 29 adults; *Monroe Co.,* Aberdeen, Aberdeen Lake, 31 Aug 1988, PEK, SEM, 59 adults; Amory, Tennessee Tombigbee, 30 Aug 1988, PEK, SEM, 43 adults; *Noxubee Co.,* Brooksville, Noxubee National Wildlife Refuge, 7 Aug, 28 Sep 1986, BKB, GCL, PEK, 64 adults; *Pearl River Co.,* Crossroads, Pearl River, 19 Jul 1990, JKN, PEK, 25 adults; *Tishomingo Co.,* Dennis, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 71 adults; High Point, Chambers Creek, 27 Sep, 10 Oct 1995, SPR, 6 adults; North Crossroads, Yellow Creek, 1 Sep 1987, 26 Aug 1988, BRM, KJT, PEK, SEM, 38 adults. NEW JERSEY, *Salem Co.,* Fort Mott, 12 Sep 1987, SKN, 46 adults. NEW YORK, *Westchester Co.,* Katonah, Croton Reservoir, 28 Jul 1987, 2 Sep 1992, PEK, 201 adults. NORTH CAROLINA, *McDowell Co.,* Marion, Lake James, 8 Jul 1988, PEK, 27 adults; *Wake Co.,* Wake Forest, Falls Lake Reservoir, 21 Sep 1987, CSA, 62 adults. SOUTH CAROLINA, *Anderson Co.,* Townville, Hartwell Lake, 28 Jun 1988, PEK, 24 adults; *Calhoun Co.,* Lone Star, Lake Marion, 13 Jul 1988, MQB, PEK, 100 adults; *Clarendon Co.,* Rimini, Lake Marion, 13 Jul 1988, MQB, PEK, 57 adults; *Jasper Co.,* Pritchardville, Savannah National Wildlife Refuge, 14 Aug 1988, PEK, 38 adults; *McCormick Co.,* Clarks Hill, Savannah River, 27 Jun 1988, PEK, 35 adults; *Sumter Co.,* Pinewood, Lake Marion, 13 Jul 1988, MQB, PEK, 50 adults. TENNESSEE, *Anderson Co.,* Oak Ridge, Melton Hill Lake, 23 Aug 1988, PEK, SEM, 48 adults; *Benton Co.,* Camden, Kentucky Lake, 23 Jul 1986, BRM, 5 adults; Eagle Creek, Tennessee River, 12 Aug 1988, BKB, PEK, 32 adults; *Decatur Co.,* Bath Springs, Tennessee River, 13 Aug 1988, BKB, PEK, 48 adults; Perryville, Tennessee River, 13 Aug 1988, BKB, PEK, 48 adults; *Henry Co.,* Oak Grove, Kentucky Lake, 12 Aug 1988, BKB, PEK, 51 adults; Springville, Big Sandy River, 12 Aug 1988, BKB, PEK, 71 adults; *Houston Co.,* McKinnon, Kentucky Lake, 12 Aug 1988, BKB, PEK, 49 adults; *Humphreys Co.,* Plant, Duck River, 12 Aug 1988, BKB, PEK, 50 adults; Trinity, Kentucky Lake, 12 Aug 1988, BKB, PEK, 48 adults; *Marion Co.,* Mineral Springs, Nickajack Lake, 25 Aug 1988, PEK, SEM, 50 adults, New Hope; Nickajack Lake, 25 Aug 1988, PEK, SEM, 49 adults; *Meigs Co.,* Birchwood, Chickamauga Lake, 9 Sep 1987, BRM, 116 adults; *Rhea Co.,* Spring City, Piney River, 24 Aug 1988, PEK, SEM, 99 adults; *Roane Co.,* Glen Alice, Watts Bar Lake, 24 Aug 1988, PEK, SEM, 26 adults; *Stewart Co.,* Land Between the Lakes, Cumberland River, Neville Creek, 16 Aug 1988, BKB, PEK, 47 adults; Kentucky Lake, Rushing Creek, 15 Aug 1988, BKB, PEK, 52 adults. TEXAS, *Brazoria Co.,* Freeport, Brazos River, 11 Aug 1990, JKN, PEK, 28 adults; *Chambers Co.,* Mont Belvieu, Trinity River, 10 Jun, 8 Jul 1986, JKO, 178 adults; *Hidalgo Co.,* Progreso, Santa Anna National Wildlife Refuge, 7 Aug 1990, JKN, PEK, 28 adults; *McLennan Co.,* Waco, Tehuacana Creek, 2 Aug 1990, JKN, PEK, 28 adults; *Refugio Co.,* Tivoli, Hynes Bay, 9 Aug 1990, JKN, PEK, 28 adults; *Sabine Co.,* Fairmount, Toledo Bend Reservoir, 21 Jul 1990, JKN, PEK, 11 adults; *Tom Green Co.,* San Angelo, Concho River, 4 Aug 1990, JKN, PEK, 28 adults; *Victoria Co.,* McFaddin, San Antonio River, 9 Aug 1990, JKN, PEK, 26 adults. WISCONSIN, *La Crosse Co.,* La Crosse, Goose Island Wildlife Preserve, 19 Aug 1987, BJS, PEK, 17 adults.

Records in literature: ALABAMA, *Montgomery Co.* (Kaiser et al. 1988b), *Marshall Co.* (Lanzaro et al. 1988), *Houston Co., Jackson Co., Limestone Co., Montgomery Co.* (Kaiser 1988), *Houston Co., Montgomery Co.* (Kaiser et al. 1988a), *Marshall Co., Montgomery Co.* (Lanzaro et al. 1990), *Houston Co., Jackson Co., Jefferson Davis Co., Limestone Co., Madison Co., Marshall Co., Montgomery Co.* (Seawright et al. 1991), *Colbert Co., Greene Co., Houston Co., Jackson Co., Lauderdale Co., Lawrence Co., Limestone Co., Madison Co., Marshall Co., Montgomery Co., Morgan Co., Pickens Co., Sumter Co.* (Seawright et al. 1992), *Limestone Co.* (Mitchell et al. 1992), *Colbert Co., Lauderdale Co., Morgan Co.* (Perera et al. 1995). ARKANSAS, *Arkansas Co.* (Kim et al. 1987a, 1987b; Kaiser 1988; Kaiser et al. 1988a; Seawright et al. 1991, 1992; Perera et al. 1995), *White Co.* (Lanzaro et al. 1988), *Arkansas Co., White Co.* (Lanzaro et al. 1990). CONNECTICUT, *Linchfield Co.* (Perera et al. 1995). FLORIDA, *Alachua Co.* (Kaiser and Seawright 1987, Kaiser et al. 1988b), *Gilchrist Co., Sumter Co.* (Lanzaro et al. 1988), *Levy Co., Sumter Co.* (Narang and Seawright 1988), *Alachua Co., Calhoun Co., Hamilton Co., Levy Co., Sarasota Co.* (Kaiser 1988), *Alachua Co.,*

Putnam Co., Sarasota Co, Sumter Co. (Kaiser et al. 1988a), *Alachua Co., Levy Co.* (Narang et al. 1989b), *Hamilton Co.* (Narang et al. 1989a), *Sumter Co.* (Cockburn 1990, Jensen et al. 1993), *Alachua Co., Gadsden Co., Gilchrist Co., Sumter Co.* (Lanzaro et al. 1990), *Gulf Co., Hamilton Co., Marion Co., Sarasota Co., Sumter Co.* (Seawright et al. 1991), *Citrus Co., Hamilton Co., Sumter Co.* (Nayar et al. 1992), *Alachua Co., Calhoun Co., Citrus Co., Collier Co., Dixie Co., Escambia Co., Flagler Co., Gilchrist Co., Glades Co., Gulf Co., Hamilton Co., Hernando Co., Holmes Co., Indian River Co., Jackson Co., Jefferson Co., LaFayette Co., Leon Co., Levy Co., Liberty Co., Marion Co., Okalsosa Co., Okeechobee Co., Orange Co., Palm Beach Co., Santa Rosa Co., Sarasota Co., Sumter Co., Suwannee Co., Taylor Co., Wakulla Co., Walton Co., Washington Co.* (Seawright et al. 1992), *Calhoun Co., Dixie Co., Hamilton Co.* (Mitchell et al. 1992), *Gilchrist Co., Sumter Co., Walton Co.* (Perera et al. 1995), *Levy Co.* (Jensen et al. 1996). GEORGIA, *Seminole Co.* (Kaiser and Seawright 1987, Kaiser 1988, Seawright et al. 1991), *Decatur Co.* (Kaiser et al. 1988a), *Brooks Co., Bryan Co., Bullock Co., Camden Co., Chatham Co., Effingham Co., Hart Co., Lanier Co., McDuffie Co., Screven Co., Seminole Co.* (Seawright et al. 1992), *Effingham Co.* (Linley et al. 1993). KENTUCKY, *Calloway Co., Marshall Co., Trigg Co.* (Seawright et al. 1992), *Marshall Co.* (Perera et al. 1995). LOUISIANA (Rutledge and Meek 1994), *Calcasieu Parish* (Kaiser 1988, Lanzaro et al. 1990), *Jefferson Davis Parish* (Seawright et al. 1991), *Jefferson Davis Parish, Vermilion Parish* (Seawright et al. 1992), *Assumption Parish, Cameron Parish, Jefferson Davis Parish* (Perera et al. 1995), *Jefferson Davis Parish, St. Landry Parish* (Rutledge et al. 1996). MASSACHUSETTS, *Worcester Co.* (Perera et al. 1995). MICHIGAN, *Ingham Co.* (Seawright et al. 1992). MINNESOTA, Northwest Territory (Say 1824), *Wabasha Co.* (Belkin et al. 1966, Seawright et al. 1992). MISSISSIPPI, *Bolivar Co.* (Lanzaro et al. 1988, 1990), *Noxubee Co.* (Narang and Seawright 1988, Kaiser 1988, Narang et al. 1989b, Apperson and Lanzaro 1991), *Bolivar Co., Noxubee Co.* (Seawright et al. 1991), *Bolivar Co., Itawamba Co., Lowndes Co., Monroe Co., Noxubee Co., Tishomingo Co.* (Seawright et al. 1992), *Noxubee Co., Tishomingo Co.* (Mitchell et al. 1992), *Noxubee Co.*; widespread throughout state (Mallet and Fritzius 1993), *Bolivar Co., Hancock Co.* (Perera et al. 1995). NEW JERSEY, *Salem Co.* (Seawright et al. 1992). NEW YORK (Cockburn 1990), *Westchester Co.* (Kaiser 1988; Seawright et al. 1991, 1992; Perera et al. 1995). NORTH CAROLINA, *Wake Co.* (Seawright et al. 1991, Perera et al. 1995), *McDowell Co., Wake Co.* (Seawright et al. 1992). SOUTH CAROLINA, *Anderson Co., Calhoun Co., Clarendon Co., Jasper Co., McCormick Co., Sumter Co.* (Seawright et al. 1992), *Calhoun Co., Jasper Co.* (Perera et al. 1995). TENNESSEE, *Benton Co., Meigs Co.* (Kaiser 1988, Seawright et al. 1991), *Meigs Co.* (Narang et al. 1989a, Perera et al. 1995), *Anderson Co., Benton Co., Decatur Co., Henry Co., Houston Co., Humphreys Co., Marion Co., Meigs Co., Rhea Co., Roane Co., Stewart Co.* (Seawright et al. 1992). TEXAS, *Brazos Co.* (Kaiser 1988, Cockburn 1990, Seawright et al. 1991), *Chambers Co.* (Seawright et al. 1992), *Brazoria Co., Hidalgo Co., McLennan Co., Refugio Co., Sabine Co., Tom Green Co.* (Perera et al. 1995). WISCONSIN (Van der Wulp 1867, Belkin 1968), *La Crosse Co.* (Seawright et al. 1992, Perera et al. 1995).

Anopheles smaragdinus

Specimens examined in morphological studies: 32 IPBs, 542 ♀pl, 49 ♀, 490 ♂pl, 46 ♂, 36 ♂g, 4 pl, 51 Pl, 3 P, 35 p, 1 l, and 641 L. FLORIDA, *Alachua Co.*, Gainesville, Paynes Prairie State Preserve, 24 Aug 1995, JFR, PEK, FL95.41 (IPB*), 47 ♀pl, 1 ♀, 54 ♂pl, 1 ♂g, 1 l, 52 L; *Hamilton Co.*, Jennings, Lake Octahatchee, 20 Oct 1987, PEK, TJZ-26 (IPB*), 7 ♀pl, 19 ♀, 7 ♂pl, 11 ♂, 10 L; TJZ-27 (IPB*), 19 ♀pl, 16 ♀, 3 ♂pl, 18 ♂, 1 ♂g, 10 L; 18 Oct 1994, JFR, PEK, FL94.23, 1 ♀pl, 4 ♀, 2 ♂pl, 17 ♂; FL94.28 (IPB*), 16 ♀pl, 1 ♀, 13 ♂pl, 1 ♂g, 5 L; FL94.29 (IPB*), 17 ♀pl, 1 ♀, 26 ♂pl, 1 ♂g, 3 L; FL94.33 (IPB*), 14 ♀pl, 1 ♀, 29 ♂pl, 1 ♂g, 2 pl, 12 L; FL94.34 (IPB*), 1 ♀pl, 1 ♀, 17 ♂pl, 2 ♂g, 4 L; FL94.35 (IPB*), 8 ♀pl, 1 ♀, 12 ♂pl, 1 ♂g, 1 pl, 2 L; FL94.36 (IPB*), 9 ♀pl, 1 ♀, 8 ♂pl, 15 L; FL94.37 (IPB*), 2 ♀pl, 1 ♀, 13 ♂pl, 15 L; FL94.39 (IPB*), 4 ♀pl, 1 ♀, 21 ♂pl, 1 ♂g, 1 Pl, 23 L; FL94.45 (IPB*), 3 ♀pl, 1 ♀, 6 ♂pl, 3 Pl, 15 L; *Levy Co.*, Chiefland, Manatee Springs State Park, 31 Aug 1995, JFR, PEK, FL95.57 (IPB*), 33 ♀pl, 25 ♂pl, 7 ♂g, 2 Pl, 23 p, 41 L; FL95.58 (IPB*), 21 ♀pl, 20 ♂pl, 2 ♂g, 12 p, 24 L; FL95.65 (IPB*), 33 ♀pl, 6 ♂pl, 3 Pl, 1 P, 70 L; FL95.67 (IPB*), 37 ♀pl, 4 ♂pl, 1 ♂g, 1 Pl, 16 L; FL95.68 (IPB*), 20 ♀pl, 25 ♂pl, 2 ♂g, 1 Pl, 17 L; *Sumter Co.*, Coleman, Coleman's Landing on Lake Panasoffkee, 21 Aug 1996, HMS, SEW, FL96.24 (IPB), 17 ♀pl, 3 ♂pl; *Walton Co.*, Bruce, Choctawhatchee River at FL-20 bridge, 11 Nov 1994, PEK, FL94.73 (IPB), 1 ♀pl, 7 ♂pl, 2 Pl. GEORGIA, *Bullock Co.*, Dover, Ogeechee River at US-301 bridge, 18 Sep 1995, PEK, GA95.32 (IPB*), 39 ♀pl, 29 ♂pl, 1 ♂g, 1 Pl, 17 L; GA95.39 (IPB*), 32 ♀pl, 15 ♂pl, 2 ♂g, 4 Pl, 22 L; GA95.41 (IPB*), 28 ♀pl, 26 ♂pl, 2 ♂g, 1 Pl, 29 L; GA95.45 (IPB*), 24 ♀pl, 8 ♂pl, 2 ♂g, 1 Pl, 18 L; GA95.46 (IPB*), 35 ♀pl, 22 ♂pl, 1 ♂g, 1 pl, 19 L. LOUISIANA, *St. Tammany Parish,* Slidell, West Pearl River at I-10 bridge, 28 Jun 1995, PEK, LA95.32 (IPB), 3 ♂p, 1 ♂g, 37 L; LA95.33 (IPB), 6 ♀pl, 17 ♂pl, 1 ♂g, 2 Pl, 42 L; LA95.34 (IPB), 21 ♀pl, 4 ♂pl, 1 ♂g, 3 Pl, 25 L; LA95.35 (IPB), 12 ♀pl, 7 ♂pl, 1 ♂g, 4 Pl, 13 L; LA95.36 (IPB), 2 ♀pl, 2 ♂pl, 1 ♂g, 6 Pl, 6 L; LA95.37 (IPB), 3 ♀pl, 1 ♂pl, 5 Pl, 2 P, 20 L; *Vermilion Parish,* Palmetto Island, 7 Nov 1994, BBB, PEK, LA94.16 (IPB*), 1

♀pl, 5 ♂pl. MISSISSIPPI, *Tishomingo Co.*, High Point, Chambers Creek at Highway 350 bridge, 27 Sep 1995, SPR, MS95.1 (IPB*), 29 ♀pl, 50 ♂pl, 2 ♂g, 59 L.

Specimens examined in biochemical, molecular, and genetic studies: ALABAMA, *Greene Co.*, Forkland, Lake Demopolis, 1 Sep 1988, PEK, SEM, 21 adults; *Houston Co.*, Pansey, Chattahoochee State Park, 27 Jun, 10 Sep, 12 Nov 1985, BKB, PEK, 12 adults; *Lauderdale Co.*, Florence, Pickwick Lake, 26 Aug 1988, BRM, PEK, SEM, 3 adults; *Limestone Co.*, Mooresville, Wheeler Lake, 8 Sep 1986, 30 Aug 1987, 9 Aug 1988, BRM, KJT, MQB, PEK, SEM, 258 adults; *Madison Co.*, Triana, Wheeler Lake, 30 Aug 1987, 9 Aug 1988, BRM, KJT, MQB, PEK, SEM, 64 adults; *Montgomery Co.*, Waugh, 23 Jul, 27 Aug, 17 Oct 1984, MQB, PEK, SEM, 203 adults; *Morgan Co.*, Decatur, Wheeler Lake, 31 Aug 1987, 10 Aug 1988, BRM, KJT, MQB, PEK, SEM, 140 adults; *Sumter Co.*, Gainesville, Tennessee Tombigbee, 1 Sep 1988, PEK, SEM, 4 adults. ARKANSAS, *Arkansas Co.*, Stuttgart, 19 Jul 1995, GCL, MQB, PEK, 4 adults. FLORIDA, *Alachua Co.*, Cross Creek, 23 Jul 1986, PEK, 6 adults; Gainesville, Kanapaha Botanical Gardens, 22 May, 5 Jun, 15 Aug, 10 Oct, 2 Dec 1985, 19 Mar, 22 May 1986, GCL, MQB, PEK, SEM, 53 adults; Paynes Prairie, 24 Aug 1995, JFR, PEK, 1 adult; High Springs, Santa Fe River, 26 May 1988, 3 Jan 1992, PEK, 3 adults; *Calhoun Co.*, Blountstown, Apalachicola River, 26 Sep 1989, PEK, 2 adults; Chippola Park, Dead Lake, 28 Sep 1987, PEK, 72 adults; *Dixie Co.*, Horseshoe Beach, 2 Jun 1987, 26 Mar 1990, BJS, PEK, 24 adults; Jena, 2 Jun 1987, BJS, PEK, 5 adults; Old Town, Guaranto Springs, 8 Jun 1988, 10 May 1991, PEK, 29 adults; *Escambia Co.*, McDavid, Escambia River, 19 Sep 1989, PEK, SEM, 6 adults; *Flagler Co.*, Palm Coast, 6 Jul 1987, BJS, PEK, 38 adults; *Gilchrist Co.*, Bell, Ginnie Springs, 17 May 1988, GCL, PEK, SEM, 2 adults; *Gulf Co.*, Wewahitchka, Dead Lake, 29 May 1987, 14 Jun 1988, PEK, 27 adults; Willis Landing, Apalachicola River, 1 Jun 1990, PEK, 1 adult; *Hamilton Co.*, Jennings, Lake Octahatchee, 12 Jun, 14 Jul, 18 Aug, 16 Sep, 27 Oct, 17 Nov 1986, 19 May, 15 Jun, 13 Jul, 4 Aug, 8 Sep, 20 Oct, 16 Nov 1987, 22 May 1990, 27 Jul 1991, 18 Oct 1994, BJS, BKB, JFR, MQB, PEK, SEM, 1,323 adults; White Springs, 12 Jun 1986, PEK, 11 adults; *Holmes Co.*, Westville, Choctawhatchee River, 28 Sep 1988, PEK, 16 adults; *Jackson Co.*, Chattahoochee, Apalachicola River, 26 Sep 1989, MQB, PEK, 7 adults; *Jefferson Co.*, Wacissa, Wacissa Springs, 28 Sep 1989, MQB, PEK, 1 adult; *Lafayette Co.*, Branford, Sawannee River, 8,21 Jun 1988, PEK, 11 adults; *Leon Co.*, Tallahassee, Lake Carr, 28 Sep 1989, MQB, PEK, 11 adults; Lake Talquin, 27 Sep 1989, MQB, PEK, 1 adult; Bradfordville, Lake Iamonia, 28 Sep 1989, MQB, PEK, 2 adults; *Levy Co.*, Chiefland, Manatee Springs State Park, 8 Jun 1988, 10 May 1989, 24 May, 16 Sep, 3 Oct, 29 Dec 1991, 26 Jun, 22 Jul, 18 Aug, 24 Sep, 13 Oct, 23 Nov 1992, 10 May 1993, 31 Aug 1995, BJS, JFR, PEK, SEM, SGS, TJ, 166 adults; Fowler Bluff, Lower Sawannee National Wildlife Refuge, 25 Jun 1992, 13 Jul 1993, BJS, PEK, TJ, 2 adults; *Liberty Co.*, Hosford, Ochlockonee River, 27 Sep 1989, MQB, PEK, 34 adults; *Marion Co.*, Eureka, Ocklawaha River, 22 May 1985, MQB, PEK, TJ, 2 adults; *Sumter Co.*, Rutland, Withlacoochee River, 12 Feb 1990, BJS, PEK, 1 adult; Tarrytown, Withlacoochee State Forest, 11 Jun 1991, TJ, 3 adults; *Suwannee Co.*, Falmouth, Suwannee River State Park, 21 Jun 1988, PEK, 5 adults; Hildreth, Ichetucknee Springs State Park, 3 Jun 1988, BJS, PEK, 6 adults; *Taylor Co.*, Smith-McCullan Creek, 9 Jun 1987, BJS, PEK, 1 adult; *Wakulla Co.*, St. Marks, St. Marks National Wildlife Refuge, 9 Jun 1987, BJS, PEK, 4 adults; *Walton Co.*, Bruce, Choctawhatchee River, 14 Jun 1988, PEK, 9 adults; Red Bay, Morrison Springs, 28 Sep 1988, PEK, SEM, 1 adult; *Washington Co.*, New Hope, Holmes Creek, 28 Sep 1988, PEK, SEM, 1 adult. GEORGIA, *Brooks Co.*, Clyattville, Withlacoochee River, 31 Aug 1989, PEK, 8 adults; *Bullock Co.*, Dover, 18 Sep 1995, PEK, 6 adults; Stilson, Ogeechee River, 11 Oct 1988, PEK, 11 adults; *Camden Co.*, Jerusalem, Satilla River, 30 Aug 1989, PEK, 72 adults; *Chatham Co.*, Bloomingdale, Ogeechee River, 14 Jul, 10 Oct 1988, 9 Jun 1992, PEK, SEM, 119 adults; *Effingham Co.*, Eden, Ogeechee River, 10 Oct 1988, PEK, 64 adults; Guyton, Ogeechee River, 14 Jul, 11 Oct 1988, 3 Aug 1989, 9 Jun 1992, PEK, SEM, SGS, TJ, 20 adults; *Glynn Co.*, Darien, Altamaha River, 27 Sep 1990, PEK, 2 adults; *Hart Co.*, Hartwell, Hartwell Lake, 28 Jun 1988, PEK, 55 adults; *Lanier Co.*, Lakeland, Banks Lake, 14 Sep 1989, PEK, 14 adults; *Long Co.*, Ludowici, Altamaha River, 10 Jun 1992, PEK, TJ, 13 adults; *Screven Co.*, Dover, Ogeechee River, 11 Oct 1988, 9 Jun 1992, 18 Sep 1995, PEK, SEM, SGS, TJ, 19 adults; Oliver, Ogeechee River, 11 Oct 1988, 9 Jun 1992, PEK, SEM, TJ, 4 adults; *Seminole Co.*, Reynoldsville, Lake Seminole, 27 Jun, 10 Sep, 12 Nov 1985, BKB, MQB, PEK, 4 adults. KENTUCKY, *Trigg Co.*, Land Between the Lakes, Lake Barkley, Energy Lake, 16 Aug 1988, BKB, PEK, 5 adults; Honker Lake, 16 Aug 1988, BKB, PEK, 6 adults. LOUISIANA, *Assumption Parish,* Napoleonville, Lake Verret, 18 Jul 1990, JKN, PEK, 15 adults; *Calcasieu Parish,* Lake Charles, 18 Aug 1993, CRR, 5 adults; *Cameron Parish,* Johnsons Bayou, 10 Aug 1993, CRR, 1 adult; Lake Arthur, 8 Jul 1993, CRR, 1 adult; Sweet Lake, 8 Jul 1993, CRR, 1 adult; *Concordia Parish,* Lettsworth, Three Rivers Wildlife Management Area, 15 Jun 1994, CRR, 2 adults; *Iberia Parish,* Delcambre, 19 Aug 1993, CRR, 2 adults; *Iberville Parish,* Baton Rouge, 13 May 1994, JT, VW, 11 adults; White Castle, 10 Jun 1994, CRR, 2 adults; *Jefferson Davis Parish,* Mermentau, Lake Mermentau, 30 Jun, 7 Jul, 6 Oct

1993, 26 May 1994, CRR, 26 adults; Pine Island, 26 Aug 1993, CRR, 7 adults; *Natchitoches Parish,* Robeline, 15 Aug 1994, CRR, 1 adult; *Ouachita Parish,* Fondale, Ouachita Wildlife Management Area, Ouachita River, 1 Jul 1993, CRR, 3 adults; Richwood, Ouachita Wildlife Management Area, Bayou Lafourche, 28 Sep 1993, CRR, 15 adults; Swartz, Russell Sage Wildlife Management Area, Bayou Lafourche, 19 Jul 1994, CRR, 1 adult; *St. Bernard Parish,* Violet, 18 Aug 1994, CRR, 1 adult; *St. James Parish,* Union, 17 Aug 1994, CRR, 2 adults; *St. Tammany Parish,* Mandeville, 4 Nov 1994, KS, 33 adults; Slidell, West Pearl River, 1 Nov 1994, 28 Jun 1995, KS, PEK, 23 adults; *Tangipahoa Parish,* Ponchatoula, Joyce Wildlife Management Area, 13 Jul 1994, CRR, 16 adults; Tangipahoa River, 3 Jun 1994, CRR, 2 adults; *Terrebonne Parish,* Chacahoula, 3 Oct 1993, EO, 19 adults; *Vermilion Parish,* Ester, Palmetto Island, 16 Jun, 7 Jul 1993, 12 May, 12 Sep, 7 Nov 1994, 26 Jul 1995, BBB, CRR, PEK, 11 adults; Kaplan, 12 Jun 1988, BBB, 1 adult; Lake Aurthur, Lake Aurthur, 4 Aug 1993, 9 Jun 1994, CRR, 2 adults. MISSISSIPPI, *Hancock Co.,* Bay St. Louis, Pearl River, 19 Jul 1990, JKN, PEK, 13 adults; *Itawamba Co.,* Fairview, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 27 adults; Fulton, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 1 adult; New Salem, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 19 adults; *Lowndes Co.,* Columbus, Aliceville Lake, 31 Aug 1988, PEK, SEM, 2 adults; Columbus Lake, 31 Aug 1988, PEK, SEM, 41 adults; *Monroe Co.,* Amory, Tennessee Tombigbee, 30 Aug 1988, PEK, SEM, 16 adults; *Noxubee Co.,* Brooksville, Noxubee National Wildlife Refuge, 7 Aug, 28 Sep 1986, BKB, GCL, PEK, 134 adults; *Tishomingo Co.,* Dennis, Tennessee Tombigbee, 29 Aug 1988, PEK, SEM, 9 adults; High Point, Chambers Creek, 27 Sep 1995, SPR, 1 adult; North Crossroads, Yellow Creek, 1 Sep 1987, 26 Aug 1988, BRM, KJT, PEK, SEM, 8 adults. NORTH CAROLINA, *Wake Co.,* Wake Forest, Falls Lake Reservoir, 21 Sep 1987, CSA, 8 adults. SOUTH CAROLINA, *Anderson Co.,* Townville, Hartwell Lake, 28 Jun 1988, PEK, 13 adults; *Calhoun Co.,* Lone Star, Lake Marion, 13 Jul 1988, MQB, PEK, 36 adults; *Clarendon Co.,* Rimini, Lake Marion, 13 Jul 1988, MQB, PEK, 5 adults; *McCormick Co.,* Clarks Hill, Savannah River, 27 Jun 1988, PEK, 2 adults. TENNESSEE, *Benton Co.,* Camden, Kentucky Lake, 23 Jul 1986, BRM, 100 adults; Eagle Creek, Tennessee River, 12 Aug 1988, BKB, PEK, 17 adults; *Decatur Co.,* Bath Springs, Tennessee River, 13 Aug 1988, BKB, PEK, 1 adult; *Henry Co.,* Springville, Big Sandy River, 12 Aug 1988, BKB, PEK, 4 adults; *Houston Co.,* McKinnon, Kentucky Lake, 12 Aug 1988, BKB, PEK, 2 adults; *Humphreys Co.,* Trinity, Kentucky Lake, 12 Aug 1988, BKB, PEK, 2 adults; *Rhea Co.,* Spring City, Piney River, 24 Aug 1988, PEK, SEM, 5 adults; *Stewart Co.,* Land Between the Lakes, Cumberland River, Neville Creek, 16 Aug 1988, BKB, PEK, 3 adults. TEXAS, *Sabine Co.,* Fairmount, Toledo Bend Reservoir, 21 Jul 1990, JKN, PEK, 17 adults.

Records in literature: ALABAMA, *Montgomery Co.* (Kaiser et al. 1988a, 1988b; Lanzaro et al. 1988, 1990), *Houston Co., Limestone Co., Montgomery Co.* (Kaiser 1988), *Houston Co., Montgomery Co.* (Kaiser et al. 1988a), *Houston Co., Limestone Co., Madison Co., Montgomery Co.* (Seawright et al. 1991), *Colbert Co., Greene Co., Houston Co., Lauderdale Co., Limestone Co., Madison Co., Montgomery Co., Morgan Co., Pickens Co., Sumter Co.* (Seawright et al. 1992), *Limestone Co.* (Mitchell et al. 1992). ARKANSAS, *Arkansas Co.* (Lanzaro et al. 1988). FLORIDA, *Alachua Co.* (Kim et al. 1987a, Kaiser et al. 1988b, Lanzaro et al. 1988, Narang et al. 1989b), Hamilton Co. (Cockburn and Seawright 1988, Narang et al. 1989a, Cockburn 1990, Nayar et al. 1992, Cornel et al. 1996), *Alachua Co., Calhoun Co., Hamilton Co., Levy Co.* (Kaiser 1988), *Alachua Co., Putnam Co.* (Kaiser et al. 1988a), *Alachua Co., Gadsden Co.* (Lanzaro et al. 1990), *Gulf Co., Hamilton Co., Marion Co.* (Seawright et al. 1991), *Alachua Co., Calhoun Co., Citrus Co., Dixie Co., Escambia Co., Flagler Co., Gilchrist Co., Gulf Co., Hamilton Co., Holmes Co., Jackson Co., Jefferson Co., LaFayette Co., Leon Co., Levy Co., Liberty Co., Marion Co., Sumter Co., Suwannee Co., Taylor Co., Wakulla Co., Walton Co., Washington Co.* (Seawright et al. 1992), *Calhoun Co., Dixie Co., Hamilton Co.* (Mitchell et al. 1992), *Levy Co.* (Linley et al. 1993, Jensen et al. 1996). GEORGIA, *Seminole Co.* (Kaiser 1988, Seawright et al. 1991), *Decatur Co.* (Kaiser et al. 1988a), *Brooks Co., Bullock Co., Camden Co., Chatham Co., Effingham Co., Hart Co., Lanier Co., Screven Co., Seminole Co.* (Seawright et al. 1992), *Screven Co.* (Linley et al. 1993). KENTUCKY, *Marshall Co., Trigg Co.* (Seawright et al. 1992). LOUISIANA (Rutledge and Meek 1994), *Calasieu Parish* (Kim et al. 1987a), *Vermilion Parish* (Seawright et al. 1992), *St. Tammany Parish, Tangipahoa Parish* (Rutledge et al. 1996). MISSISSIPPI, *Noxubee Co.* (Narang and Seawright 1988, Kaiser 1988, Narang et al. 1989b, Seawright et al. 1991, Apperson and Lanzaro 1991), *Itawamba Co., Lowndes Co., Monroe Co., Noxubee Co., Tishomingo Co.* (Seawright et al. 1992), *Noxubee Co., Tishomingo Co.* (Mitchell et al. 1992), *Noxubee Co.*; restricted to relatively undisturbed forest habitats near major rivers to east and south of Mississippi Delta (Mallet and Fritzius 1993). NORTH CAROLINA, *Wake Co.* (Seawright et al. 1991, 1992). SOUTH CAROLINA, *Anderson Co., Calhoun Co., Clarendon Co., McCormick Co.* (Seawright et al. 1992). TENNESSEE, *Benton Co.* (Kaiser 1988, Seawright et al. 1991), *Benton Co., Decatur Co., Henry Co., Houston Co., Humphreys Co., Rhea Co., Stewart Co.* (Seawright et al. 1992).

HYBRIDIZATION STUDIES

Hybridization studies between suspected sibling species were an important part of identifying the validity of the species. Crosses of sibling species resulted in embryonic mortality, larval and pupal mortality, and atrophied or dysfunctional ovaries and testes, and the hybrid progeny of sibling species usually had asynaptic polytene chromosomes. Experimentation with hybrids of the 5 species of the Quadrimaculatus Complex produced results consistent with previous observations recorded for other anopheline species complexes (see review by Narang and Seawright 1991). Field-collected mosquitoes of the *An. quadrimaculatus* species complex would not mate in laboratory cages; therefore, crosses were done with a modified version of the induced-mating technique of Baker et al. (1962). Field-collected females were identified by isozyme analysis using the keys of Narang et al. (1989a, 1990a), and their F_1 progeny were used for the hybrid crosses. In many of the studies, our USDA laboratory-colonized strain (designated ORLANDO) of *An. quadrimaculatus s.s.* was used as a "tester" for crosses with field material. Fertile progeny were obtained from reciprocal crosses between ORLANDO and *An. quadrimaculatus* from Montgomery Co., AL, Marshall Co., AL, Arkansas Co., AR, Alachua Co., FL, Sumter Co., FL, Jefferson Davis Parish, LA, and Bolivar Co., MS (Kaiser et al. 1988b, Lanzaro et al. 1988). Fertile progeny were also obtained from crosses of ORLANDO with *An. quadrimaculatus s.s.* from Westchester Co., NY, and Meigs Co., TN (unpublished data).

Anopheles quadrimaculatus (ORLANDO) were crossed with *An. smaragdinus* from Montgomery Co., AL, Arkansas Co., AR, and Alachua Co., FL. Results varied with the direction of the cross (Kaiser et al. 1988b, Lanzaro et al. 1988). The fertility of the F_1 eggs was normal for both crosses. For crosses with *An. smaragdinus* females, the F_1 survival and sex ratio were normal, but the F_1 females had low fertility (10–15%), and F_1 males were completely sterile. For crosses with *An. quadrimaculatus* females, the results were more variable and ranged from progeny broods with survival greater than 65% and a normal sex ratio to progeny broods with survival of less than 40% and no F_1 adult males. Complete asynapsis of the X chromosomes and partial asynapsis of the autosomes were observed in the preparations of polytene chromosomes from F_1 females.

Anopheles diluvialis from Levy Co., FL, were crossed with *An. quadrimaculatus* from Arkansas Co., AR, and *An. smaragdinus* from Alachua Co., FL (Kaiser et al. 1988c). In the crosses with *An. quadrimaculatus,* only sterile males with atrophied testes survived. The matings with *An. smaragdinus* again produced varied results. For *An. smaragdinus* female × *An. diluvialis* male, some F_1 progeny broods consisted of only sterile females, some consisted of only sterile males, and some consisted of both males and females with normal-appearing ovaries and testes. For the reciprocal cross, *An. diluvialis* female × *An. smaragdinus* male, a few progeny broods had very low survival (less than 2%) and only sterile female adults; other progeny broods consisted of male and female adults with normal-appearing ovaries and testes. The F_1 progeny from the broods exhibiting normal-appearing testes and ovaries were backcrossed to parental types. These backcross progeny were abnormal with either 100% immature mortality or only sterile males.

Anopheles diluvialis from Dixie Co., FL, and *An. inundatus* from Walton Co., FL, were force-mated in reciprocal crosses, and the results were distorted F_1 sex ratios and sterile progeny. The polytene X chromosomes of the hybrids were either partly or completely asynaptic, and asynaptic regions were also observed on the autosomes (Seawright, Kaiser, and Mitchell, unpublished data).

Anopheles quadrimaculatus (ORLANDO) was crossed with *An. maverlius* from Tishomingo Co., MS. Eggs were obtained from the *An. maverlius* females, but the egg hatch was low (40.6 ± 21.7%), and all the F_1 hybrid progeny died in the 1st larval stage. *Anopheles maverlius* males failed to fertilize ORLANDO females (Narang et al. 1989a).

CYTOLOGY STUDIES

Cytotaxonomy has been used to distinguish morphologically similar species (Coluzzi and Sabatini 1967, 1968, 1969) and to demonstrate similarities between related species (Kitzmiller et al. 1967). The polytene chromosomes of *An. quadrimaculatus* from salivary glands of 4th-instar larvae were described by Klassen et al. (1965). Kaiser and Seawright (1987) provided a polytene chromosome map for the ovarian nurse cells of *An. quadrimaculatus.* The banding patterns in *An. smaragdinus* were almost identical to those in *An. quadrimaculatus.* There were 3 fixed inversions (one each on X, 2L, and 3R) and 2 large floating inversions (on 2L and 3R) in *An. smaragdinus* (Kaiser et al. 1988a). Three floating inversions were observed on 3R of *An. quadrimaculatus.* Chromosome 3L was unusual in both species, because there were 2 distinct banding patterns, $3L_1$ and $3L_2$ (Seawright et al. 1991). From the work of Kitzmiller et al. (1967), the banding patterns for 3L of the other members of the Nearctic Maculipennis Complex were more similar to $3L_2$. The ovarian polytene chromosomes of *An. diluvialis, An. inundatus,* and *An. maverlius* were of poor quality making it difficult to make comparisons with *An. quadrimaculatus* and *An. smaragdinus.*

A technique developed by Conn (1990) for salivary gland polytene chromosomes of South American anophelines was used at our laboratory to compare the chromosomes of the 5 sibling species

of the Quadrimaculatus Complex. All salivary gland dissections were made on reared F_1 progeny from field-collected adults for which the parental female had been identified electrophoretically. Sample sizes were low so results are not indicative of inversion frequencies of the populations used. Salivary gland polytene chromosome preparations, using the technique described, are illustrated for *An. quadrimaculatus* (Fig. 20A) and *An. smaragdinus* (Fig. 20B). It was difficult to discern the homozygous inversions in *An. quadrimaculatus* and the fixed inversions in *An. smaragdinus*. The X chromosome puff that was a discriminating feature in the ovarian polytenes was not present; the puff shown here in both species (Figs. 20A, 20B) was different and distal to the inversion. However, the size and location of the polymorphic inversions on 3R in *An. quadrimaculatus* and on 2L and 3R in *An. smaragdinus* are helpful diagnostic characteristics.

No chromosome maps have been prepared for the salivary gland polytene chromosomes of the other 3 species; therefore, no detailed comparisons with the chromosomes of *An. quadrimaculatus* and *An. smaragdinus* were made. The X chromosome of *An. diluvialis* (Fig. 20C) was shorter than the X chromosomes of *An. quadrimaculatus* and *An. smaragdinus*; also, a small section of the chromosome adjacent to the centromere appeared to have been inserted on the other side. There was no puff on the terminal end, as seen in *An. quadrimaculatus* and *An. smaragdinus*. Two polymorphic inversions were seen on 2L, a large one covering ⅔ of the arm with a breakpoint next to the centromere and a smaller one within the larger inversion. Two inversions were noted on 2R, one situated in the proximal half of 2R and a larger one that had the same distal breakpoint and the proximal break near the centromere. Inversions on 2R had not been detected in *An. quadrimaculatus* or *An. smaragdinus* (Kaiser et al. 1988a). The $3L_1$ and $3L_2$ dimorphism was not observed, and the only form of this arm corresponded to $3L_2$, but a floating inversion covering the middle ⅓ of this arm was observed.

The F_1 progeny of adults collected in Walton Co., FL, Screven Co., GA, and Iberia Parish, LA, were used for the *An. inundatus* salivary gland dissections. The X chromosome was easily distinguishable from those of the other species (Fig. 20D), but the banding quality in the distal half of the X chromosome was always poorly defined. In hybrid crosses to *An. diluvialis,* the X chromosomes from the salivary glands of the F_1 progeny were variable in terms of synapsis, and ranged from almost total asynapsis to *ca.* 75% synapsis (Seawright, Kaiser, and Mitchell, unpublished data). There was a small floating inversion on chromosome 2R, located in the centromeric half of the arm, with breakpoints in regions similar to those seen in the small In(2R) in *An. diluvialis.* No 3L chromosomal dimorphism was seen in *An. inundatus,* and only the $3L_2$ form depicted on the *An. quadrimaculatus* chromosome map was observed. A small floating inversion was seen in the middle ⅓ of 3L that appeared to have breakpoints identical to the In(3L) inversion described for *An. diluvialis.*

The F_1 progeny of field-collected *An. maverlius* adults from Walton Co., FL, Screven Co., GA, and Tishomingo Co., MS, were used for observations on salivary gland polytene chromosomes. The general quality of the chromosomal banding was poor, with the X chromosome (Fig. 20E) showing the least definitive banding. No inversions were observed on either of the autosomes, but the lack of inversions in the small sample size examined should not be taken as definitive for this species.

ELECTROPHORESIS STUDIES

Horizontal starch gel electrophoretic methods (Steiner and Joslyn 1979) were used to differentiate the 5 sibling species (Narang et al. 1989a, 1989b, 1990a). Individual homogenates of parental females (grinding buffer: 1.4 µl 2-mercaptoethanol/1 ml H_2O) were loaded via paper wicks into a 12.5% starch gel made with a CA-8 buffer system (Steiner and Joslyn 1979). Typically, 7 1-mm-thick slices from each gel were stained for 10 enzyme systems, and 15 loci were used for diagnostic analysis. The enzymes and respective loci were: aconitase (*Acon-1*), aldehyde oxidase (*Ao-1*), esterases (*Est-2, Est-5, Est-6*), glutamate oxaloacetate transaminase (*Got-1, Got-2*), hydroxy acid dehydrogenase (*Had-3*), isocitrate dehydrogenase (*Idh-1, Idh-2*), malic enzyme (*Me-1*), mannose phosphate isomerase (*Mpi-1*), peptidase (*Pep-2, Pep-4*), and phosphate glucose isomerase (*Pgi-1*). Isozyme results for specimens taken from the progeny broods, from which the types of the 5 sibling species were selected, are given in Table 32 and Figs. 21 and 22. An inbred colony strain of *An. quadrimaculatus* (Q_2), homozygous for these loci, was used as a standard and assigned a mobility value (*Rf*) of 100.

Narang et al. (1989a) provided an electrophoretic key for distinguishing species *An. quadrimaculatus, An. smaragdinus, An. diluvialis,* and *An. maverlius,* and later used 5 enzymes and 6 loci to differentiate *An. diluvialis* from *An. inundatus* (Narang et al. 1990a). A few loci were diagnostic for each species. For example, *Got-1* (*Rf* = 0.89), *Me-1* (*Rf* = 1.08) and *Mpi-1* (*Rf* = 0.62) were all diagnostic for *An. maverlius. Anopheles diluvialis* and *An. inundatus* were difficult to distinguish from each other but were easily separated from the other 3 species by using *Had-3* (*Rf* = 0.5), *Mpi-1* (*Rf* = 1.06) or *Pgi-1* (*Rf* = 0.95). Narang et al. (1990a) documented differences between *An. diluvialis* and *An. inundatus* in allelic frequencies at 5 loci (*Acon-1, Ao-1, Est-5, Est-6,* and *Me-1*). Differentiating *An. quadrimaculatus* from *An. smaragdinus* required using 2 or more loci with high diagnostic values (see Ayala and Powell 1972 for calculation of di-

agnostic values). The *Idh-1* and *Idh-2* loci were the most diagnostic for *An. quadrimaculatus* and *An. smaragdinus,* but some populations of *An. quadrimaculatus* were polymorphic for the *Idh* loci, and a third locus, *Est-2,* was used to identify *An. smaragdinus.*

MOLECULAR STUDIES

The mitochondrial DNA genome of *An. quadrimaculatus* was cloned by Cockburn et al. (1990) and sequenced completely by Mitchell et al. (1993a). Mitchell et al. (1992) also cloned the ribosomal gene from *An. smaragdinus.* This information and the clones were used to analyze the members of the Quadrimaculatus Complex in terms of endonuclease-restriction-enzyme site differences and variability in field populations (Mitchell et al. 1992, Perera et al. 1995). They were able to identify the mtDNA of 4 of the species (*An. quadrimaculatus, An. smaragdinus, An. maverlius,* and either *An. diluvialis* or *An. inundatus)* by using *Ava I, HindIII,* and *Pvu II* in combination (Cockburn et al. 1990). All 5 species were identified using *Ava I, Acc I, Ban I, Bcl I, HindIII,* or *Spe I* to digest the rDNA (Mitchell, personal communication). The polymerase chain reaction was used by Cornel et al. (1996) to analyze the second internal transcribed spacer (ITS2) of nuclear ribosomal DNA from the 5 sibling species. They were able to identify the same species previously identified with mtDNA. The bands observed for *An. diluvialis* and *An. inundatus* were too similar for a clear identification.

Species-specific DNA clones of *An. quadrimaculatus* (Cockburn 1990) were used as probes with a convenient quick-blot technique (Johnson et al. 1993) for the analysis of a field population composed of *An. quadrimaculatus, An. smaragdinus,* and *An. diluvialis* (Jensen et al. 1993, 1996). The combination of the probes and the quick-blot enabled the rapid analysis and identification of several thousand mosquitoes per week. The lack of probes for *An. maverlius, An. diluvialis,* and *An. inundatus* precluded the accurate analysis of all field populations. However, since *An. diluvialis* and *An. inundatus* have a limited distribution and occur parapatrically, the 2 probes could be used for the analysis of the Quadrimaculatus Complex throughout much of its range.

CUTICULAR HYDROCARBON ANALYSIS

An analysis of the hydrocarbons extracted from the cuticles of females of the Quadrimaculatus Complex was reported by Carlson et al. (1997). Extracts were prepared from adults that were uniformly reared and processed. Specimens were selected for testing from IPBs that included the type series of the 4 new species and the neotype of *An. quadrimaculatus.* Results were compared with those of specimens from IPBs obtained from other geographic localities of the species. An analysis of the data showed that *An. quadrimaculatus, An. smaragdinus,* and *An. maverlius* could be separated at 100% from each other and the combined *An. diluvialis* and *An. inundatus*; however, separation of *An. diluvialis* from *An. inundatus* was 80%. All specimens used in this analysis were also examined in the morphological studies and members of each brood were evaluated by starch gel electrophoresis.

MULTIPLE-TECHNIQUE STUDIES

Narang et al. (1993) showed that it was possible to analyze the ovarian nurse cell polytene chromosomes, cuticular hydrocarbons, isozymes, mtDNA, and rDNA from single females of *An. quadrimaculatus* and *An. smaragdinus.* For those 2 species, this technique would be of considerable value for the analysis of field populations. For the other species in the complex, the procedures would work except for the polytene chromosomes. By using individually reared adults with their associated pupal and 4th-instar larval exuviae, morphological identification could be included with the other identification techniques on the adult specimens. In the present study, multiple evaluations (i.e., anatomical morphology, polytene chromosomes, isozymes, and cuticular hydrocarbons) were selectively employed to analyze specimens taken from the same F_1 IPB.

COLOR AND PATTERN VARIATIONS OF LARVAE AND PUPAE

During laboratory rearing of the mosquitoes used in this study, it was noted that the various pigments found in larvae and pupae of the members of the Quadrimaculatus Complex were quite variable. For field-collected larvae and pupae, we also observed considerable variability in coloration. With the naked eye, several color variants were obvious, but under a stereomicroscope, the variety of pigments increased considerably. The pigments were associated with the cuticle, fat body, and other internal organs. Various colors observed included green, yellow, pink, white, purple, blue, red, brown, and black, and these pigments were often mixed (e.g., greenish brown or other combinations). Bellamy (1942) reported the majority of *An. quadrimaculatus s.l.* larvae to be yellow, green or greenish, but many were dark brown or black. Warren et al. (1975) reported color phenotypes for *An. albimanus* Wiedemann pupae and provided color photographs of the green and brown alleomorphs. We noted the occurrence of a mixture of individuals with different pigments in the progeny from a single female of species in the Quadrimaculatus Complex. The lone exception was *An. smaragdinus,* which was always an emerald green (or a shade close to emerald green) that was visible to the naked eye in the 4th-instar larvae and early pupae. In the other members of the complex, pale green larvae were

occasionally observed, but they were always present as a minor variant and never occurred in Mendelian ratios. The lack of fit to Mendelian ratios was true for most of the other colors, except as noted below.

The phenomenon of homochromy occurs when insects produce pigments in response to the color and shade of pigmentation in the environment. When *An. quadrimaculatus* and *An. albimanus* larvae were reared in a black container, they responded by producing dark (mostly brown and black) pigments (Benedict and Seawright 1987), apparently in an effort to blend in with the background color, which might provide protection from predators. This ability seems to be a common trait among anopheline mosquitoes (Benedict and Chang 1996). Eye color mutants did not respond to the shade or color of the environment. Benedict and Seawright (1987) noted that the *green larva, yellow larva, ebony,* and *amber* mutants of *An. albimanus* did not produce dark pigments in response to being reared in a black container. Instead, they produced a more intense shade of green or yellow, or another pigment associated with the mutant condition. Recently, we found the same situation for *golden,* an EMS-induced mutant of *An. quadrimaculatus.* Homochromy could have an effect on mosquitoes of the Quadrimaculatus Complex collected in the field. If only emerald green (or a shade close to emerald green) 4th-instar larvae and early pupae are observed in a progeny of a single female reared in the laboratory in a white container, then those mosquitoes most probably are *An. smaragdinus.* The exception could be a naturally occurring variant such as *green larva,* a recessive trait found in *An. albimanus* (Seawright et al. 1979). The key would be whether the progeny brood fits a Mendelian ratio for the inheritance of either a recessive or dominant trait, e.g., either 25%, 50%, 75%, or 100% of the progeny would be green. Care should be exercised in attributing black or dark brown larvae from field collections to the homochromy phenomenon, because *black larva,* a recessive lethal mutant that mimics homochromy, was discovered in a field collection of *An. quadrimaculatus* and described by Seawright and Anthony (1972). The *brown larva* mutant (mapped to 2L by Mitchell and Seawright 1984a) was found in the ORLANDO strain of our laboratory colony of *An. quadrimaculatus,* and this very dark variant could be mistaken for homochromy. As an example, there were 4 naturally occurring dark mutants (i.e., *black larva* [Rabbani et al. 1976], *ebony* [Benedict et al. 1979], *brown larva,* and *sable* [Seawright et al. 1985]) in *An. albimanus* that were very dark and could easily be confused with homochromy.

In larvae and pupae of members of the Quadrimaculatus Complex, the presence or absence of a dorsal stripe on the thorax/cephalothorax and on most of the abdominal segments was very obvious in field-collected and laboratory-reared specimens. There were 3 natural variants, of which 2 were quite common (white *stripe* and absence of stripe), and the other, *red stripe,* was quite rare. Larvae with no stripe or with the white *stripe* trait were reported by Coggeshall (1941) in late-instar larvae of *An. quadrimaculatus.* The white *stripe* was a dominant trait and was later mapped to 3R of *An. quadrimaculatus* (Mitchell and Seawright 1984a). The chromosomal location in the other species of the complex remains unknown, but the presumed location should be similar to that in *An. quadrimaculatus.* The *red stripe* trait (Mitchell and Seawright 1984b) was found at a gene frequency of about 3% in a population from Stuttgart, AR. Genetic crosses confirmed that *red stripe* was a codominant allele at the same locus as white *stripe,* and both of these monofactorial traits were dominant over the lack of a stripe. The *red stripe* trait was also expressed in adults reared from *red stripe* larvae. Benedict et al. (1996) showed that white stripe and collarless traits in anopheline mosquitoes were apparently the result of uric acid deposition. The possible nature of the red stripe on larvae remains unknown. Burgess (1946) noted pigmentation patterns of pupae in several North American anopheline species, including *An. quadrimaculatus s.l.* Warren et al. (1975) discussed and published color photographs of striped and nonstriped forms of *An. albimanus* pupae.

ACKNOWLEDGMENTS

We express grateful appreciation to Donald R. Barnard and Herbert Oberlander (CMAVE) for providing support and laboratory facilities to the senior author; to Taina R. Litwak (Litwak Illustration Studio, Gaithersburg, MD) for preparing Figs. 1–17; to Susan E. White (CMAVE), for constructing the maps in Figs. 23–27; to Thomas J. Zavortink (Department of Biology, University of San Francisco, San Francisco, CA) for the gift of 7 reared IPBs of the Quadrimaculatus Complex; to Peter J. Van Helsdingen (National Museum of Natural History, Leiden, the Netherlands) for arranging the loan of the holotype of *An. annulimanus*; to Karl R. Kangas, Marlene M. Falkner, and Heather M. Scheer (CMAVE) for laboratory assistance; and to the editor (American Mosquito Control Association) for permission to use photographs of eggs in Figs. 18 and 19. We give special thanks to Bruce A. Harrison (North Carolina Department of Environment, Health and Natural Resources, Winston-Salem, NC), E. L. Peyton (Walter Reed Biosystematics Unit, Smithsonian Institution, Washington, DC), and Ralph E. Harbach (The Natural History Museum, London, United Kingdom), for reviewing the manuscript. We acknowledge the following, in alphabetical order, for assistance in making field collections of specimens: C. S. Apperson, M. Q. Benedict, J. S. Billodeaux, B. K. Birky, B. B. Broussard, T. Fukuda, T. Jensen, D. O. Joslyn, K. R. Kan-

gas, G. C. Lanzaro, B. R. McDuff, C. L. Meek, S. E. Mitchell, S. K. Narang, J. K. Nayar, J. K. Nesheim, J. K. Olson, E. Ostheimer, S. P. Robertson, C. R. Rutledge, K. Samui, H. M. Scheer, B. J. Smittle, E. L. Snoddy, S. G. Straub, J. Tard, K. J. Tennessen, J. Tessmer, E. D. Walker, S. E. White, O. R. Willis, V. Wright, and the rangers and biologists at the parks. Special recognition is given to C. Roxanne Rutledge (Department of Entomology, Louisiana State University, Baton Rouge, LA) who generously provided much of the distribution data for Louisiana.

REFERENCES CITED

Apperson, C. S. and G. C. Lanzaro. 1991. Comparison of host-feeding patterns between *Anopheles quadrimaculatus* sibling species A and B. J. Am. Mosq. Control Assoc. 7:507–508.

Ayala, F. J. and J. R. Powell. 1972. Allozymes as diagnostic characters in sibling species of *Drosophlia.* Proc. Natl. Acad. Sci. 69:1094–1096.

Baker, R. H., W. L. French and J. B. Kitzmiller. 1962. Induced copulation in *Anopheles* mosquitoes. Mosq. News 22:16–17.

Belkin, J. N. 1962. The mosquitoes of the South Pacific (Diptera, Culicidae). Volume I. Univ. of Calif. Press, Berkeley and Los Angeles, CA.

Belkin, J. N. 1968. Mosquito studies (Diptera, Culicidae) IX. The type specimens of New World mosquitoes in European museums. Contrib. Am. Entomol. Inst. (Ann Arbor) 3(4):1–69.

Belkin, J. N., R. X. Schick and S. J. Heinemann. 1966. Mosquito studies (Diptera, Culicidae) VI. Mosquitoes originally described from North America. Contrib. Am. Entomol. Inst. (Ann Arbor) 1(6):1–39.

Belkin, J. N., C. L. Hogue, P. Galindo, T. H. G. Aitken, R. X. Schick and W. A. Powder. 1965. Mosquito studies (Diptera, Culicidae). II. Methods for the collection, rearing and preservation of mosquitoes. Contrib. Am. Entomol. Inst. (Ann Arbor) 1(2):19–78.

Bellamy, R. E. 1942. Observations on the macroscopic species-identification of larval *Anopheles* in Georgia. J. Parasitol. 28:299–310.

Benedict, M. Q. and H. Chang. 1996. Rapid isolation of anopheline mosquito eye-colour mutants based on larval colour change. Med. Vet. Entomol. 10:93–96.

Benedict, M. Q. and J. A. Seawright. 1987. Changes in pigmentation in mosquitoes (Diptera: Culicidae) in response to color of environment. Ann. Entomol. Soc. Am. 80:55–61.

Benedict, M. Q., A. Cohen, A. J. Cornel and D. L. Brummett. 1996. Uric acid in *Anopheles* mosquitoes (Diptera: Culicidae): effects of *collarless, stripe* and *white* mutations. Ann. Entomol. Soc. Am. 89:261–265.

Benedict, M. Q., J. A. Seawright, D. W. Anthony and S. W. Avery. 1979. *Ebony,* a semidominant lethal mutant in the mosquito *Anopheles albimanus.* Can. J. Genet. Cytol. 21:193–200.

Blanchard, R. 1905. Les moustiques, histoire naturelle et medicale. F. R. de Rudeval, Imprimeur-Editeur, Paris, France.

Boromisa, R. D. and P. R. Grimstad. 1986. Virus-vector-host relationships of *Aedes stimulans* and Jamestown Canyon virus in a northern Indiana enzootic focus. Am. J. Trop. Med. Hyg. 35:1285–1295.

Bray, R. L. and B. C. Walton. 1961. The life cycle of *Dirofilaria uniformis* Price and transmission to wild and laboratory rabbits. J. Parasitol. 47:13–22.

Brook, J. H., C. A. Genese, P. B. Bloland, J. R. Zucker and K. C. Spitalny. 1994. Brief report: malaria probably locally acquired in New Jersey. N. Engl. J. Med. 331:22–23.

Burgess, R. W. 1946. Pigmentation as a specific character in certain anopheline pupae. J. Natl. Malaria Soc. 5: 189–191.

Burton, G. J. 1953. Some techniques for mounting mosquito eggs, larvae, pupae and adults on slides. Mosq. News 13:7–15.

Calisher, C. H., D. B. Francy, G. C. Smith, D. J. Muth, J. S. Lazuick, N. Karabatsos, W. L. Jakob and R. G. McLean. 1986. Distribution of Bunyamwera serogroup viruses in North America, 1956–1984. Am. J. Trop. Med. Hyg. 35:429–443.

Carlson, D. A., J. F. Reinert, U. R. Bernier, B. D. Sutton and J. A. Seawright. 1997. Analysis of the cuticular hydrocarbons among species of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 13(Suppl.):103–111.

Carpenter, S. J. and W. J. LaCasse. 1955. Mosquitoes of North America (north of Mexico). Univ. of Calif. Press, Berkeley and Los Angeles, CA.

Centers for Disease Control and Prevention. 1991. Mosquito-transmitted malaria—California and Florida, 1990. Morbid. Mortal. Wkly. Rep. 40(6):106–108.

Centers for Disease Control and Prevention. 1995. Local transmission of *Plasmodium vivax* malaria—Houston, Texas, 1994. Morbid. Mortal. Wkly. Rep. 44(15):295, 301–303.

Centers for Disease Control and Prevention. 1996. Mosquito-transmitted malaria—Michigan, 1995. Morbid. Mortal. Wkly. Rep. 45(19):398–400.

Chamberlain, R. W. 1963. Anophelines as arbovirus vectors. Proc. and Pap. 7th Int. Congr. Trop. Med. Malaria, Rio de Janeiro, Brazil 3:160–161.

Chamberlain, R. W., W. D. Sudia and P. H. Coleman. 1969. Isolations of an arbovirus of the Bunyamwera group (Tensaw virus) from mosquitoes in the southeastern United States, 1960–1963. Am. J. Trop. Med. Hyg. 18:92–97.

Chamberlain, R. W., R. K. Sikes, D. B. Nelson and W. D. Sudia. 1954. Studies on the North American arthropod-borne encephalitides VI. Quantitative determinations of virus–vector relationships. Am. J. Hyg. 60:278–285.

Chamberlain, W. F. 1956. An improved ethyl acetate jar for trap light collecting. J. Econ. Entomol. 49:702.

Cockburn, A. F. 1990. A simple and rapid technique for identification of large numbers of individual mosquitoes using DNA hybridization. Arch. Insect Biochem. Physiol. 14:191–199.

Cockburn, A. F. and S. E. Mitchell. 1989. Repetitive DNA interspersion patterns in Diptera. Arch. Insect Biochem. Physiol. 10:105–113.

Cockburn, A. F. and J. A. Seawright. 1988. Techniques for mitochondrial and ribosomal DNA analysis of anopheline mosquitoes. J. Am. Mosq. Control Assoc. 4: 261–265.

Cockburn, A. F., S. E. Mitchell and J. A. Seawright. 1990. Cloning of the mitochondrial genome of *Anopheles quadrimaculatus.* Arch. Insect Biochem. Physiol. 14: 31–36.

Cockburn, A. F., C. A. Tarrant and S. Mitchell. 1988. Use of DNA probes to distinguish sibling species of the

Anopheles quadrimaculatus complex. Fla. Entomol. 71: 299–302.

Coggeshall, L. T. 1941. Strains of *Anopheles quadrimaculatus,* inheritance of color patterns in the larvae of *Anopheles quadrimaculatus.* Am. J. Trop. Med. 21: 755–765.

Collins, F. H., C. H. Porter and S. E. Cope. 1990. Comparison of $_{R}$DNA and $_{MT}$DNA in the sibling species *Anopheles freeborni* and *A. hermsi.* Am. J. Trop. Med. Hyg. 42:417–423.

Collins, W. E. and L. E. Chester. 1963. Studies on the transmission of Semliki Forest virus by anopheline mosquitoes. Am. J. Hyg. 77:109–113.

Collins, W. E., A. J. Harrison and J. C. Skinner. 1964. The use of a membrane feeding technique to determine infection and transmission thresholds of Semliki Forest virus in *Anopheles quadrimaculatus* and *Anopheles albimanus.* Mosq. News 24:25–27.

Coluzzi, M. and A. Sabatini. 1967. Cytogenetic observations on species A and B of the *Anopheles gambiae* complex. Parassitologia 9:73–88.

Coluzzi, M. and A. Sabatini. 1968. Cytogenetic observations on species C of the *Anopheles gambiae* complex. Parassitologia 10:155–166.

Coluzzi, M. and A. Sabatini. 1969. Cytogenetic observations on the salt water species, *Anopheles merus* and *Anopheles melas,* of the *gambiae* complex. Parassitologia 11:177–187.

Conn, J. 1990. A genetic study of the malaria vector *Anopheles nuneztovari* from western Venezuela. J. Am. Mosq. Control Assoc. 6:400–405.

Cornel, A. J., C. H. Porter and F. H. Collins. 1996. Polymerase chain reaction species diagnostic assay for *Anopheles quadrimaculatus* cryptic species (Diptera: Culicidae) based on ribosomal DNA ITS2 sequences. J. Med. Entomol. 33:109–116.

Crans, W. J. 1996a. Northeastern States Region, New Jersey. Vector Ecol. Newsl. 27(3):8–10.

Crans, W. J. 1996b. The ten most important mosquito species in New Jersey. Proc. NJ Mosq. Control Assoc. 83:103–105.

Daggy, R. H., O. J. Muegge and W. A. Riley. 1941. A preliminary survey of the anopheline mosquito fauna of southeastern Minnesota and adjacent Wisconsin areas. Public Health Rep. 56:883–895.

Darsie, R. F., Jr. and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Mosq. Syst. 1(Suppl.):1–313.

DeFoliart, G. R., D. M. Watts and P. R. Grimstad. 1986. Changing patterns in mosquito-borne arboviruses. J. Am. Mosq. Control Assoc. 2:437–455.

Duxbury, R. E., A. P. Moon and E. H. Sadun. 1961. Susceptibility and resistance of *Anopheles quadrimaculatus* to *Dirofilaria uniformis.* J. Parasitol. 47:687–691.

Dyar, H. G. 1928. The mosquitoes of the Americas. Carnegie Institute, Publ. No. 387, Washington, DC.

Edwards, F. W. 1925. Mosquito notes.—V. Bull. Entomol. Res. 15:257–270.

Edwards, F. W. 1932. Genera Insectorum. Diptera, Fam. Culicidae. Fascicle 194, Desmet-Verteneuil, Imprimeur-Editeur, Belgium.

Fairchild, G. B. and M. Hertig. 1948. An improved method for mounting small insects. Science 108:20–21.

Foote, R. H. 1952. A method of making whole mounts of mosquito larvae for special study. J. Parasitol. 38: 494–495.

French, W. L., R. H. Baker and J. B. Kitzmiller. 1962. Preparation of mosquito chromosomes. Mosq. News 22: 377–383.

Giles, G. M. 1900. A handbook of the gnats or mosquitoes giving the anatomy and life history of the Culicidae. John Bale, Sons and Danielsson, Ltd., London, United Kingdom.

Giles, G. M. 1902. A handbook of the gnats or mosquitoes giving the anatomy and life history of the Culicidae together with descriptions of all species noticed up to the present date. John Bale, Sons and Danielsson, Ltd., London, United Kingdom.

Harbach, R. E. 1994. Review of the internal classification of the genus *Anopheles* (Diptera: Culicidae): the foundation for comparative systematics and phylogenetic research. Bull. Entomol. Res. 84:331–342.

Harbach, R. E. and K. L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Plexus Publishing, Inc., Marlton, NJ.

Harbach, R. E. and K. L. Knight. 1982. Corrections and additions to Taxonomists' Glossary of Mosquito Anatomy. Mosq. Syst. (1981) 13:201–217.

Howard, L. O., H. G. Dyar and F. Knab. 1917. The mosquitoes of North and Central America and the West Indies, Volume 4. Systematic description (in two parts), Part II. Carnegie Institute, Washington, DC.

Jensen, T., P. E. Kaiser and D. R. Barnard. 1993. Short-term changes in the abundance and parity rate of *Anopheles quadrimaculatus* species C (Diptera: Culicidae) in a central Florida swamp. J. Med. Entomol. 30: 1038–1042.

Jensen, T., P. E. Kaiser and D. R. Barnard. 1994. Adaptation to intermittently flooded swamps by *Anopheles quadrimaculatus* species C1 (Diptera: Culicidae). Environ. Entomol. 23:1150–1154.

Jensen, T., A. E. Cockburn, P. E. Kaiser and D. R. Barnard. 1996. Human blood-feeding rates among sympatric sibling species of *Anopheles quadrimaculatus* mosquitoes in northern Florida. Am. J. Trop. Med. Hyg. 54:523–525.

Jensen, T., P. E. Kaiser, T. Fukuda and D. R. Barnard. 1995. *Anopheles perplexens* from artificial containers and intermittently flooded swamps in northern Florida. J. Am. Mosq. Control Assoc. 11:141–144.

Johnson, D. W., A. F. Cockburn and J. A. Seawright. 1992. Quick blots and nonradioactive detection of DNA probes for the identification of mosquitoes. J. Am. Mosq. Control Assoc. 8:231–236.

Johnson, D. W., A. F. Cockburn and J. A. Seawright. 1993. Sequence of a DNA probe specific for *Anopheles quadrimaculatus* species A (Diptera: Culicidae). J. Med. Entomol. 30:939–942.

Kaiser, P. E. 1988. Cytotaxonomy as a tool for identification of siblings of the *Anopheles quadrimaculatus* complex. Fla. Entomol. 71:311–323.

Kaiser, P. E. 1994. Paul Kaiser's biosynopsis of. . . the "Quads" *Anopheles quadrimaculatus* Say. Wing Beats 5:8–9.

Kaiser, P. E. 1995. Mosquitoes (Diptera: Culicidae). p. 32. *In*: J. H. Frank and E. D. McCoy. Precinctive insect species in Florida. Fla. Entomol. 78:21–35.

Kaiser, P. E. and J. A. Seawright. 1987. The ovarian nurse cell polytene chromosomes of *Anopheles quadrimaculatus,* species A. J. Am. Mosq. Control Assoc. 3:222–230.

Kaiser, P. E., J. A. Seawright and B. K. Birky. 1988a. Chromosome polymorphism in natural populations of

Anopheles quadrimaculatus Say species A and B. Genome 30:138–146.

Kaiser, P. E., S. E. Mitchell, G. C. Lanzaro and J. A. Seawright. 1988b. Hybridization of laboratory strains of sibling species A and B of *Anopheles quadrimaculatus*. J. Am. Mosq. Control Assoc. 4:34–38.

Kaiser, P. E., S. K. Narang, J. A. Seawright and D. L. Kline. 1988c. A new member of the *Anopheles quadrimaculatus* complex, species C. J. Am. Mosq. Control Assoc. 4:494–499.

Kim, S. S., S. K. Narang and J. A. Seawright. 1987a. Genetic polymorphism, mapping, and characterization of isocitrate dehydrogenase in *Anopheles quadrimaculatus*. J. Hered. 78:187–190.

Kim, S. S., J. A. Seawright and P. E. Kaiser. 1987b. A genetic sexing strain of *Anopheles quadrimaculatus*, species A. J. Am. Mosq. Control Assoc. 3:50–53.

Kitzmiller, J. B. 1982. Anopheline names: their derivations and histories, Volume VIII. Thomas Say Foundation, Entomological Society of America, College Park, MD.

Kitzmiller, J. B., G. Frizzi and R. H. Baker. 1967. Evolution and speciation within the *Maculipennis* complex of the genus *Anopheles*, pp. 151–210. *In*: J. W. Wright and R. Pal (eds.). Genetics of insect vectors of disease. Elsevier Publishing Co., New York, NY.

Klassen, W., W. L. French, H. Laven and J. B. Kitzmiller. 1965. The salivary chromosomes of *Anopheles quadrimaculatus* Say. Mosq. News 25:328–334.

Kline, D. L., D. A. Dame and M. V. Meisch. 1991. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas. J. Am. Mosq. Control Assoc. 7:165–169.

Knight, K. L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae), Volume VI. Thomas Say Foundation, Entomological Society of America, College Park, MD.

Kokernot, R. H., J. Hayes, C. H. Tempelis, D. H. M. Chan, K. R. Boyd and R. J. Anderson. 1969. Arbovirus studies in the Ohio-Mississippi Basin, 1964–1967 IV. Cache Valley virus. Am. J. Trop. Med. Hyg. 18:768–773.

Lanzaro, G. C. 1987. Use of enzyme polymorphism and hybridization crosses to identify sibling species of the mosquito, *Anopheles quadrimaculatus* (Say). Diss. Abstr. Int. B 48(4):0955.

Lanzaro, G. C., S. K. Narang and J. A. Seawright. 1990. Speciation in an anopheline (Diptera: Culicidae) mosquito: enzyme polymorphism and the genetic structure of populations. Ann. Entomol. Soc. Am. 83:578–585.

Lanzaro, G. C., S. E. Mitchell, S. K. Narang and J. A. Seawright. 1991. Assignment of two enzyme loci to the X chromosome of *Anopheles quadrimaculatus* species A. J. Hered. 82:349–351.

Lanzaro, G. C., S. K. Narang, S. E. Mitchell, P. E. Kaiser and J. A. Seawright. 1988. Hybrid male sterility in crosses between field and laboratory strains of *Anopheles quadrimaculatus* (Say) (Diptera: Culicidae). J. Med. Entomol. 25:248–255.

Layton, M., M. E. Parise, C. C. Campbell, R. Advani, J. D. Sexton, E. M. Bosler and J. R. Zucker. 1995. Mosquito-transmitted malaria in New York City, 1993. Lancet 346(8977):729–731.

Linley, J. R., P. E. Kaiser and A. F. Cockburn. 1993. A description and morphometric study of the eggs of species of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). Mosq. Syst. 25:124–147.

Mallet, J. and R. S. Fritzius. 1993. Genetic evidence for insecticide resistance in sibling species of the mosquito *Anopheles quadrimaculatus*. Resist. Pest Manage. 5: 25–26.

Martini, E. 1933. The hypopygia of certain anophelines (Diptera: Culicidae). Proc. Entomol. Soc. Wash. 35:61–67.

Meek, C. L., M. V. Meisch and T. W. Walker. 1985. Portable battery-powered aspirators for collecting adult mosquitoes. J. Am. Mosq. Control Assoc. 1:102–105.

Mitchell, S. E. and J. A. Seawright. 1984a. Chromosome-linked group correlation in *Anopheles quadrimaculatus* (Say). J. Hered. 75:341–344.

Mitchell, S. E. and J. A. Seawright. 1984b. A red stripe mutant and its relationship in an allelic series in *Anopheles quadrimaculatus*. J. Hered. 75:421–422.

Mitchell, S. E. and J. A. Seawright. 1989a. EMS-induced mutations in *Anopheles quadrimaculatus* (Say), species A. J. Hered. 80:58–61.

Mitchell, S. E. and J. A. Seawright. 1989b. Recombination between the X and Y chromosomes in *Anopheles quadrimaculatus* species A. J. Hered. 80:496–499.

Mitchell, S. E., A. F. Cockburn and J. A. Seawright. 1993a. The mitochondrial genome of *Anopheles quadrimaculatus* species A: complete nucleotide sequence and gene organization. Genome 36:1058–1073.

Mitchell, S. E., J. Seawright and S. D. Narang. 1993b. Linkage map of the mosquito (*Anopheles quadrimaculatus* species A) (2N=6) August 1992, pp. 3.273–3.276. *In*: S. J. O'Brien (ed.), Genetic maps, locus maps of complex genomes. 6th ed. Cold Spring Harbor Laboratory Press, Plainview, NY.

Mitchell, S. E., S. K. Narang, A. F. Cockburn, J. A. Seawright and M. Goldenthal. 1992. Mitochondrial and ribosomal DNA variation among members of the *Anopheles quadrimaculatus* (Diptera: Culicidae) species complex. Genome 35:939–950.

Morris, C. D. 1992. Eastern equine encephalitis. J. Fla. Mosq. Control Assoc. 63:23–34.

Narang, S. K. and J. A. Seawright. 1988. Electrophoretic method for recognition of sibling species of anopheline mosquitoes a practical approach. Fla. Entomol. 71:303–311.

Narang, S. K. and J. A. Seawright. 1990. Hexane preserves biological activity of isozymes and DNA. J. Am. Mosq. Control Assoc. 6:533–534.

Narang, S. K. and J. A. Seawright. 1991. Genetic differentiation among members of species complexes in anopheline mosquitoes (Diptera: Culicidae). pp. 59–96. *In*: R. C. Sobti and G. Obe (eds.). Eukaryotic chromosomes, structure and function aspects. Norosa Publishing House, New Delhi, India.

Narang, S. K., P. E. Kaiser and J. A. Seawright. 1989a. Identification of species D, a new member of the *Anopheles quadrimaculatus* species complex: a biochemical key. J. Am. Mosq. Control Assoc. 5:317–324.

Narang, S. K., J. A. Seawright and P. E. Kaiser. 1990a. Evidence for microgeographic genetic subdivision of *Anopheles quadrimaculatus* species C. J. Am. Mosq. Control Assoc. 6:179–187.

Narang, S. K., J. A. Seawright and S. E. Mitchell. 1990b. Linkage map of the mosquito (*Anopheles quadrimaculatus* species A) (2N=6) August 1989, pp. 3.194–3.197. *In*: S. J. O'Brien (ed.). Genetic maps, locus maps of complex genomes. 5th ed. Cold Springs Harbor Laboratory Press, Plainview, NY.

Narang, S. K., S. R. Toniolo, J. A. Seawright and P. E. Kaiser. 1989b. Genetic differentiation among sibling

species A, B, and C of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). Ann. Entomol. Soc. Am. 82:508–515.

Narang, S. K., J. A. Seawright, S. E. Mitchell, P. E. Kaiser and D. A. Carlson. 1993. Multiple-technique identification of sibling species of the *Anopheles quadrimaculatus* complex. J. Am. Mosq. Control Assoc. 9:463–464.

Nayar, J. K. and J. W. Knight. 1991. Nutritional factors and antimicrobials on development of infective larvae of subperiodic *Brugia malayi* (Nematoda: Filarioidea) in *Anopheles quadrimaculatus* and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 28:275–279.

Nayar, J. K. and D. M. Sauerman, Jr. 1973. Appraisal of our present knowledge of enzootic filariae in the southeastern United States. Rep. Fla. Anti-Mosq. Assoc. 44: 69–71.

Nayar, J. K. and D. M. Sauerman, Jr. 1975. Physiological basis of host susceptibility of Florida mosquitoes to *Dirofilaria immitis*. J. Insect Physiol. 21:1965–1975.

Nayar, J. K., J. C. Djam and J. W. Knight. 1984. Susceptibility of *Anopheles quadrimaculatus* and other mosquitoes to *Brugia patei* (Nematoda: Filarioidea). Mosq. News 44:417–419.

Nayar, J. K., J. W. Knight and A. C. Vickery. 1990. Susceptibility of *Anopheles quadrimaculatus* (Diptera: Culicidae) to subperiodic *Brugia malayi* and *Brugia pahangi* (Nematoda: Filarioidea) adapted to nude mice and jirds. J. Med. Entomol. 27:409–411.

Nayar, J. K., J. W. Knight, P. E. Kaiser, J. A. Seawright and S. K. Narang. 1992. Comparative susceptibility of species A, B and C of *Anopheles quadrimaculatus* complex to infection with subperiodic *Brugia malayi* and *Brugia pahangi* (Nematoda: Filarioidea). J. Am. Mosq. Control Assoc. 8:61–64.

Perera, O. P., S. E. Mitchell, A. F. Cockburn and J. A. Seawright. 1995. Variation in mitochondrial and ribosomal DNA of *Anopheles quadrimaculatus* species A (Diptera: Culicidae) across a wide geographic range. Ann. Entomol. Soc. Am. 88:836–845.

Rabbani, M. G., J. A. Seawright, and J. B. Kitzmiller. 1976. The genetics of *black larva,* an autosomal recessive lethal mutation located on chromosome 3 in the mosquito *Anopheles albimanus*. Can. J. Genet. Cytol. 18:51–56.

Rattanarithikul, R. 1983. A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys, biological notes and preservation and mounting techniques. Mosq. Syst. (1982) 14:139–208.

Reid, J. A. 1968. Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. Med. Res. Malaya 31:1–520.

Reinert, J. F. 1974. Terminology and preparation techniques of the female genitalia of aedine mosquitoes (Diptera: Culicidae). Mosq. Syst. 6:46–56.

Reinert, J. F. 1990. Medical entomology studies—XVII. Biosystematics of *Kenknightia,* a new subgenus of the mosquito genus *Aedes* Meigen from the Oriental Region (Diptera: Culicidae). Contrib. Am. Entomol. Inst. 26(2):1–119.

Reinert, J. F. 1997. Bibliography of *Anopheles quadrimaculatus* Say *sensu lato* (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 13(Suppl.):112–161.

Reinert, J. F., P. E. Kaiser and J. A. Seawright. 1996. Unusual features of pupal exuviae noted in the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 12:310–311.

Reinert, J. F., S. E. White and O. R. Willis. 1997. Immatures of *Anopheles quadrimaculatus* species A collected from atypical habitats. J. Fla. Mosq. Control Assoc. 66:1–2.

Rutledge, C. R. and C. L. Meek. 1994. Record of *Anopheles quadrimaculatus* species C in Louisiana. J. Am. Mosq. Control Assoc. 10:585–586.

Rutledge, C. R., A. J. Cornel, C. L. Meek and F. H. Collins. 1996. Validation of a ribosomal DNA-polymerase chain reaction species diagnostic assay for the common malaria mosquito (Diptera: Culicidae) sibling species complex. J. Med. Entomol. 33:952–954.

Saliba, E. K., G. R. DeFoliart, T. M. Yuill and R. P. Hanson. 1973. Laboratory transmission of Wisconsin isolates of a Cache Valley-like virus by mosquitoes. J. Med. Entomol. 10:470–476.

Say, T. 1824. Appendix. Part I.—Natural history, 1. Zoology, E. Class Insecta, order Diptera, pp. 268–378. *In*: W. H. Keating (ed.), Narrative of an expedition to the source of St. Peter's River, Volume 2. Philadelphia, PA.

Schacher, J. F. 1962. Developmental stages of *Brugia pahangi* in the final host. J. Parasitol. 48:693–706.

Schiefer, B. A. and J. R. Smith. 1974. Comparative susceptibility of eight mosquito species to Sindbis virus. Am. J. Trop. Med. Hyg. 23:131–134.

Seawright, J. A. and D. W. Anthony. 1972. Black body, a lethal mutant in *Anopheles quadrimaculatus* Say. Mosq. News 32:47–50.

Seawright, J. A., M. Q. Benedict and S. Narang. 1985. Color mutants in *Anopheles albimanus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 78:177–181.

Seawright, J. A., L. V. Childress and M. Q. Benedict. 1979. Genetics of *green larva,* a recessive mutant on chromosome 2 in *Anopheles albimanus* Wiedemann. Mosq. News 39:55–58.

Seawright, J. A., P. E. Kaiser and S. K. Narang. 1991. A unique chromosomal dimorphism in species A and B of the *Anopheles quadrimaculatus* complex. J. Hered. 82:221–227.

Seawright, J. A., P. E. Kaiser, S. K. Narang, K. J. Tennessen and S. E. Mitchell. 1992. Distribution of sibling species A, B, C, and D of the *Anopheles quadrimaculatus* complex. J. Agric. Entomol. 9:289–300.

Shetty, N. J., M. D. Young, S. K. Narang and J. A. Seawright. 1987. Genetic selection of a *Plasmodium yoelii*–refractory strain of the malaria vector *Anopheles quadrimaculatus* (Say). Genetics 116(Suppl., No. 1, Part 2):s9.

Steiner, W. W. M. and D. J. Joslyn. 1979. Electrophoretic techniques for the genetic study of mosquitoes. Mosq. News 39:35–54.

Stone, A., K. L. Knight and H. Starcke. 1959. A synoptic catalog of the mosquitoes of the world (Diptera, Culicidae), Volume VI. Thomas Say Foundation, Entomological Society of America, Washington, DC.

Sudia, W. D., P. H. Coleman and R. W. Chamberlain. 1969. Experimental vector–host studies with Tensaw virus, a newly recognized member of the Bunyamwera arbovirus group. Am. J. Trop. Med. Hyg. 18:98–102.

Sudia, W. D., P. H. Coleman, R. W. Chamberlain, J. S. Wiseman and T. H. Work. 1967. St. Louis encephalitis vector studies in Houston, Texas, 1964. J. Med. Entomol. 4:32–36.

Takken, W. and D. L. Kline. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. J. Am. Mosq. Control Assoc. 5:311–316.

Theobald, F. V. 1901. A monograph of the Culicidae or mosquitoes. Mainly compiled from the collections re-

ceived at the British Museum from various parts of the world in connection with the investigation into the cause of malaria conducted by the Colonial Office and the Royal Society, Volume I. British Museum of Natural History, London, United Kingdom.

Theobald, F. V. 1907. A monograph of the Culicidae or mosquitoes. Mainly compiled from collections received at the British Museum, Volume IV. British Museum of Natural History, London, United Kingdom.

Theobald, F. V. 1910. A monograph of the Culicidae or mosquitoes. Mainly compiled from collections received at the British Museum. Volume V. British Museum of Natural History, London, United Kingdom.

Todaro, W. S., C. D. Morris and N. A. Heacock. 1977. *Dirofilaria immitis* and its potential mosquito vectors in central New York State. Am J. Vet. Res. 38:1197–1200.

Van der Wulp, F. M. 1867. Eenige Noord-Americaansche Diptera. Tijdschr. Entomol. 10:125–130.

Warren, M., B. B. Richardson and W. E. Collins. 1975. Pupal pleomorphism in a strain of *Anopheles albimanus* from El Salvador. Mosq. News 35:549–551.

Webster, L. T., A. D. Clow and J. H. Bauer. 1935. Experimental studies on encephalitis III. Survival of encephalitis virus (St. Louis type) in *Anopheles quadrimaculatus*. J. Exp. Med. 61:479–487.

Weiner, D. J. and R. E. Bradley. 1970. Ability of some mosquitoes to transmit *Dirofilaria immitis* in Florida. Mosq. News 30:406–410.

Willis, N. L., S. K. Narang, S. R. Toniolo and N. J. Shetty. 1987. Electrophoretic analysis of temporal changes in frequency distribution of sympatric sibling species of *Anopheles quadrimaculatus* (Diptera: Culicidae). Genetics 116(Suppl., No. 1, Part 2):s45.

Wing, S. R., M. D. Young, S. E. Mitchell and J. A. Seawright. 1985. Comparative susceptibilities of *Anopheles quadrimaculatus* mutants to *Plasmodium yoelii*. J. Am. Mosq. Control Assoc. 1:511–513.

Wong, Y. W., J. A. Rowe, D. C. Dorsey, M. J. Humphreys and W. J. Hausler, Jr. 1971. Arboviruses isolated from mosquitoes collected in southeastern Iowa in 1966. Am. J. Trop. Med. Hyg. 20:726–729.

World Health Organization. 1995. Malaria, local transmission of *Plasmodium vivax* malaria, Houston, Texas, 1994. Wkly. Epidemiol. Rec. 70:257–259.

LIST OF FIGURES

Fig. 1. Adult female (lateral view). A. *Anopheles quadrimaculatus.* Female wing (dorsal view). B. *Anopheles quadrimaculatus.* Female femora I–III and tibiae I–III (anterior view). C. *Anopheles quadrimaculatus.* D. *Anopheles smaragdinus.* E. *Anopheles diluvialis.* F. *Anopheles inundatus.* G. *Anopheles maverlius.*

Fig. 2. Dorsal view female scuti and scutelli (scales on left half and setae on right half). A. *Anopheles quadrimaculatus.* B. *Anopheles smaragdinus.* C. *Anopheles maverlius.* D. *Anopheles diluvialis.* E. *Anopheles inundatus.* Proepisterna with upper setae. F. *Anopheles quadrimaculatus.* G. *Anopheles inundatus.*

Fig. 3. Male genitalia (prerotation aspect). *Anopheles quadrimaculatus.* A. Sternum VIII (ventral view, flattened). B. Sternum IX (ventral view, flattened). C. Gonostylus, gonocoxite, phallosome, and claspette (dorsal view, flattened).

Fig. 4. Male genitalia (prerotation aspect); gonostylus, phallosome, and claspette (dorsal view, flattened). A. *Anopheles maverlius.* B. *Anopheles smaragdinus.* C. *Anopheles quadrimaculatus.* D. *Anopheles diluvialis.* E. *Anopheles inundatus.*

Fig. 5. Male genitalia (prerotation aspect); tergum IX (dorsal view, flattened). A. *Anopheles maverlius.* B. *Anopheles smaragdinus.* C. *Anopheles quadrimaculatus.* D. *Anopheles diluvialis.* E. *Anopheles inundatus.*

Fig. 6. Male genitalia (prerotation aspect); tergum VIII (dorsal view, flattened). A. *Anopheles quadrimaculatus.* B. *Anopheles smaragdinus.* C. *Anopheles maverlius.* D. *Anopheles diluvialis.* E. *Anopheles inundatus.*

Fig. 7. Dimensions for measurements. A. Larval antenna. B. Pupal trumpet. C. Male genital lobe of pupa. D. Pupal paddle.

Fig. 8. Pupal exuviae, *Anopheles diluvialis.* A. Cephalothorax (outer view). B. Anterior angle of scutum. C. Metanotum, abdominal segments I-IX (dorsal view on left half and ventral view on right half), paddle. D. Dorsal apotome. E. Male genital lobe (ventral view).

Fig. 9. Pupal exuviae, *Anopheles inundatus.* A. Cephalothorax (outer view). B. Anterior angle of scutum. C. Metanotum, abdominal segments I–IX (dorsal view on left half and ventral view on right half), paddle. D. Dorsal apotome. E. Male genital lobe (ventral view).

Fig. 10. Pupal exuviae, *Anopheles maverlius.* A. Cephalothorax (outer view). B. Anterior angle of scutum. C. Metanotum, abdominal segments I–IX (dorsal view on left half and ventral view on right half), paddle. D. Dorsal apotome. E. Male genital lobe (ventral view).

Fig. 11. Pupal exuviae, *Anopheles quadrimaculatus.* A. Cephalothorax (outer view). B. Anterior angle of scutum. C. Metanotum, abdominal segments I–IX (dorsal view on left half and ventral view on right half), paddle. D. Dorsal apotome; E. Male genital lobe (ventral view).

Fig. 12. Pupal exuviae, *Anopheles smaragdinus.* A. Cephalothorax (outer view). B. Anterior angle of scutum. C. Metanotum, abdominal segments I–IX (dorsal view on left half and ventral view on right half), paddle. D. Dorsal apotome. E. Male genital lobe (ventral view).

Fig. 13. Fourth-instar larva, *Anopheles diluvialis.* A. Cranium (dorsal view on left half and ventral view on right half). B. Antennal seta 3-A. C. Head seta 3-C. D. Thorax and abdominal segments I–VI (dorsal view on left half and ventral view on right half). E. Abdominal segments VII, VIII, and X (lateral view). F. Dorsomentum (ventral view). G. Pecten plate (lateral view).

Fig. 14. Fourth-instar larva, *Anopheles inundatus.* A. Cranium (dorsal view on left half and ventral view on right half). B. Antennal seta 3-A. C. Head seta 3-C. D. Thorax and abdominal segments I–VI (dorsal view on left half and ventral view on right half). E. Abdominal segments VII, VIII, and X (lateral view). F. Dorsomentum (ventral view). G. Pecten plate (lateral view).

Fig. 15. Fourth-instar larva, *Anopheles maverlius.* A. Cranium (dorsal view on left half and ventral view on right half). B. Antennal seta 3-A. C. Head seta 3-C. D. Thorax and abdominal segments I–VI (dorsal view on left half and ventral view on right half). E. Abdominal segments VII, VIII, and X (lateral view). F. Dorsomentum (ventral view). G. Pecten plate (lateral view).

Fig. 16. Fourth-instar larva, *Anopheles quadrimaculatus.* A. Cranium (dorsal view on left half and ventral view on right half). B. Antennal seta 3-A. C. Head seta 3-C. D. Thorax and abdominal segments I–VI (dorsal view on left half and ventral view on right half). E. Abdominal segments VII, VIII, and X (lateral view). F. Dorsomentum (ventral view). G. Pecten plate (lateral view).

Fig. 17. Fourth-instar larva, *Anopheles smaragdinus.* A. Cranium (dorsal view on left half and ventral view on right half). B. Antennal seta 3-A. C. Head seta 3-C. D. Thorax and abdominal segments I–VI (dorsal view on left half and ventral view on right half). E. Abdominal segments VII, VIII, and X (lateral view). F. Dorsomentum (ventral view); G. Pecten plate (lateral view).

Fig. 18. Eggs (ventral view). A. *Anopheles maverlius.* B. *Anopheles inundatus.* C. *Anopheles diluvialis.* D. *Anopheles smaragdinus.* E. *Anopheles quadrimaculatus.*

Fig. 19. Eggs; row 1A–E, cells of dorsal plaston; row 2A–E, tubercles of middle anterior deck; 3, entire egg (lateral view, ventral surface at top, anterior end at left); 4, entire egg (ventral view, anterior end at left). 1A, 2A, 3, 4, *Anopheles quadrimaculatus*; 1B, 2B, *Anopheles smaragdinus*; 1C, 2C, *Anopheles diluvialis*; 1D, 2D, *Anopheles inundatus*; 1E, 2E, *Anopheles maverlius.*

Fig. 20. Salivary gland polytene X chromosomes. A. *Anopheles quadrimaculatus*. B. *Anopheles smaragdinus*. C. *Anopheles diluvialis*. D. *Anopheles inundatus*. E. *Anopheles maverlius.*

Figs. 21 and 22. Electromorphs of various loci of specimens from type series. 1 and 7, Q_2 strain of *Anopheles quadrimaculatus*. 2, *Anopheles quadrimaculatus*. 3, *Anopheles smaragdinus*. 4, *Anopheles diluvialis*. 5, *Anopheles inundatus*. 6, *Anopheles maverlius*. A. Enzyme *Acon-1*. B. Enzyme *Ac-1*. C. Enzyme *Mpi-1*. D. Enzymes *Pep-2* and *Pep-4*. E. Enzymes *Est-2, Est-5,* and *Est-6*. F. Enzymes *Got-1* and *Got-2*. G. Enzymes *Had-3* and *Me-1*. H. Enzymes *Idh-1, Idh-2,* and *Pgi-1*. Full names of the enzymes are included in the text in the electrophoresis studies section.

Fig. 23. Map showing distribution of *Anopheles diluvialis* by county

Fig. 24. Map showing distribution of *Anopheles inundatus* by county/parish.

Fig. 25. Map showing distribution of *Anopheles maverlius* by county/parish.

Fig. 26. Map showing distribution of *Anopheles quadrimaculatus* by county/parish.

Fig. 27. Map showing distribution of *Anopheles smaragdinus* by county/parish.

LIST OF FIGURE ABBREVIATIONS

Male Genitalia

Ae	=	aedeagus
BP	=	basal piece
BMS	=	basal median seta
Cl	=	claspette
Gc	=	gonocoxite
Gs	=	gonostylus
InS	=	internal seta
Par	=	paramere
PBL	=	parabasal lobe
PBS	=	parabasal seta
PH	=	phallosome
PlL	=	posterolateral lobe
S-IX	=	sternum IX
S-VIII	=	sternum VII
Te-IX	=	tergum IX
Te-VIII	=	tergum VIII

Pupa

CT	=	cephalothorax
DAp	=	dorsal apotome
GL	=	genital lobe
I–IX	=	abdominal segments I–IX
Mr	=	midrib
Mtn	=	metathoracic wing
MW	=	mesothoracic wing
Pa	=	paddle
PsA	=	postscutal area
T	=	trumpet

Larva

A	=	antenna
C	=	cranium
Dm	=	dorsomentum
FEL	=	frontal ecdysial line
Fl	=	flagellum
I–VIII, X	=	abdominal segments I–VIII, X
M	=	mesothorax
MATP	=	median accessory tergal plate
Mdp	=	median plate (spiracular apparatus)
Mx	=	maxilla
NSG	=	Nuttall and Shipley's organ
P	=	prothorax
PP	=	pecten plate
PTP	=	posterior tentorial pit
Sa	=	saddle
Sc	=	scape
StP	=	sternal plate
T	=	metathorax
TP	=	tergal plate

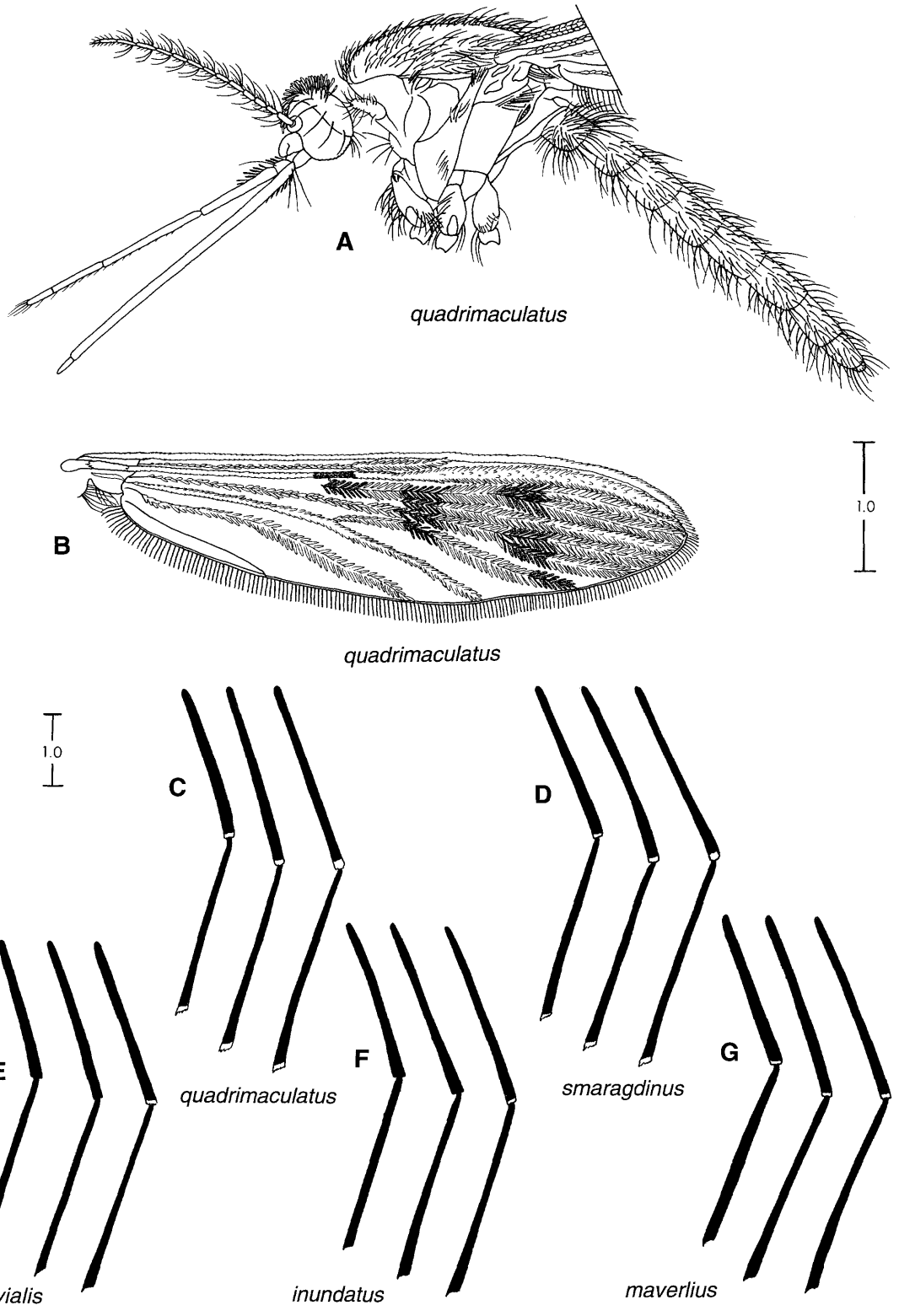

A
quadrimaculatus
B
1.0
quadrimaculatus
1.0
C
D
E
F
G
quadrimaculatus
smaragdinus
diluvialis
inundatus
maverlius

Fig. 1.

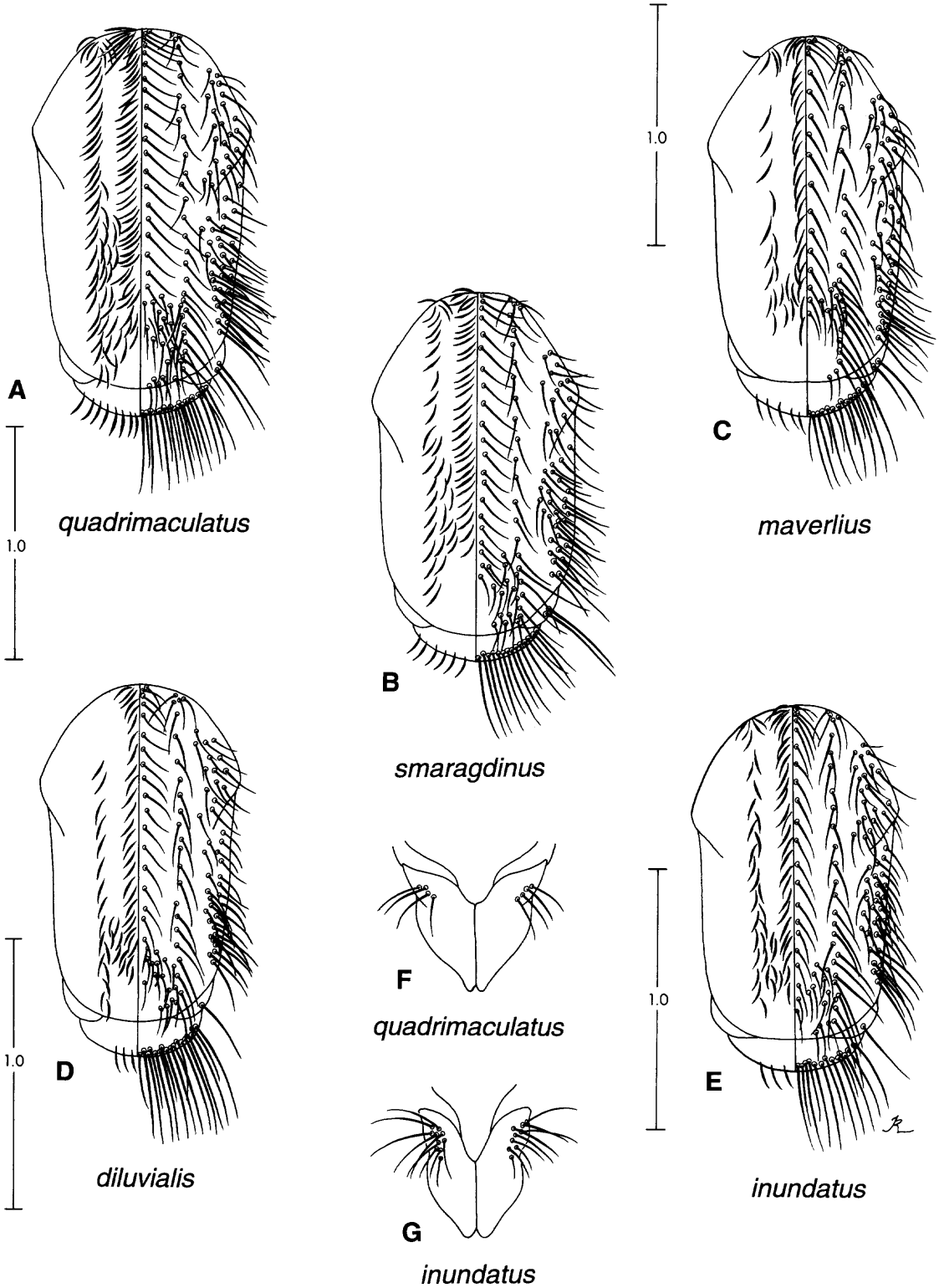

A
quadrimaculatus
1.0
B
smaragdinus
C
maverlius
1.0
D
diluvialis
1.0
F
quadrimaculatus
G
inundatus
E
inundatus
1.0

Fig. 2.

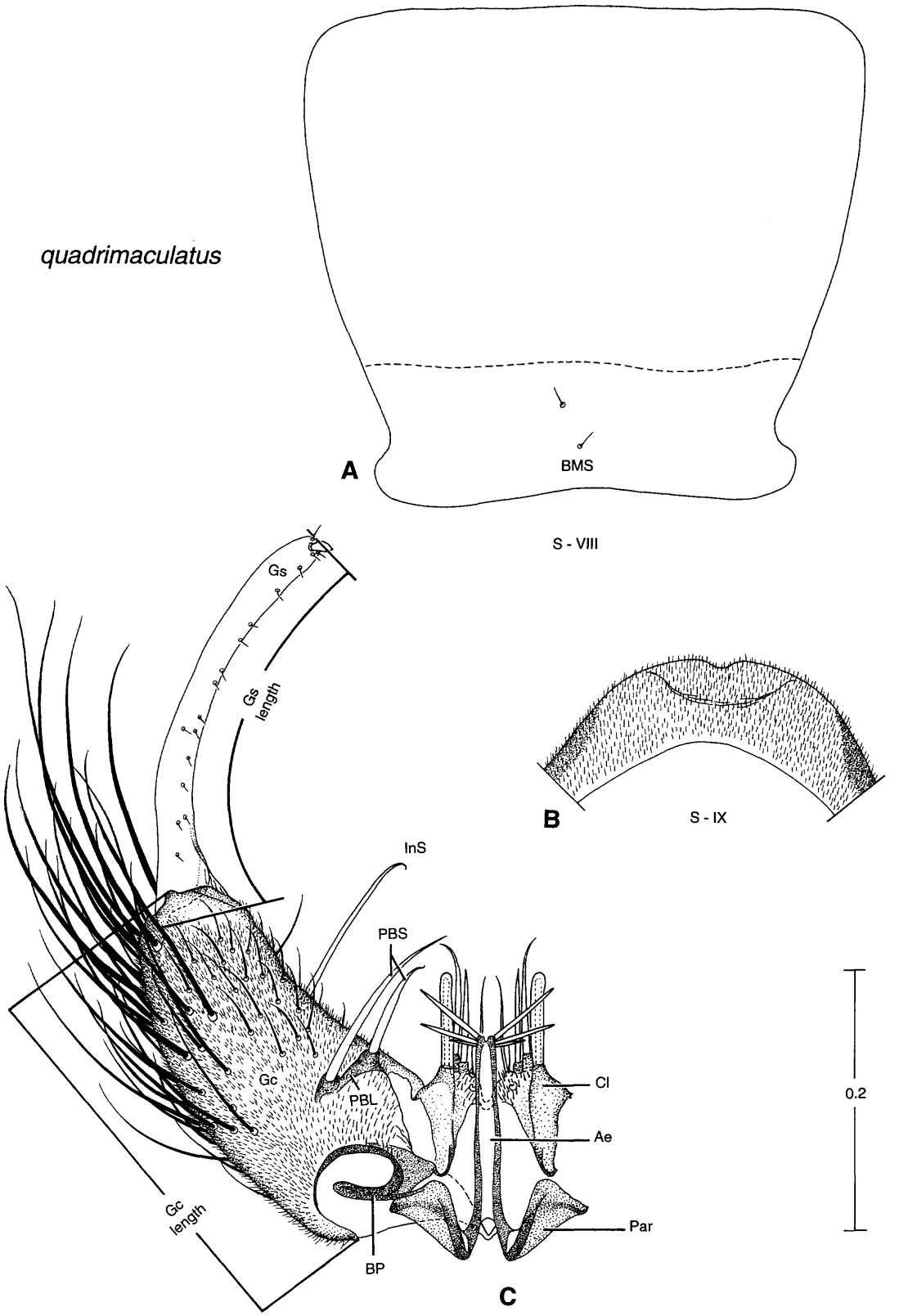

quadrimaculatus
A
BMS
S - VIII
Gs
Gs length
B
S - IX
InS
PBS
Gc
PBL
Cl
Ae
Par
Gc length
BP
C
0.2

Fig. 3.

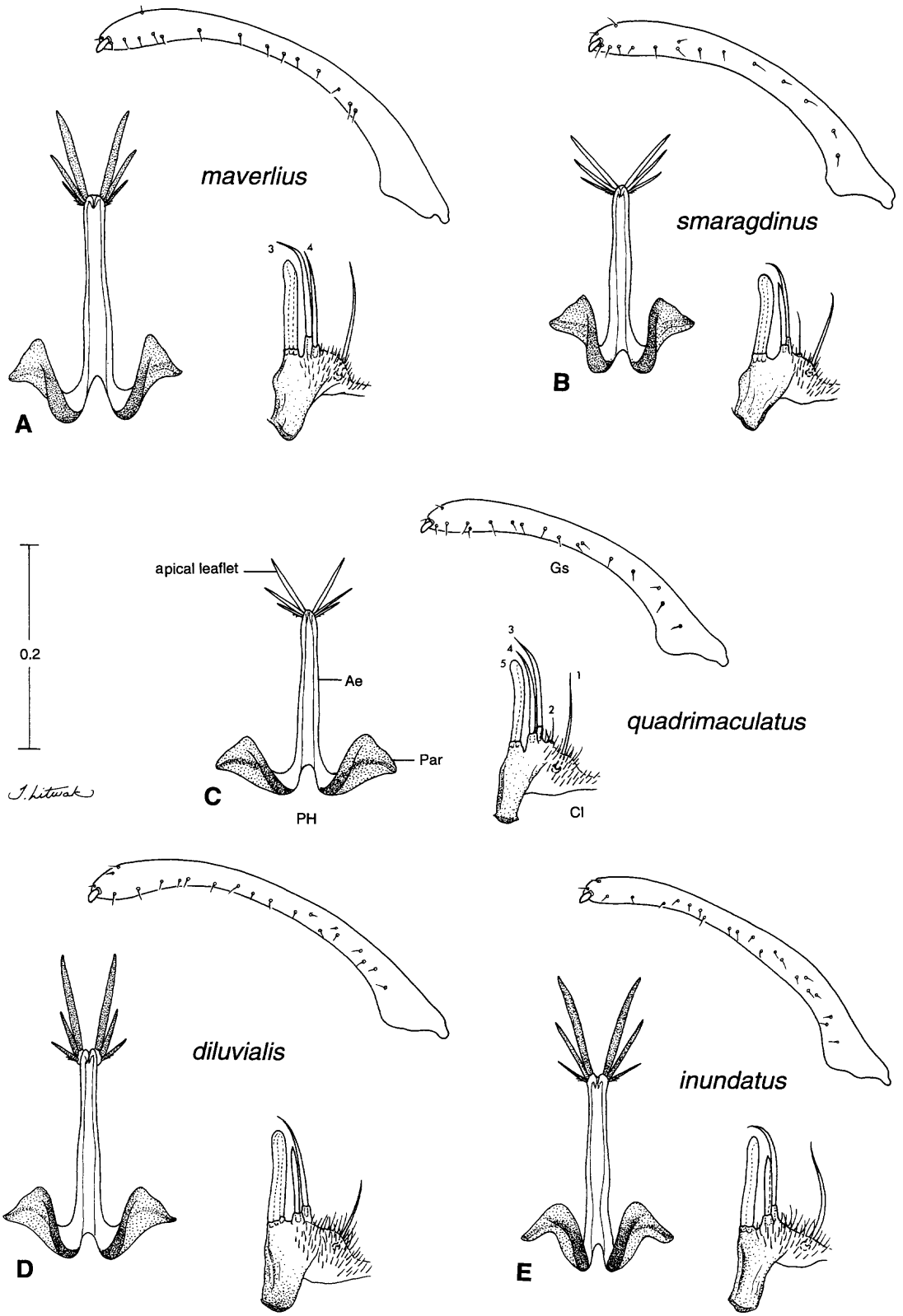

maverlius
A
3
4
smaragdinus
B
0.2
J. Litwak
apical leaflet
Ae
Par
C
PH
Gs
3
4
5
1
2
Cl
quadrimaculatus
diluvialis
D
inundatus
E

Fig. 4.

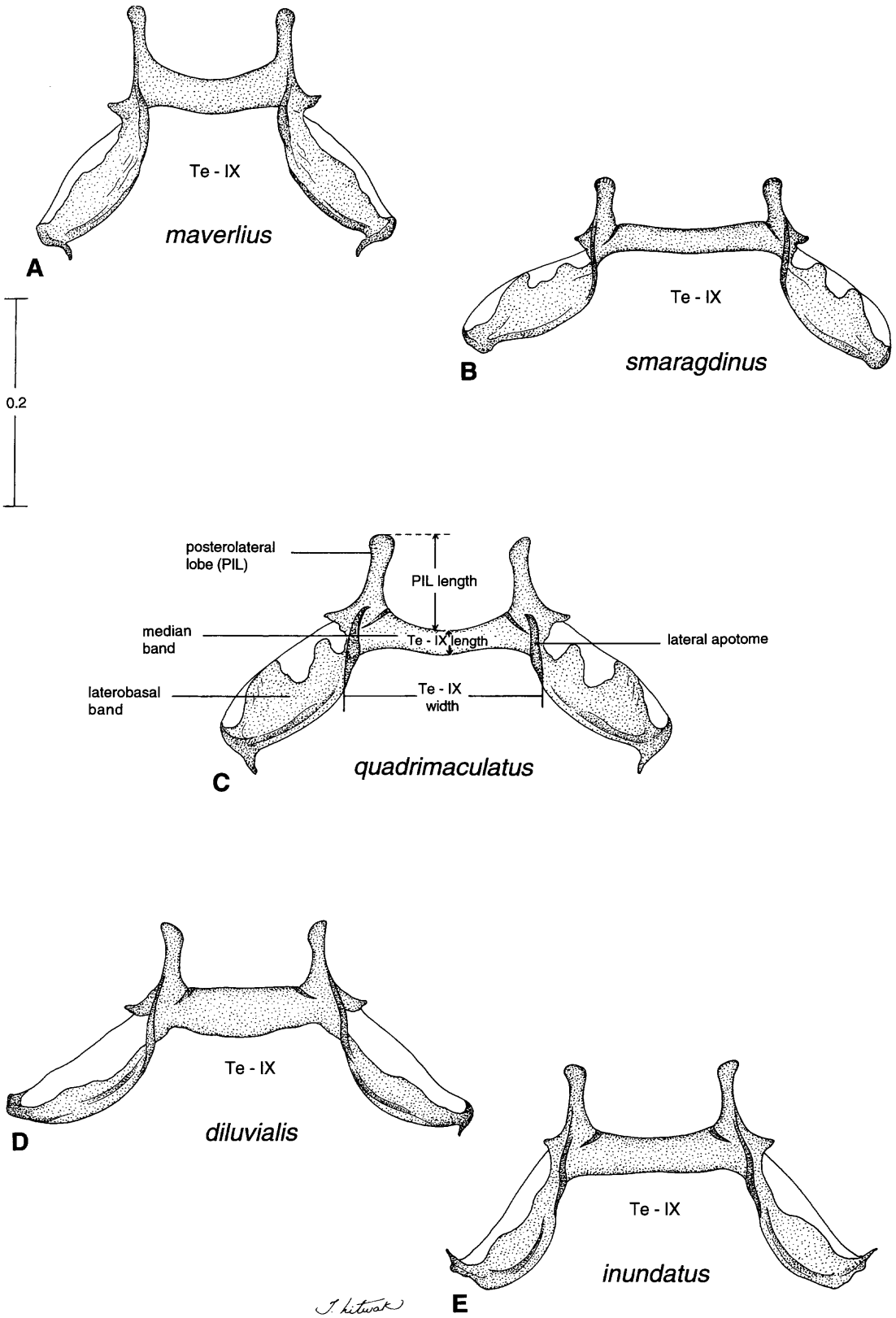

Te - IX
maverlius
A
0.2
Te - IX
smaragdinus
B
posterolateral lobe (PIL)
PIL length
median band
Te - IX length
lateral apotome
laterobasal band
Te - IX width
quadrimaculatus
C
Te - IX
diluvialis
D
Te - IX
inundatus
E
J. Kitwak

Fig. 5.

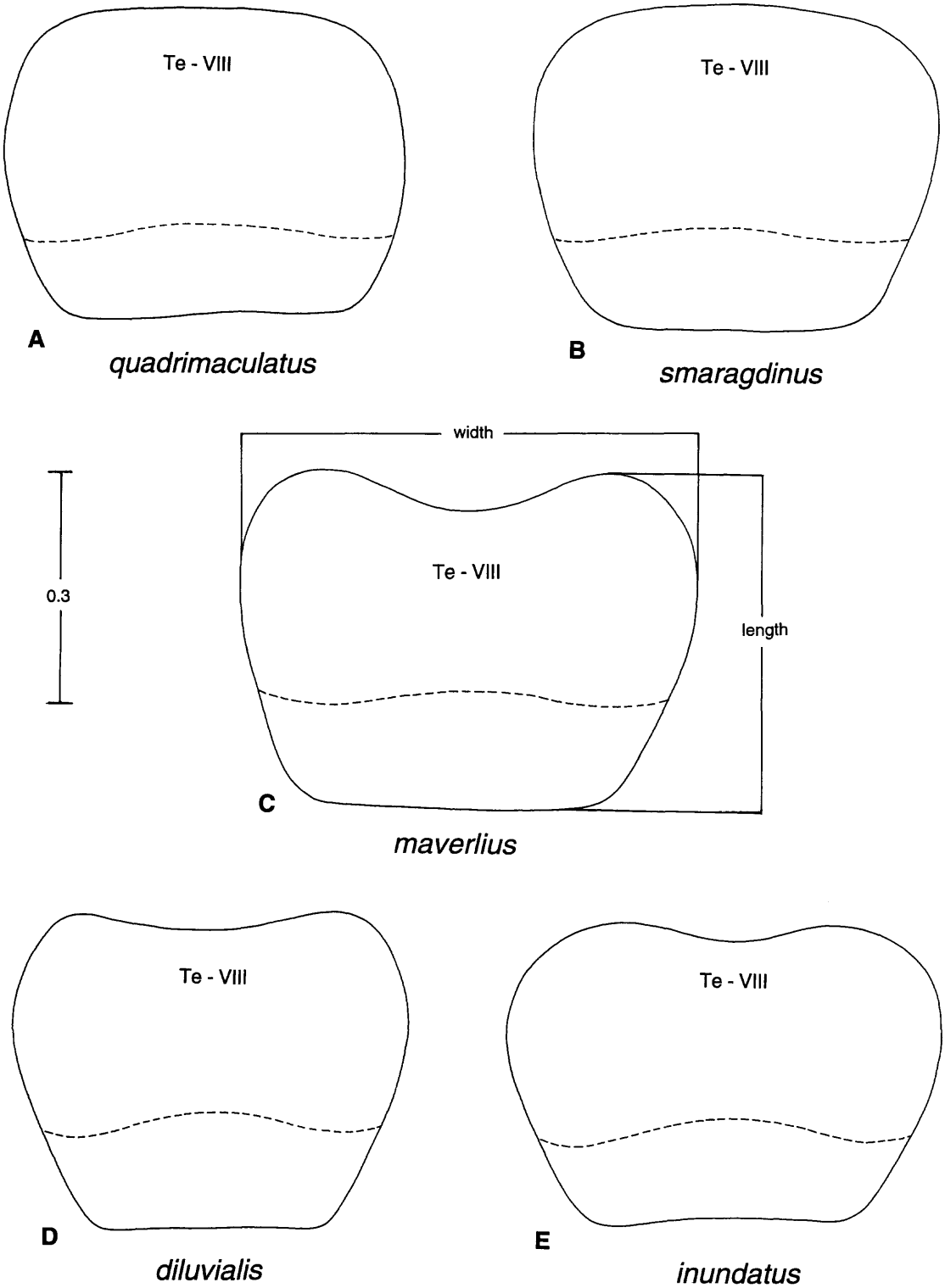
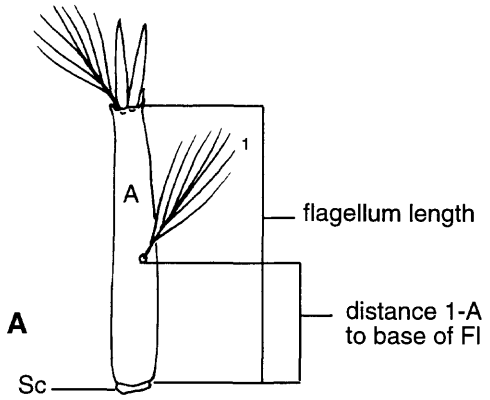
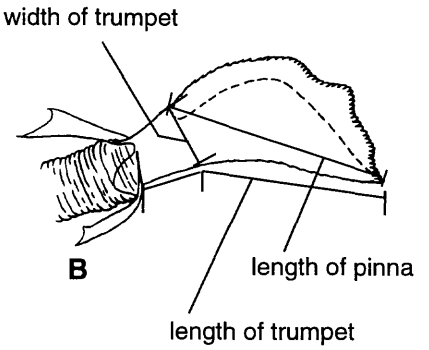

Te - VIII
A
quadrimaculatus
Te - VIII
B
smaragdinus
width
0.3
Te - VIII
length
C
maverlius
Te - VIII
D
diluvialis
Te - VIII
E
inundatus

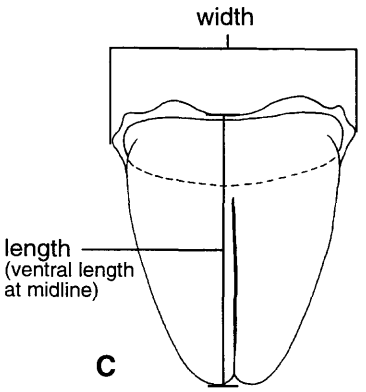
Fig. 6.



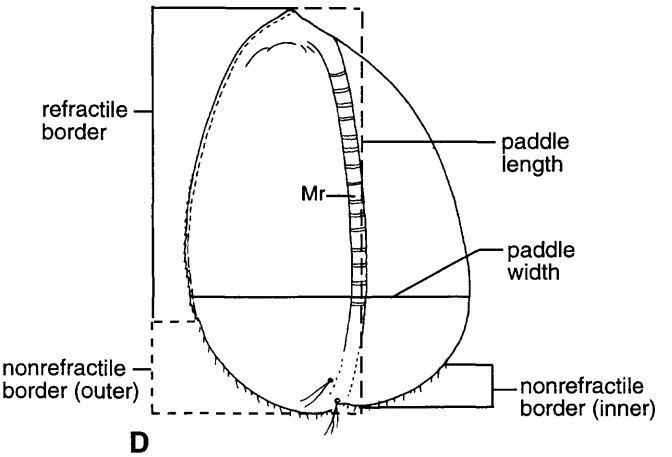
LARVAL ANTENNA



PUPAL TRUMPET



PUPAL ♂ GENITAL LOBE



PUPAL PADDLE

Fig. 7.

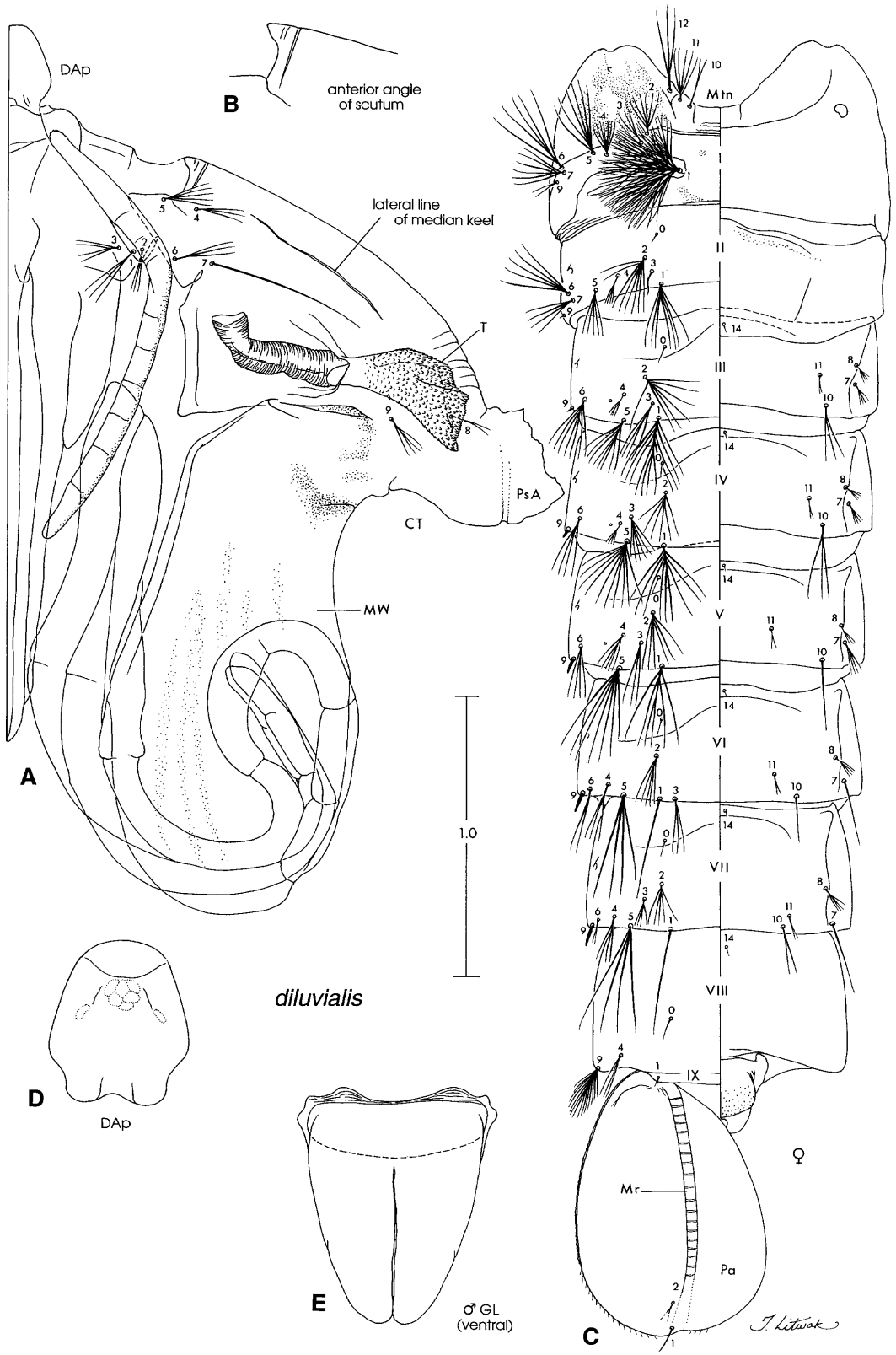

DAp
B
anterior angle of scutum
lateral line of median keel
T
PsA
CT
MW
A
1.0
diluvialis
D
DAp
E
♂ GL (ventral)
Mtn
I
II
III
IV
V
VI
VII
VIII
IX
♀
Mr
Pa
C
J. Litwak

Fig. 8.

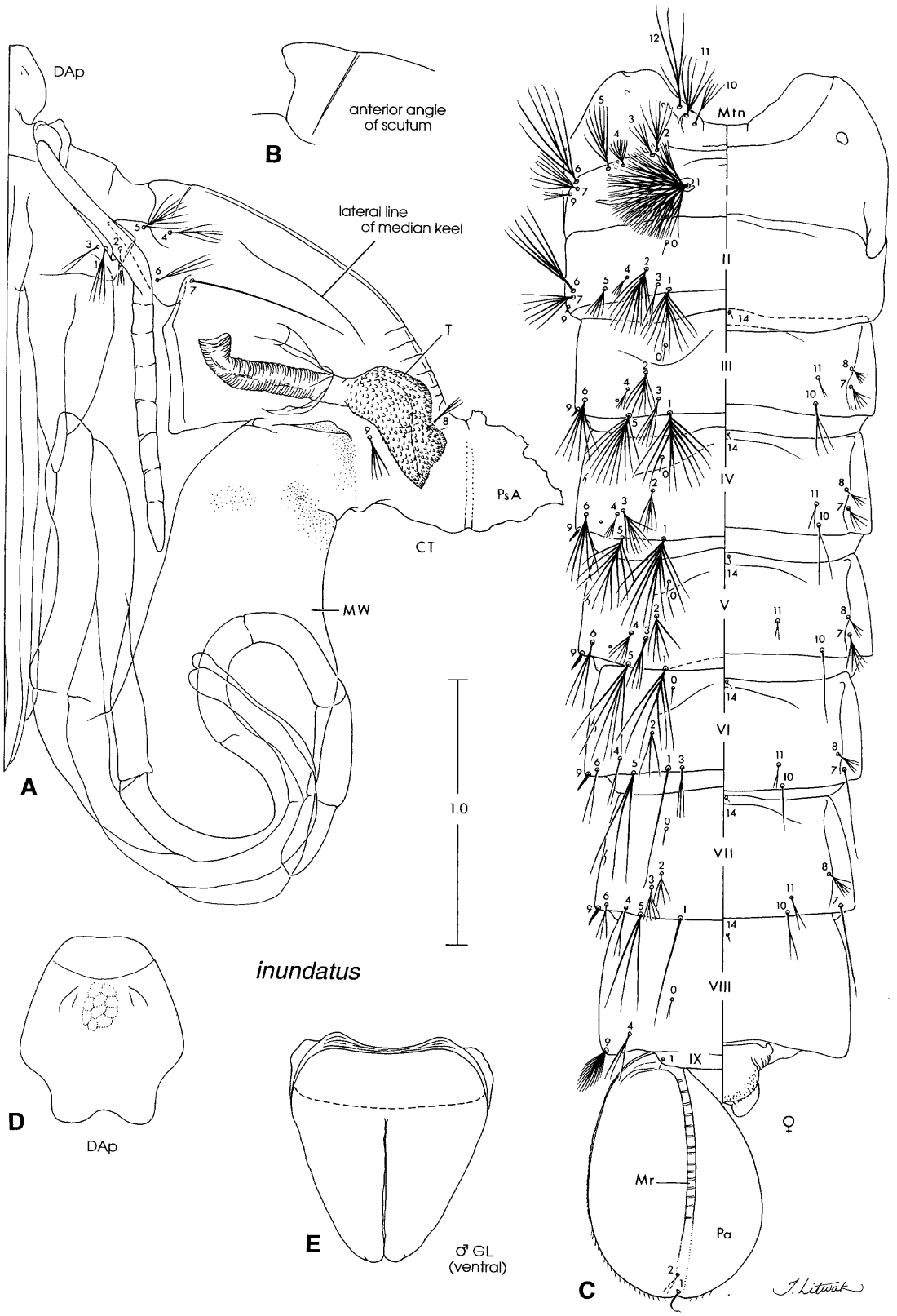

DAp
anterior angle of scutum
B
lateral line of median keel
T
PsA
CT
MW
A
1.0
inundatus
D
DAp
E
♂ GL (ventral)
Mtn
II
III
IV
V
VI
VII
VIII
IX
♀
Mr
Pa
C
J. Litwak

Fig. 9.

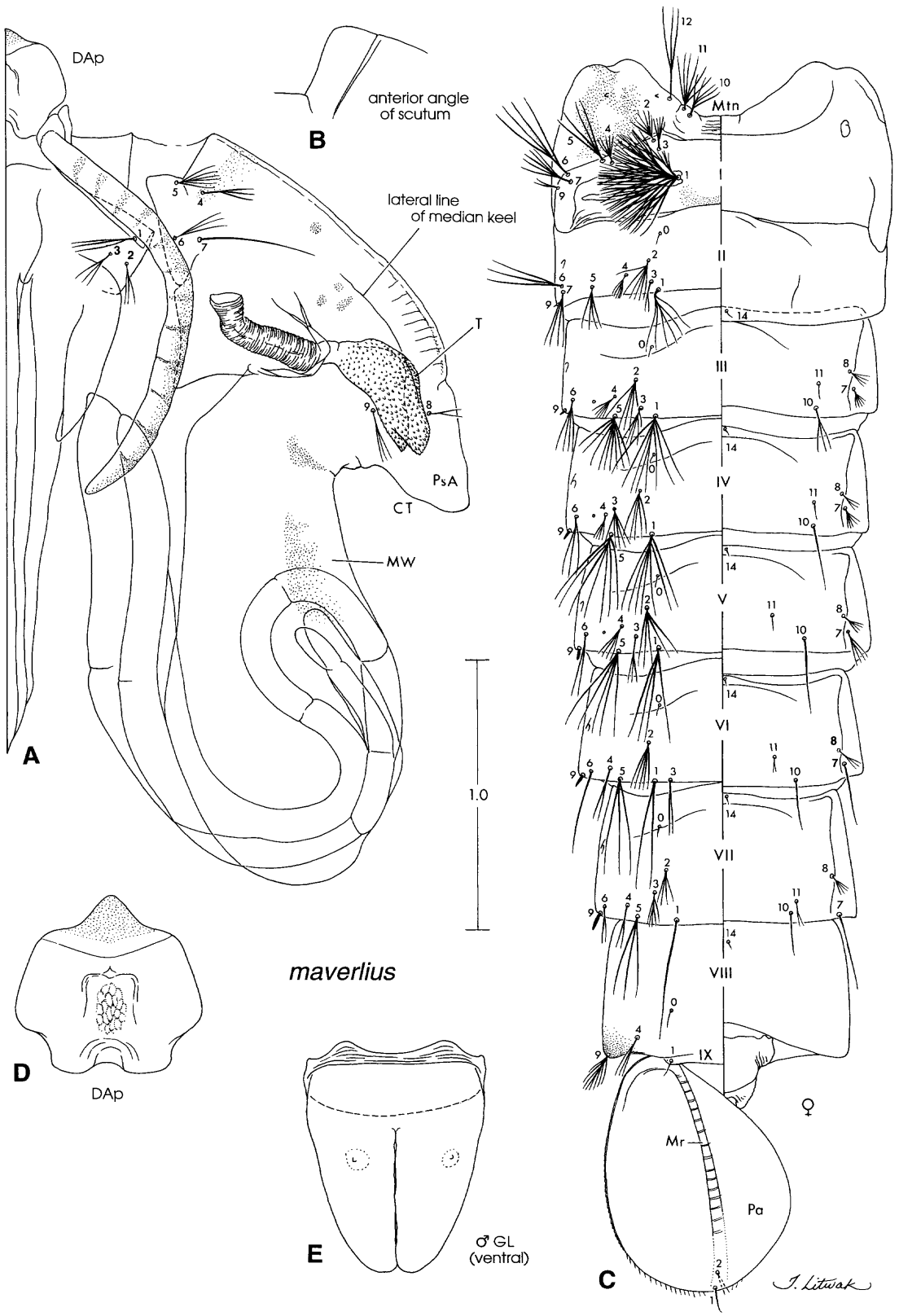

DAp
anterior angle of scutum
B
lateral line of median keel
T
PsA
CT
MW
A
1.0
maverlius
D
DAp
E
♂ GL (ventral)
Mtn
I
II
III
IV
V
VI
VII
VIII
IX
♀
Mr
Pa
C
I. Litwak

Fig. 10.

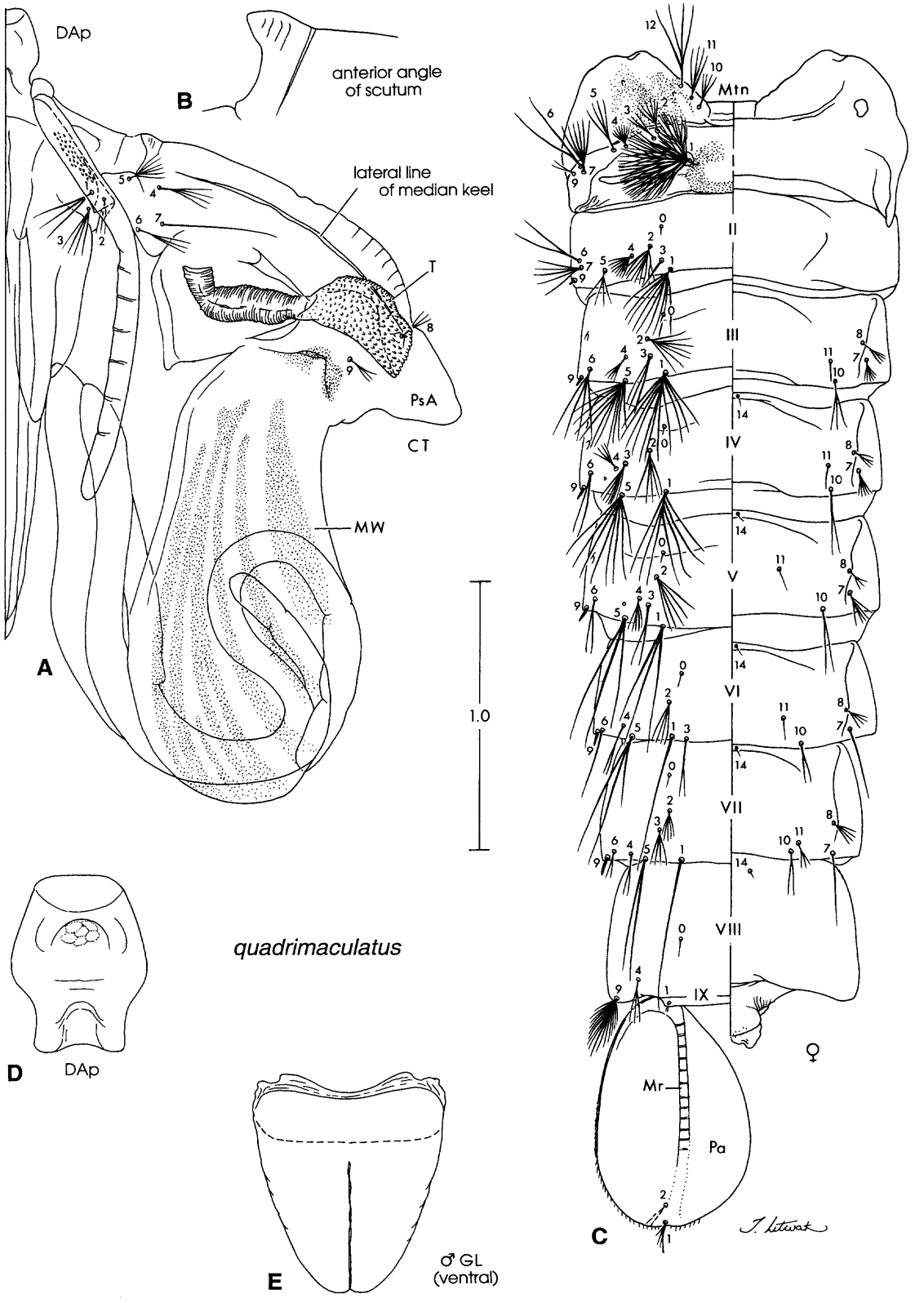

DAp
B
anterior angle of scutum
lateral line of median keel
T
PsA
CT
MW
A
1.0
quadrimaculatus
D
DAp
E
♂ GL (ventral)
Mtn
I
II
III
IV
V
VI
VII
VIII
IX
Mr
Pa
C
♀

Fig. 11.

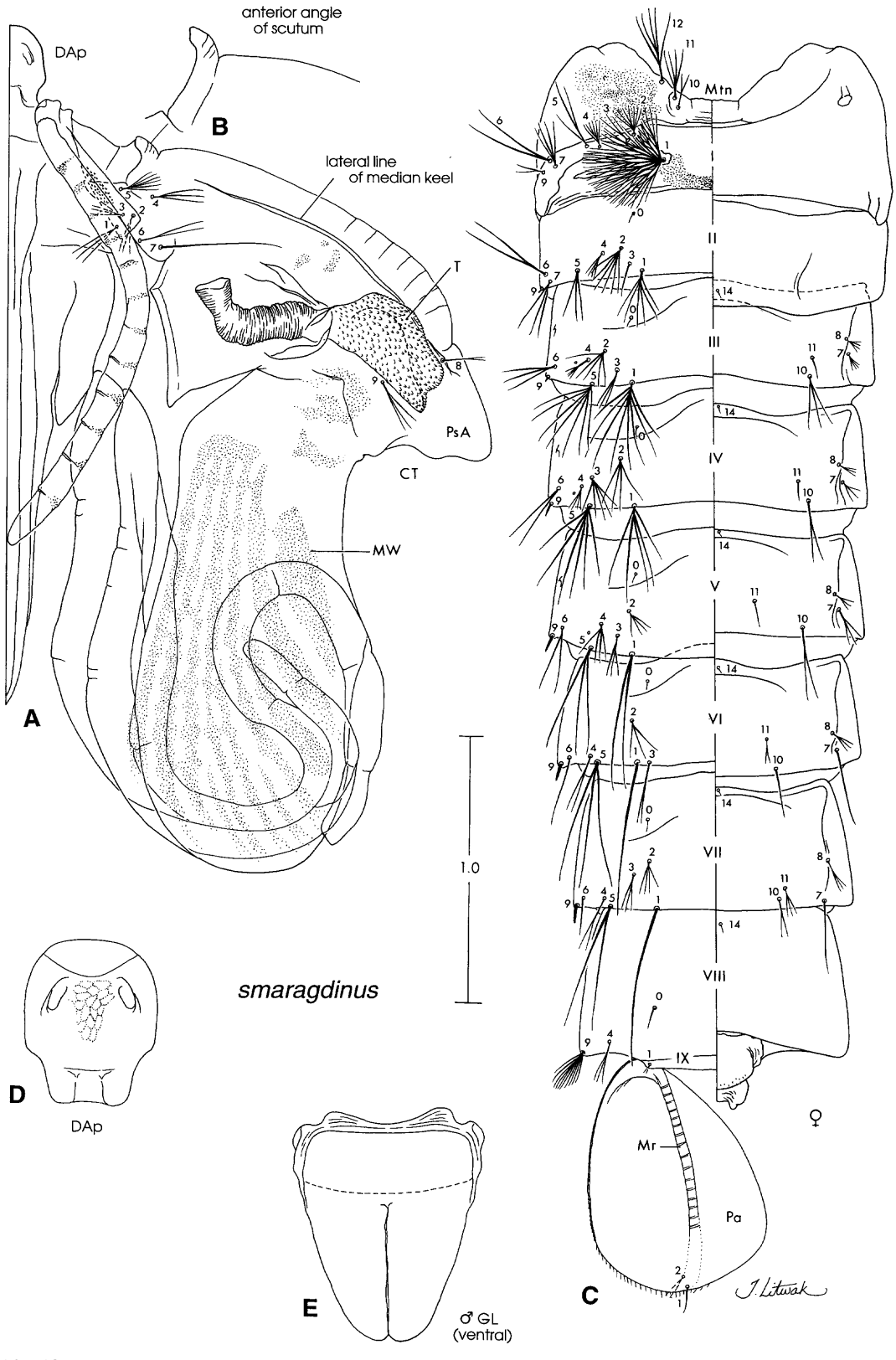

anterior angle
of scutum
DAp
B
lateral line
of median keel
T
PsA
CT
MW
A
1.0
smaragdinus
D
DAp
E
♂ GL
(ventral)
Mtn
I
II
III
IV
V
VI
VII
VIII
IX
Mr
Pa
C
♀
J. Litwak

Fig. 12.

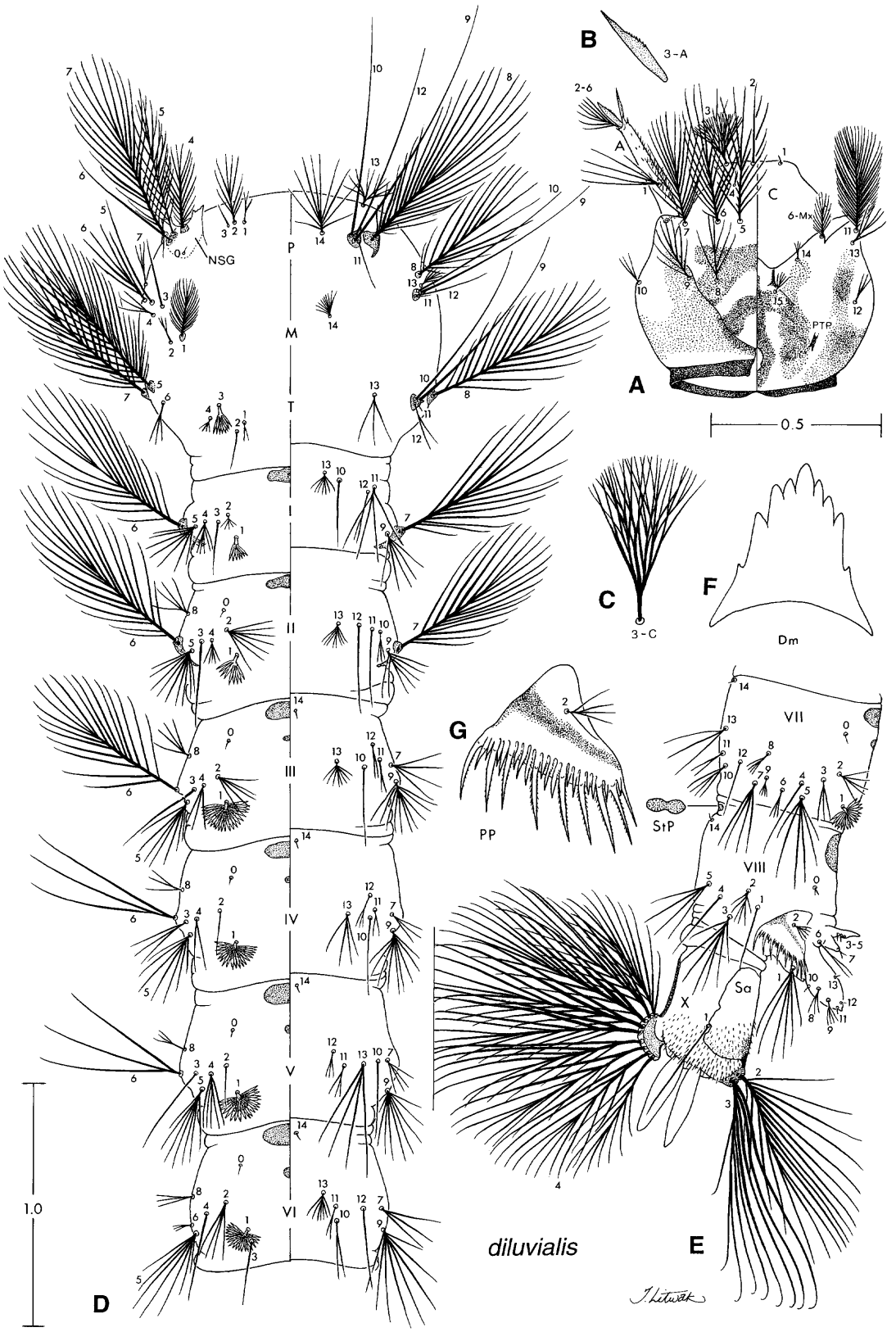

B
3-A
2-6
A
C
6-Mx
PTP
0.5
NSG
P
M
T
I
II
III
IV
V
VI
VII
VIII
X
Sa
3-5
C
3-C
F
Dm
G
PP
StP
1.0
D
diluvialis
E

Fig. 13.

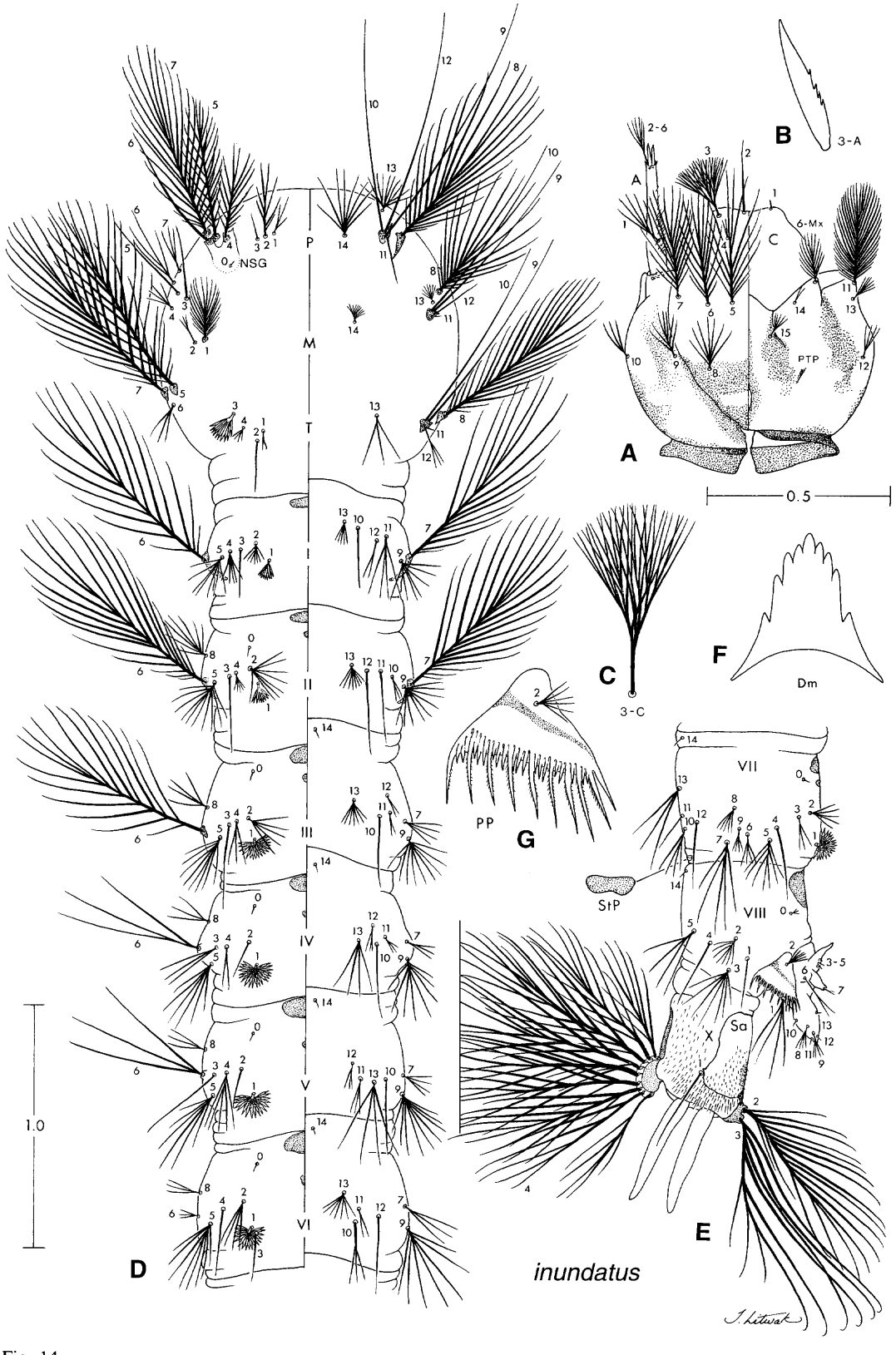

P
M
T
I
II
III
IV
V
VI
VII
VIII
X
NSG
PP
StP
Sa
PTP
Dm
A
B
C
D
E
F
G
3-A
3-C
6-Mx
3-5
0.5
1.0
inundatus

Fig. 14.

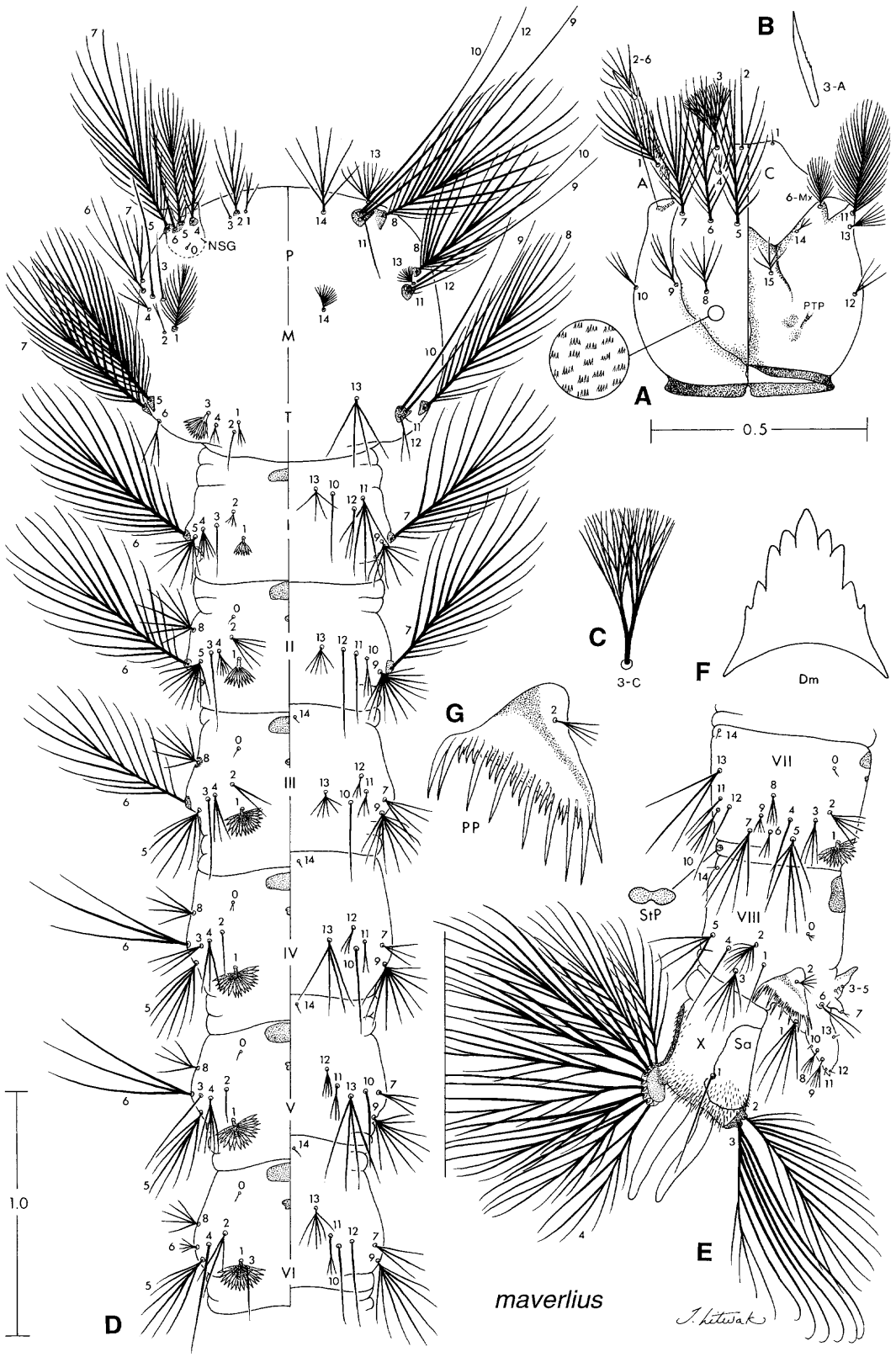

B
2-6
3-A
A
C
6-Mx
PTP
0.5
A
C
3-C
F
Dm
G
PP
NSG
P
M
T
I
II
III
IV
V
VI
VII
VIII
StP
X
Sa
3-5
1.0
D
E
maverlius

Fig. 15.

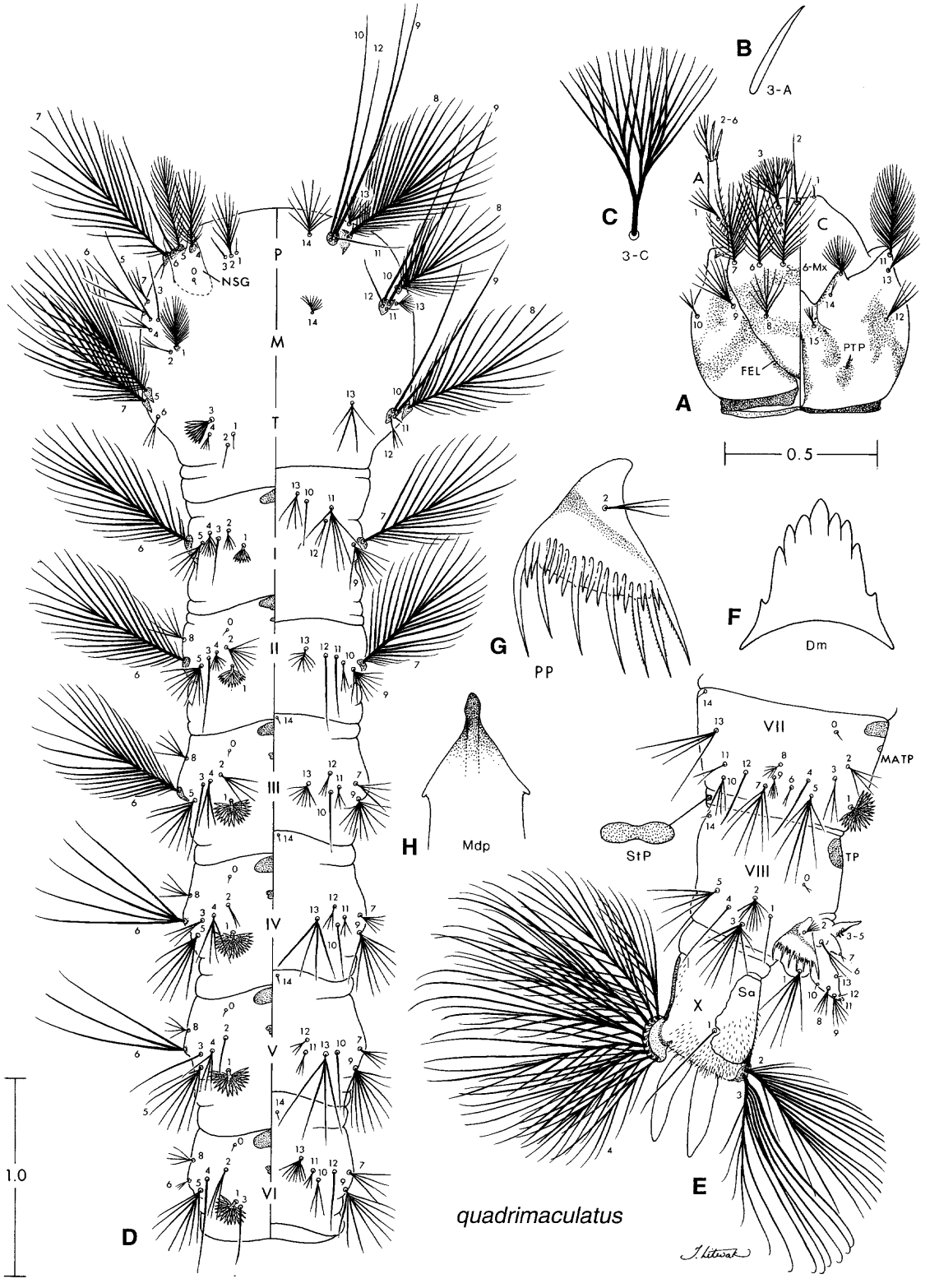

P
M
T
I
II
III
IV
V
VI
NSG
1.0
D
A
B
3-A
C
3-C
2-6
6-Mx
FEL
PTP
0.5
G
PP
F
Dm
H
Mdp
VII
MATP
StP
TP
VIII
3-5
Sa
X
E
quadrimaculatus

Fig. 16.

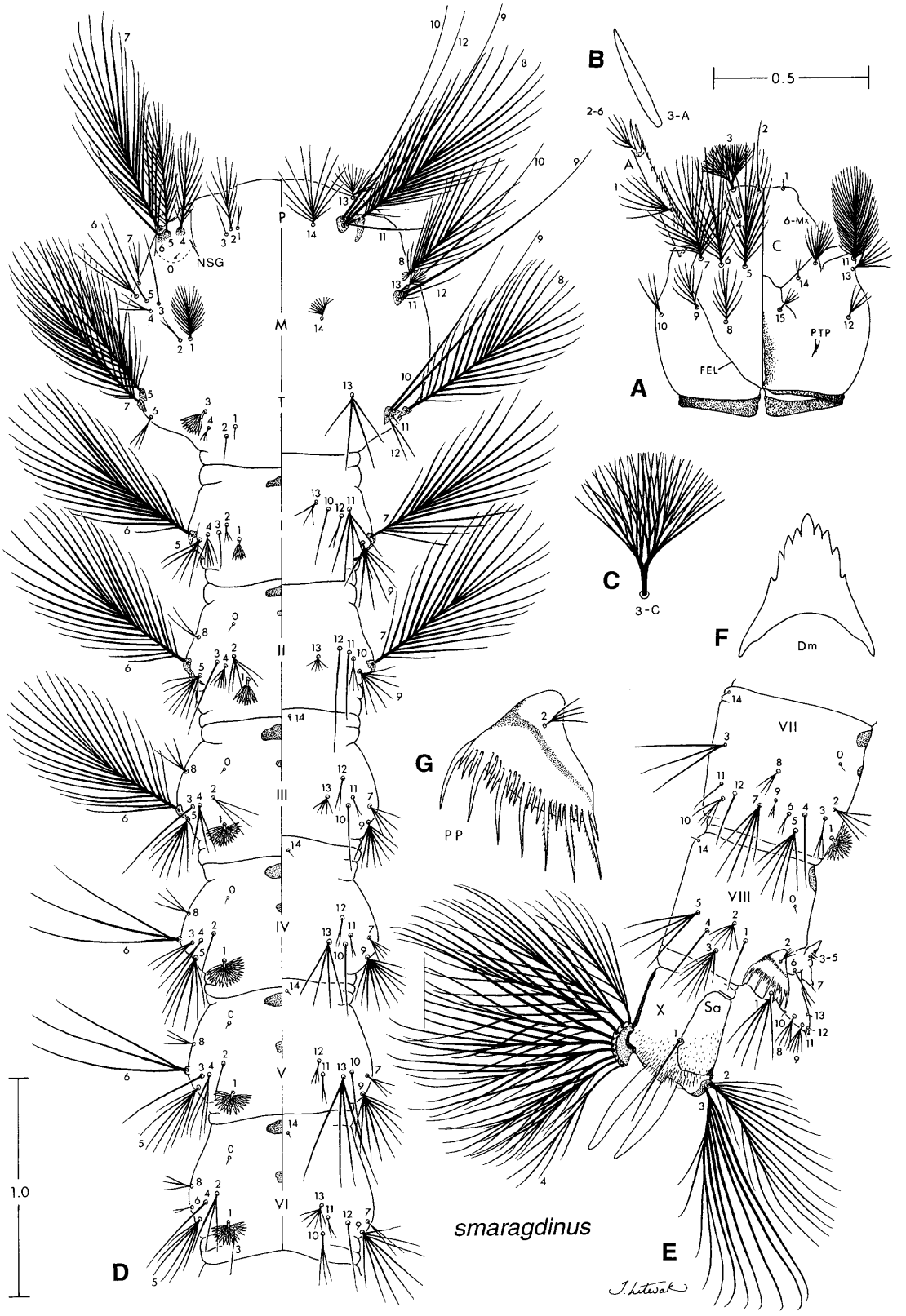

B
0.5
2-6
3-A
A
6-Mx
C
PTP
FEL
A
C
3-C
F
Dm
G
PP
VII
VIII
X
Sa
3-5
P
M
T
I
II
III
IV
V
VI
NSG
1.0
D
smaragdinus
E
J. Litwak

Fig. 17.

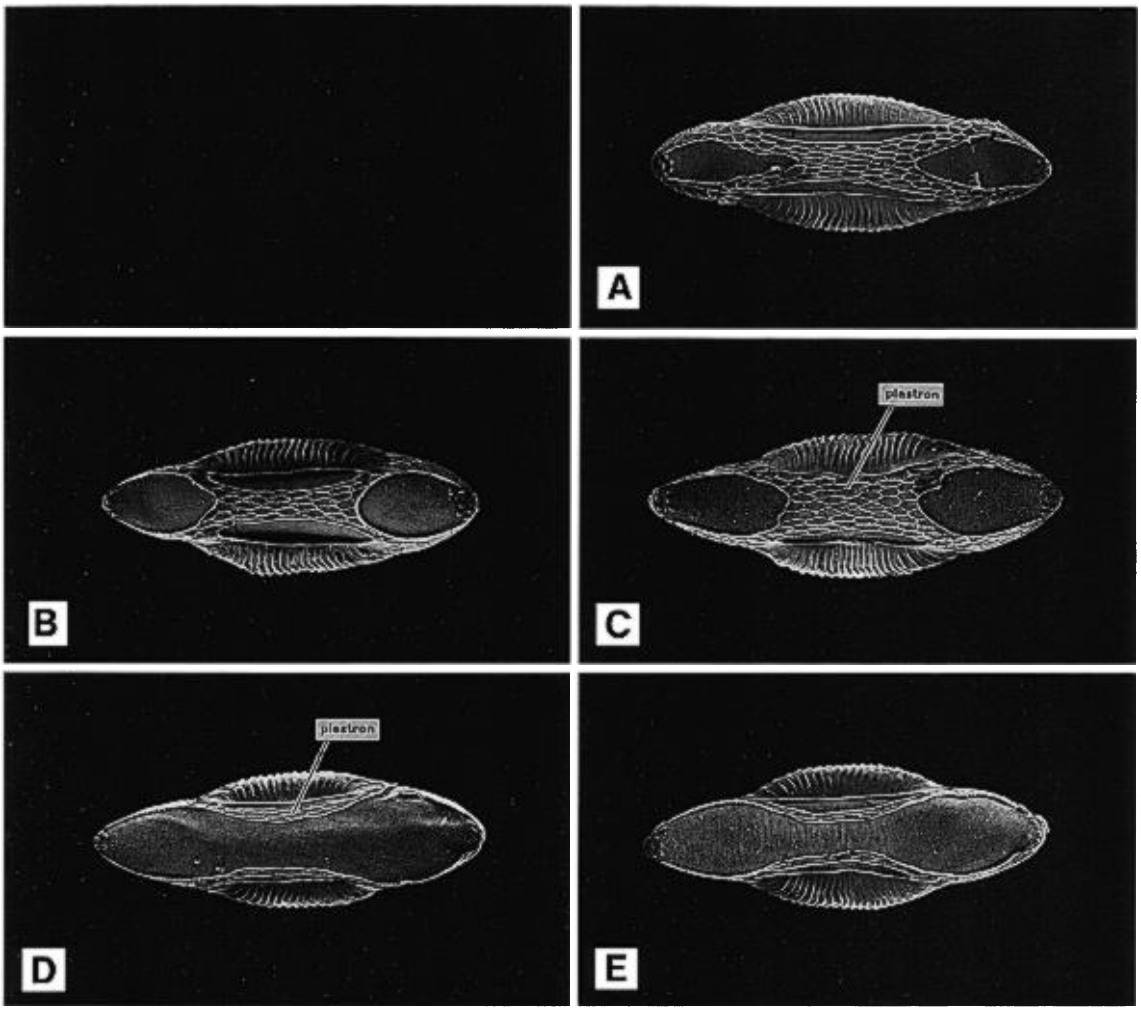


Fig. 18.

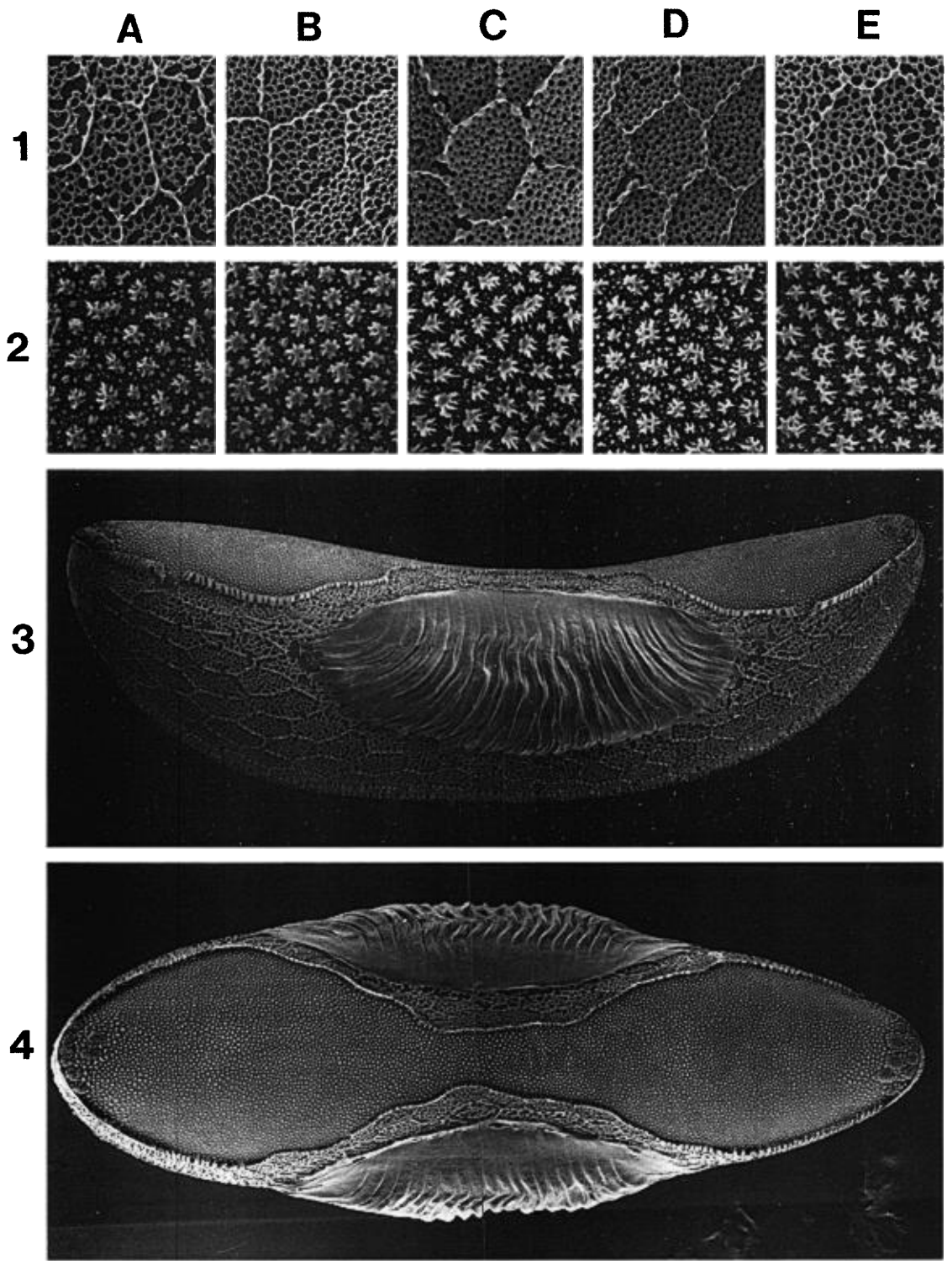


Fig. 19.

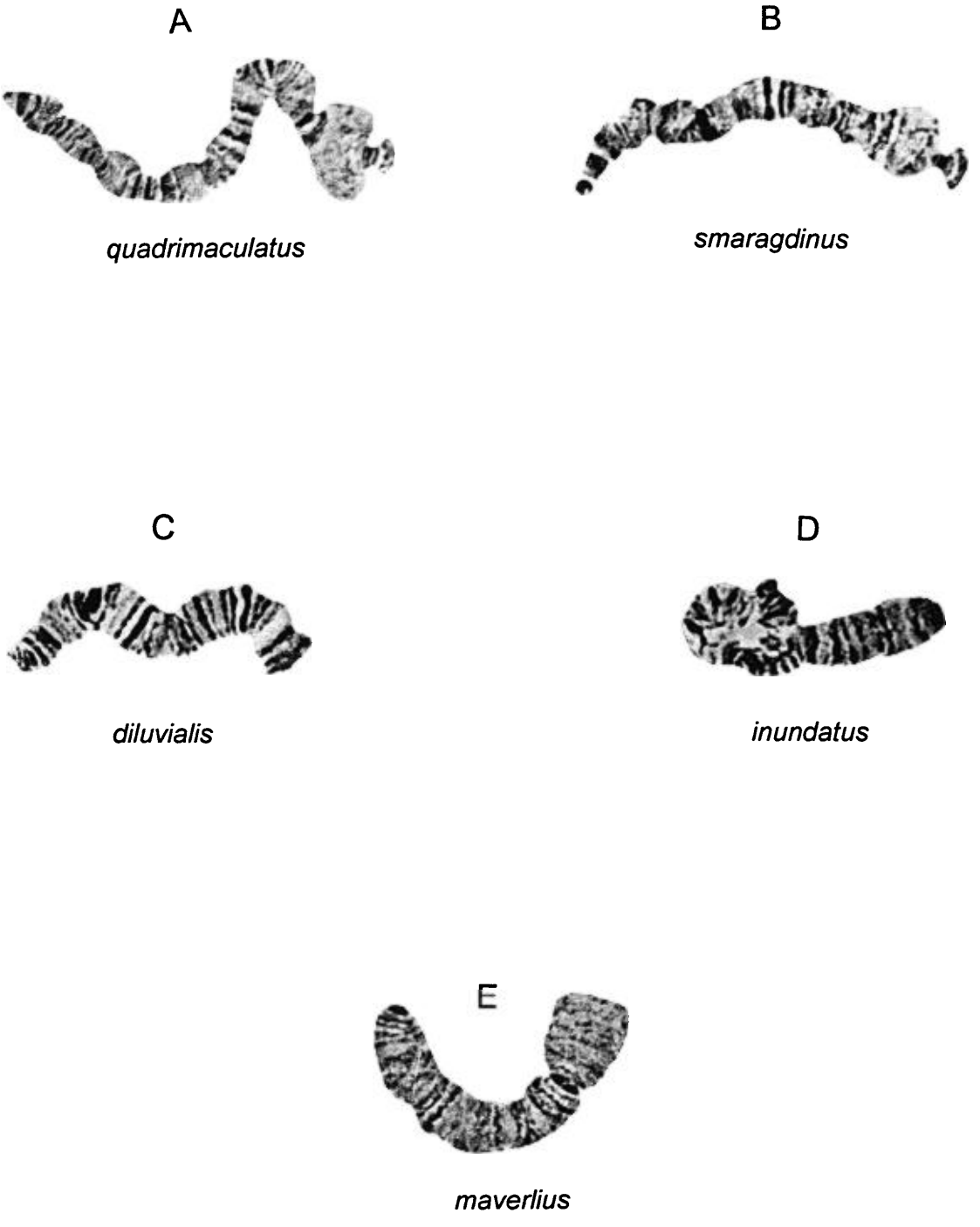

A
quadrimaculatus
B
smaragdinus
C
diluvialis
D
inundatus
E
maverlius

Fig. 20.

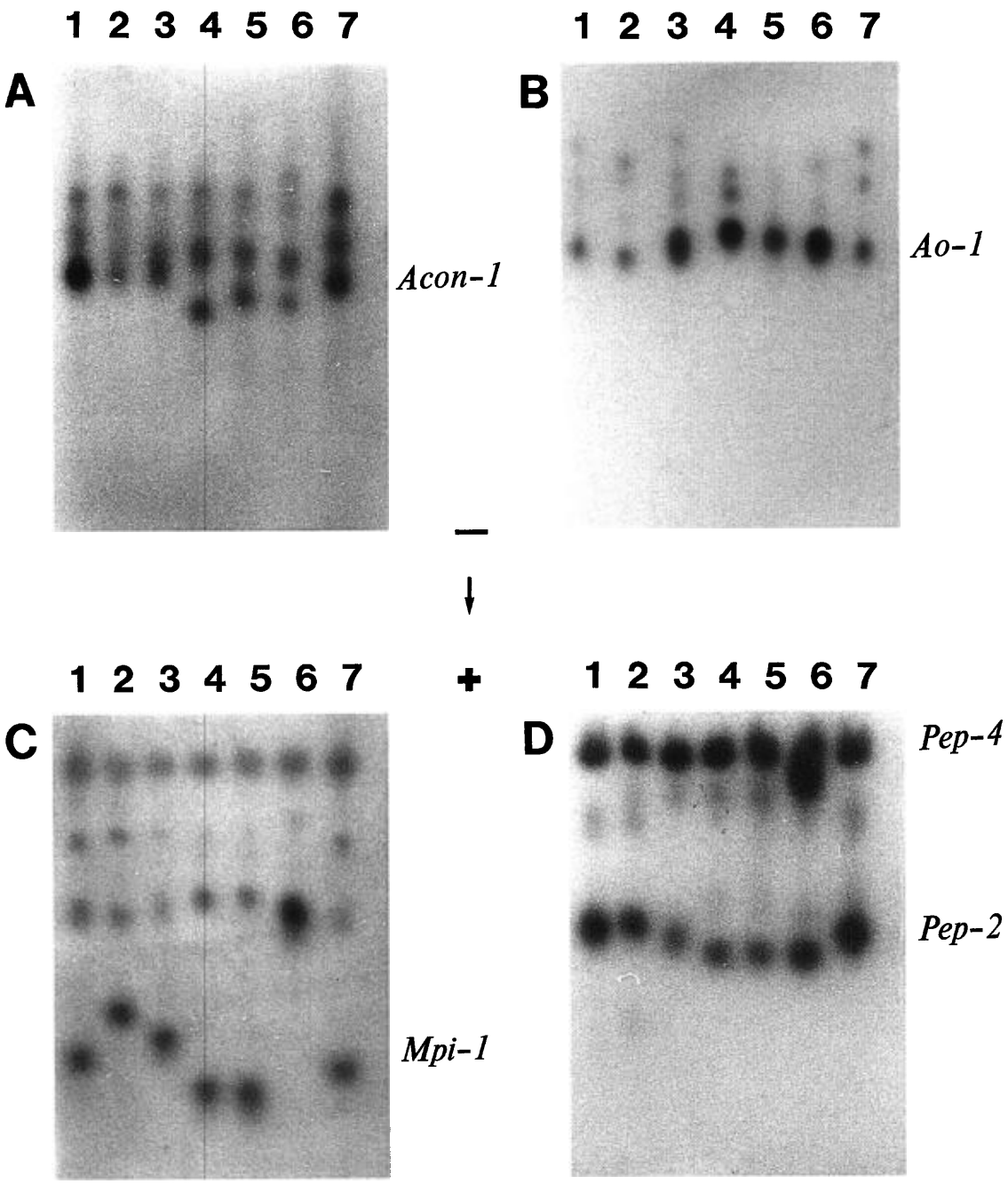

1 2 3 4 5 6 7
A
Acon-1
1 2 3 4 5 6 7
B
Ao-1
—
+
1 2 3 4 5 6 7
C
Mpi-1
1 2 3 4 5 6 7
D
Pep-4
Pep-2

Fig. 21.

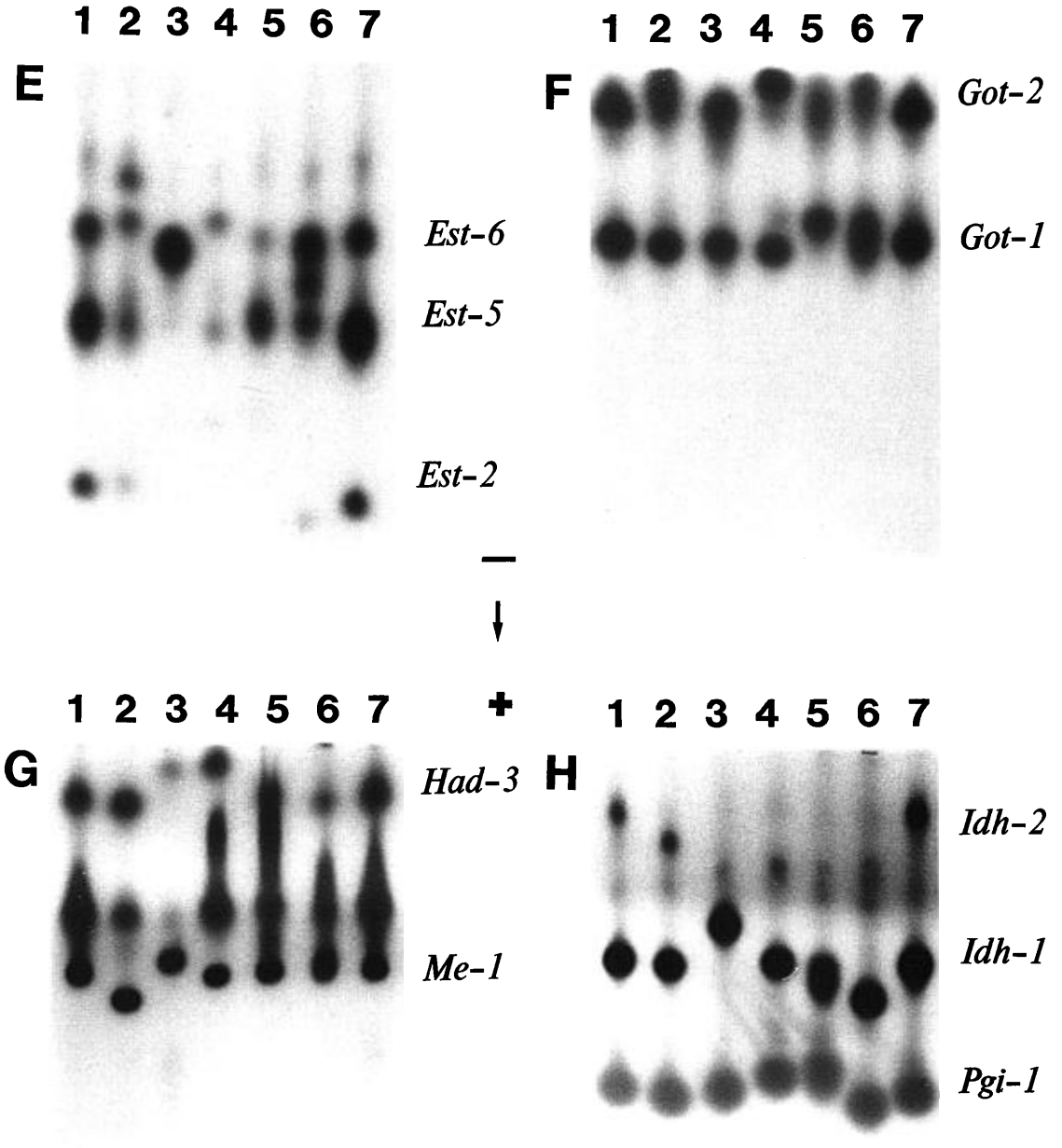

1 2 3 4 5 6 7
E
Est-6
Est-5
Est-2
1 2 3 4 5 6 7
F
Got-2
Got-1
1 2 3 4 5 6 7
G
Had-3
Me-1
1 2 3 4 5 6 7
H
Idh-2
Idh-1
Pgi-1

Fig. 22.

Fig. 23.

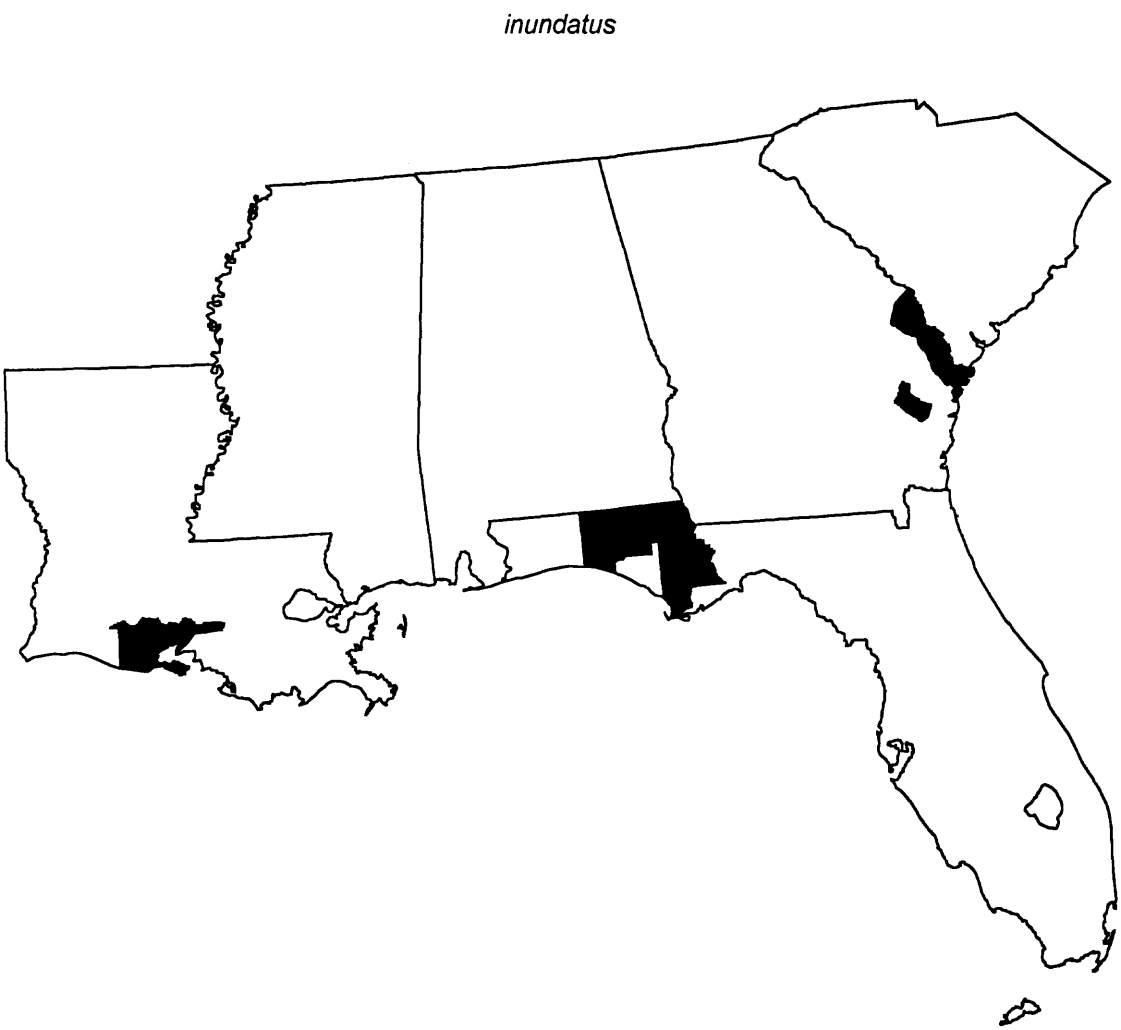


Fig. 24.

Fig. 25.

quadrimaculatus

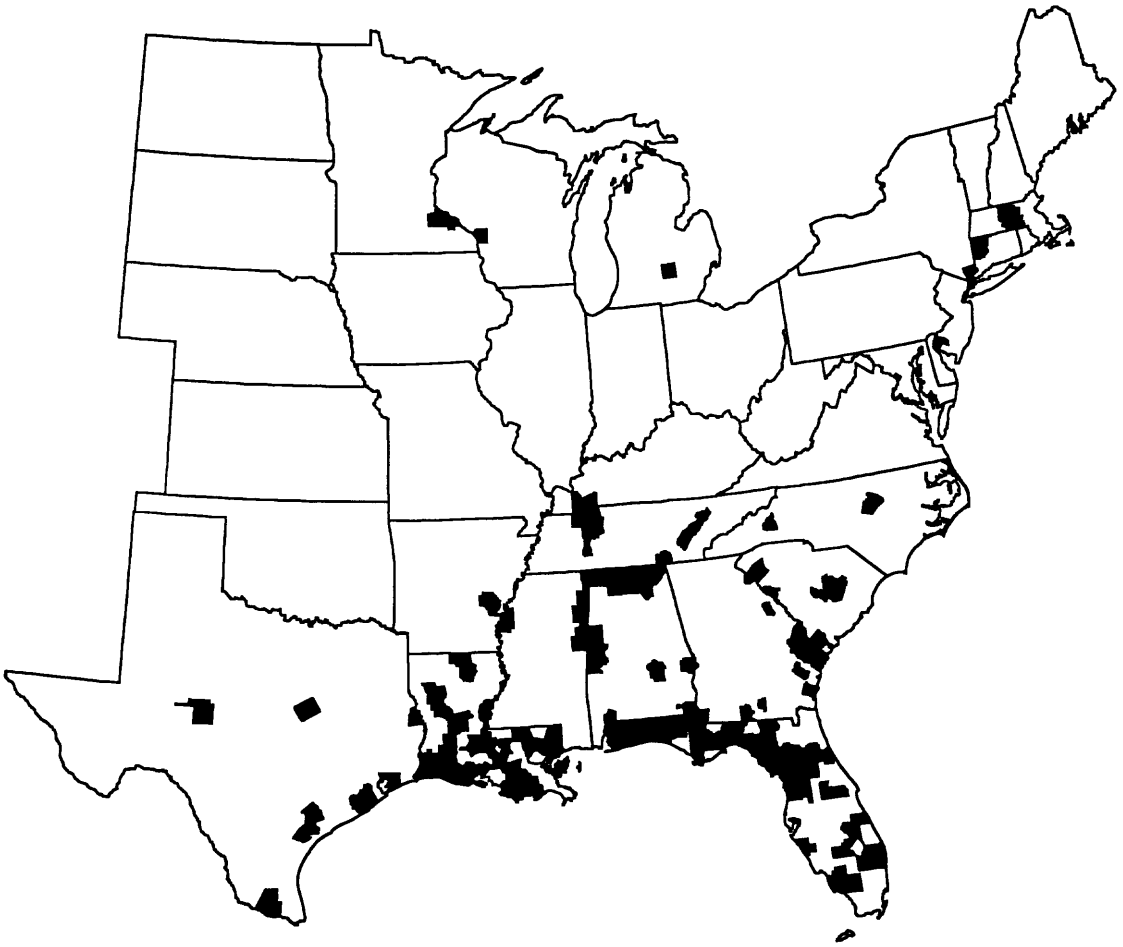


Fig. 26.

smaragdinus

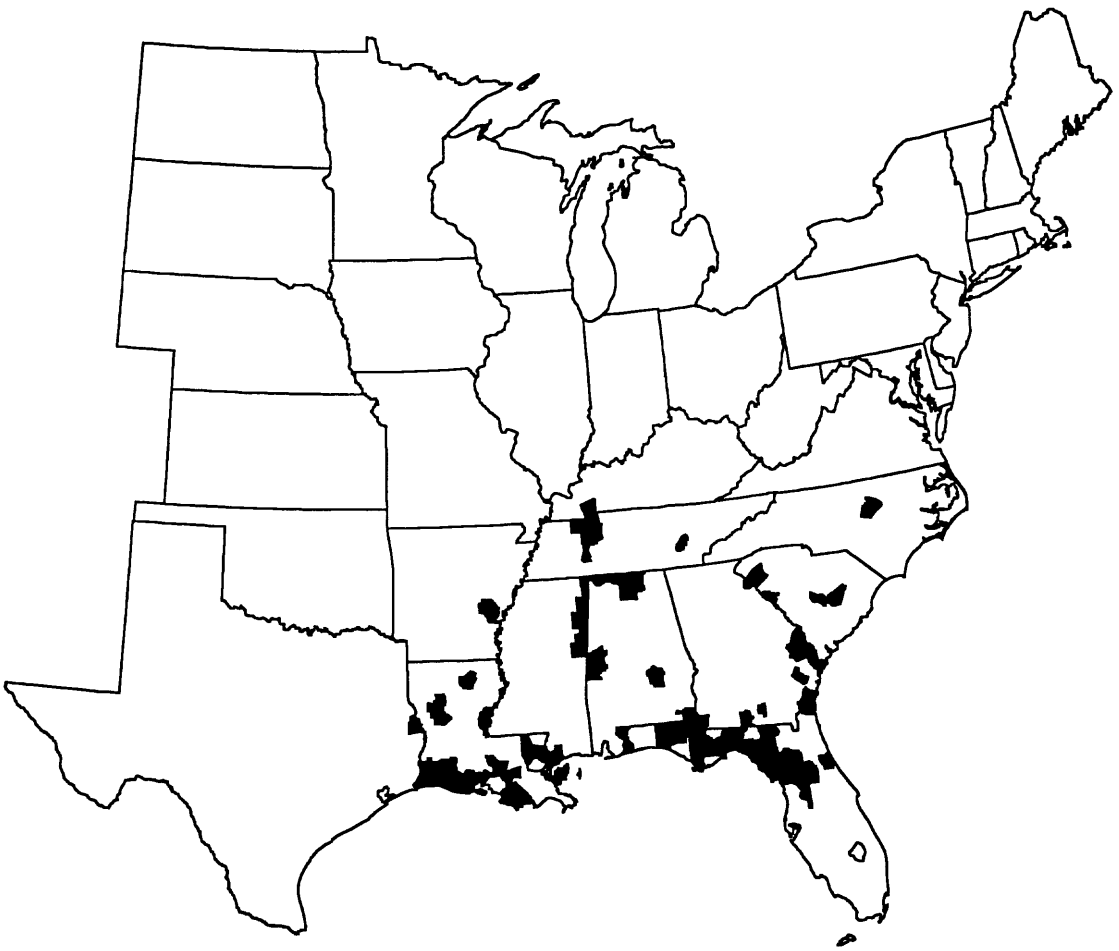


Fig. 27.

Table 1. Percentage of females with numbers of setae on indicated structure.

Character[1]	Species	No. examined	No. of setae											
			1	2	3	4	5	6	7	8	9	10	11	12
ISe	*diluvialis*	320			3.4	30.6	34.4	25.3	4.4	1.9				
	inundatus	320					10.1	51.6	22.5	13.8	2.2			
	maverlius	304			19.1	38.5	25.0	14.1	2.6	0.7				
	quadrimaculatus	320						1.9	26.6	29.3	28.4	9.4	3.4	0.9
	smaragdinus	393				2.0	26.5	51.9	15.8	3.8				
PaS	*diluvialis*	548	1.1	12.6	31.2	27.4	21.7	4.4	1.6					
	inundatus	548		6.4	18.4	30.3	27.0	13.3	3.3	0.9	0.4			
	maverlius	367		0.5	8.2	15.3	31.6	25.3	10.9	6.0	1.6		0.5	
	quadrimaculatus	548			0.4	1.5	11.1	29.7	27.9	17.7	6.2	4.0	1.3	0.2
	smaragdinus	652	2.5	8.6	23.9	30.1	22.5	8.6	3.6	0.8				
PsA	*diluvialis*	418	8.9	32.8	39.0	13.9	5.5							
	inundatus	473	11.2	33.0	33.0	16.9	4.7	0.8						
	maverlius	418	1.4	22.2	43.8	25.8	5.7	1.0						
	quadrimaculatus	418	1.2	10.5	24.4	29.7	19.4	9.8	3.6	1.0	0.2	0.2		
	smaragdinus	418	2.2	17.9	28.2	28.7	16.5	4.5	1.4	0.2	0.2			
MkSU	*diluvialis*	352	0.6	21.3	49.1	23.0	5.7	0.3						
	inundatus	352	8.2	37.5	36.6	15.9	1.4	0.3						
	maverlius	352	10.8	51.1	29.8	6.5	1.4	0.3						
	quadrimaculatus	352		1.1	5.1	17.9	27.6	30.4	14.2	3.4	0.3			
	smaragdinus	352		2.0	17.0	28.4	31.8	15.1	4.3	1.4				
MkSL	*diluvialis*	348		2.0	20.4	37.4	32.5	6.6	1.1					
	inundatus	348		0.3	8.6	33.6	36.5	14.7	6.3					
	maverlius	348		2.3	12.9	41.4	30.2	10.9	2.3					
	quadrimaculatus	348			1.7	7.8	26.1	33.6	19.3	8.0	2.6	0.9		
	smaragdinus	348		9.5	27.0	34.8	21.3	6.9	0.6					

[1] ISe, interocular seta; MkSL, lower mesokatepisternal seta; MkSU, upper mesokatepisternal seta; PaS, prealar seta; PsA, prespiracular seta.

Table 2. Percentage of females with numbers of setae on indicated structure.

Character[1]	Species	No. examined	No. of setae											
			2	3	4	5	6	7	8	9	10	11	12	13
PeSU	*diluvialis*	612					1.1	9.0	13.1	13.9	19.3	11.3	10.3	5.2
	inundatus	676		0.3	0.1	0.7	3.0	13.5	28.0	28.6	13.5	7.5	3.6	0.7
	maverlius	644			0.2	1.1	4.5	25.5	33.9	21.3	8.5	3.1	1.4	0.5
	quadrimaculatus	1,479	5.7	35.0	35.9	16.6	5.4	1.3	0.1					
	smaragdinus	867	4.4	22.6	33.2	26.2	12.1	1.4		0.1				
MeSU	*diluvialis*	327			0.6	4.3	9.8	13.8	23.9	18.7	12.2	9.5	4.3	2.1
	inundatus	327				1.8	5.2	10.1	15.6	21.7	16.2	13.8	8.0	6.1
	maverlius	327				0.3	1.8	6.4	16.2	20.2	21.7	13.8	11.6	4.3
	quadrimaculatus	327				0.6	0.6	2.1	4.3	13.1	22.0	20.8	15.9	10.4
	smaragdinus	327		0.3	2.1	7.3	9.5	17.1	17.4	16.2	9.8	9.5	5.8	2.1

[1] MeSU, upper mesepimeral seta; PeSU, upper proepisternal seta.

Table 3. Percentage of females with numbers of setae on scutal fossal area (1 side).

Species	No. examined	No. of setae														
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
diluvialis	817		0.2	1.8	5.9	7.3	11.8	13.6	15.1	14.2	12.1	7.7	3.7	3.3	1.8	0.7
inundatus	862								0.6	1.0	1.9	3.5	4.4	5.9	14.3	14.7
maverlius	717		0.1	0.1	0.2	1.0	2.0	5.3	10.7	9.6	13.4	14.6	14.1	11.0	7.3	4.3
quadrimaculatus	848								0.1	0.2	0.2	0.8	1.9	3.7	8.5	11.6
smaragdinus	848	0.2	0.5	1.8	2.2	5.4	10.8	11.8	16.7	12.3	11.1	9.3	6.8	5.0	2.5	1.5

Table 4. Percentage of females with numbers of setae for the sum of upper and lower mesokatepisternum.

Species	No. examined	No. of setae															
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
diluvialis	346		1.4	5.5	21.7	25.7	26.0	12.4	6.6	0.6							
inundatus	346		0.6	8.7	17.9	26.6	25.1	11.0	8.4	1.7		0.3					
maverlius	346	0.3	2.6	13.0	26.3	29.8	18.8	6.4	2.6	0.3							
quadrimaculatus	346				0.6	2.6	4.9	6.6	18.5	19.1	19.4	13.6	9.0	3.8	0.6	1.2	0.3
smaragdinus	346		1.4	2.6	8.4	17.9	24.0	22.3	11.6	6.9	2.3	2.0	0.6				

Table 2. Extended.

No. of setae												
14	15	16	17	18	19	20	21	22	23	24	25	26
4.7	2.9	2.1	2.1	1.8	0.8	0.7	0.5	0.2	0.2	0.2	0.2	0.5
0.3	0.3											
0.2												
0.3	0.3	0.3										
0.9	0.6											
2.4	1.2											
5.2	2.8	1.2	0.9									
1.8	0.6		0.3									

Table 3. Extended.

No. of setae																						
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
0.5	0.1	0.1																				
12.8	10.7	9.6	8.0	5.0	3.7	2.0	1.0	0.7	0.2													
3.3	0.7	1.3	0.8																			
10.4	10.5	10.1	11.3	7.5	6.0	4.5	3.7	2.4	1.7	1.9	0.8	0.2	0.6	0.5	0.4	0.2		0.2				0.1
1.3	0.4	0.1	0.1		0.1																	

Table 5. Percentage of females with indicated numbers of setae for the sum of both scutal fossal areas.

Species	No. examined	No. of setae																												
		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
diluvialis	437				0.5	1.4	2.1	2.5	5.3	3.9	6.4	5.0	8.2	8.5	8.9	6.9	9.6	6.6	6.9	3.9	2.5	3.0	1.4	2.1	1.6	0.7	0.7	0.5	0.5	0.2
inundatus	481															0.2	0.4	0.4	0.8	0.2	2.0	1.2	3.1	1.9	2.9	4.8	7.5	5.2	10.6	6.2
maverlius	361						0.3	0.3	0.3	0.3	1.1	0.6	1.9	1.9	7.2	4.7	6.4	5.8	7.5	7.5	9.7	8.0	7.8	5.8	4.4	4.2	3.3	4.2	0.6	3.0
quadrimaculatus	429																			0.5	0.2		0.5	0.5	1.4	2.3	3.0	5.6	7.9	5.6
smaragdinus	429	0.2	0.2	0.2	0.7	0.2	0.7	2.6	1.2	2.1	5.6	5.1	8.2	7.9	8.4	8.6	9.1	6.3	6.1	4.0	5.4	3.5	3.5	2.3	1.6	1.4	1.6	0.9	0.5	0.5

Table 5. Continued.

Species	No. of setae																													
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	74	75	76
diluvialis	0.5																													
inundatus	9.4	5.6	8.1	3.7	5.2	3.5	3.7	2.0	4.2	2.7	1.9	0.8	1.2	0.2																
maverlius	1.4	0.6	0.3	1.1																										
quadrimaculatus	5.4	6.1	5.6	4.7	4.0	5.6	7.0	5.8	4.4	3.0	4.0	1.4	2.3	2.3	2.1	0.5	1.2	1.4	1.2	0.2	0.7	0.5	0.5	0.2	0.2	0.5	0.7	0.5	0.2	0.5
smaragdinus	0.5	0.5	0.2			0.2																								

Table 6. Record of branching of setae on pupae of *Anopheles diluvialis* (20 specimens).

Seta no.	Cephalothorax	Abdominal segment I	II	III	IV	V	VI	VII	VIII	IX	Paddle
0			1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (1)	1–3 (1)		
1	2–5 (3)	13–40 (19)	6–17 (7)	7–13 (11)	6–12 (9)	2–10 (5)	1–3 (1)	1–4 (1)		1–4 (2)	1, 2 (1)
2	2–5 (3)	3–8 (6)	6–11 (8)	5–11 (7)	3–8 (6)	3–8 (5)	2–7 (5)	3–6 (5)			1–3 (2)
3	2–6 (3)	2–6 (3)	1–3 (1)	3–7 (4)	4–6 (6)	2–6 (3)	2–5 (3)	3–5 (4)			
4	2–6 (3)	6–12 (7)	3–8 (4)	1–9 (4)	2–6 (4)	2–8 (4)	2–5 (3)	1–4 (2)	2–5 (3)		
5	4–10 (5)	3–10 (5)	3–9 (5)	8–13 (8)	6–14 (10)	4–9 (7)	3–7 (4)	2–6 (4)			
6	2–5 (3)	2–9 (5)	2–6 (4)	3–11 (7)	3–6 (4)	2–5 (3)	1, 2 (2)	1–4 (2)			
7	1	4–12 (6)	4–12 (6)	2–7 (4)	2–6 (5)	2–7 (4)	1, 2 (1)	1–4 (1)			
8	1–4 (2)			2–6 (3)	1–6 (3)	1–5 (3)	2–7 (3)	3–5 (3)			
9	3–6 (4)	1–4 (2)	1	1	1	1	1	1	8–21 (14)		
10	1–5 (2)			1–5 (3)	1–4 (3)	1–3 (1)	1, 2 (1)	1–3 (2)			
11	4–7 (5)			1–4 (1)	1–3 (1)	1–3 (2)	1–4 (2)	1–3 (2)			
12	3–6 (3)										
13											
14				1	1	1	1	1	1		

Table 7. Record of branching of setae on pupae of *Anopheles inundatus* (20 specimens).

Seta no.	Cephalothorax	Abdominal segment I	II	III	IV	V	VI	VII	VIII	IX	Paddle
0			1, 2 (1)	1	1, 2 (1)	1, 2 (1)	1–3 (1)	1–3 (1)	1–3 (1)		
1	2–5 (4)	16–37 (20)	4–22 (10)	7–13 (10)	7–13 (9)	2–7 (6)	1–4 (1)	1–3 (1)		1–4 (2)	1
2	2–5 (3)	5–10 (6)	6–12 (10)	3–11 (7)	3–7 (5)	3–10 (5)	2–6 (3)	4–7 (5)			1–4 (2)
3	2–4 (3)	2–5 (4)	1–3 (1)	3–7 (5)	4–9 (7)	2–5 (3)	2–4 (3)	2–6 (5)			
4	2–8 (5)	6–11 (8)	2–7 (4)	3–6 (4)	2–6 (4)	3–6 (5)	1–4 (2)	1–4 (2)	2–5 (3)		
5	4–11 (6)	4–11 (6)	4–7 (5)	5–15 (10)	8–15 (10)	3–9 (5)	3–8 (3)	2–6 (3)			
6	2–4 (3)	3–8 (5)	2–8 (4)	4–11 (6)	2–6 (4)	2–5 (3)	1, 2 (1)	1–4 (3)			
7	1	4–12 (6)	3–7 (5)	4–7 (5)	3–6 (4)	3–6 (4)	1	1, 2 (1)			
8	1–4 (3)			2–7 (4)	2–5 (3)	2–5 (3)	1–5 (4)	2–7 (5)			
9	2–6 (4)	1–4 (2)	1	1	1	1	1	1	7–23 (14)		
10	1–8 (3)			1–5 (3)	1–3 (2)	1, 2 (1)	1, 2 (1)	1–4 (2)			
11	3–7 (5)			1–3 (2)	1–3 (2)	1–3 (2)	1–3 (2)	1–3 (2)			
12	2–6 (3)										
13											
14				1	1	1	1	1	1–5 (1)		

Table 8. Record of branching of setae on pupae of *Anopheles maverlius* (20 specimens).

Seta no.	Cephalothorax	Abdominal segment I	II	III	IV	V	VI	VII	VIII	IX	Paddle
0			1, 2 (1)	1	1	1	1	1, 2 (1)	1–3 (1)	1, 2 (1)	
1	2–4 (3)	15–44 (19)	3–11 (6)	6–12 (8)	5–10 (7)	2–10 (3)	1–3 (1)	1–3 (1)			1–3 (1)
2	2–4 (3)	5–8 (6)	3–9 (6)	3–7 (5)	2–6 (5)	3–7 (5)	2–6 (4)	3–5 (4)			1–3 (2)
3	2–5 (3)	2–4 (3)	1–4 (2)	3–6 (4)	3–7 (5)	1–6 (3)	1–4 (2)	3–6 (5)			
4	2–4 (3)	5–14 (5)	3–7 (5)	3–7 (5)	3–6 (5)	3–6 (4)	1–3 (2)	1–3 (2)	2–4 (3)		
5	4–8 (6)	2–5 (3)	3–6 (4)	6–14 (9)	5–12 (7)	3–7 (5)	2–4 (3)	1–4 (3)			
6	2–4 (3)	1–6 (3)	1–4 (3)	2–8 (4)	2–4 (3)	1–3 (2)	1, 2 (1)	1–4 (1)			
7	1	3–9 (5)	2–6 (4)	3–7 (5)	2–7 (5)	2–5 (5)	1, 2 (1)	1			
8	1–4 (2)			3–7 (4)	2–5 (3)	2–5 (3)	2–4 (3)	2–5 (4)			
9	2–6 (3)	1–3 (2)	1	1	1	1	1	1	4–15 (10)		
10	2–10 (5)		0–4 (0)	1–4 (3)	1–3 (1)	1	1	1–3 (1)			
11	3–7 (5)			1, 2 (1)	1, 2 (1)	1, 2 (1)	1–3 (1)	1–3 (2)			
12	2–5 (3)										
13				0, 1 (0)							
14				1	1	1	1	1	1		

Table 9. Record of branching of setae on pupae of *Anopheles quadrimaculatus* (20 specimens).

Seta no.	Cephalothorax	Abdominal segment I	II	III	IV	V	VI	VII	VIII	IX	Paddle
0			1, 2 (1)	1, 2 (1)	1–3 (1)	1–4 (1)	1–4 (1)	1–3 (1)	1–3 (1)		
1	2–5 (3)	17–46 (19)	3–17 (8)	4–15 (8)	4–10 (7)	1–5 (3)	1–3 (1)	1, 2 (1)		1–3 (2)	1–6 (3)
2	2–4 (3)	3–9 (5)	5–10 (8)	3–10 (6)	3–6 (5)	3–7 (5)	2–6 (3)	3–7 (5)			1–4 (2)
3	3–6 (4)	2–6 (3)	1–4 (1)	1–7 (3)	3–7 (6)	1–3 (2)	1–3 (2)	3–6 (4)			
4	2–5 (4)	5–10 (8)	3–8 (5)	1–6 (4)	2–6 (4)	3–6 (5)	1–3 (2)	1–4 (2)	2–4 (3)		
5	4–8 (6)	3–7 (4)	3–6 (4)	5–13 (10)	5–12 (7)	2–7 (3)	2–5 (3)	1–3 (2)			
6	2–4 (3)	2–5 (3)	1–5 (2)	2–9 (4)	2–4 (2)	1–3 (2)	1, 2 (1)	1–3 (2)			
7	1, 2 (1)	3–9 (5)	3–7 (5)	2–10 (6)	2–7 (5)	3–6 (5)	1, 2 (1)	1, 2 (1)			
8	2–5 (3)		0–5 (0)	2–8 (6)	2–6 (3)	2–4 (3)	2–5 (4)	3–6 (4)			
9	2–4 (3)	2–5 (2)	1	1	1	1	1	1	8–23 (13)		
10	1–11 (3)	0, 1 (0)	0, 1 (0)	2–5 (3)	1–3 (2)	1–4 (2)	1–3 (2)	1–4 (2)			
11	3–6 (4)	0–2 (0)	0–4 (0)	1–4 (1)	1–3 (1)	1, 2 (1)	1–3 (1)	2–4 (3)			
12	2–5 (3)										
13						0–3 (0)	0–6 (0)	0, 1 (0)	0–2 (0)		
14				1	1	1	1, 2 (1)	1	1		

Table 10. Record of branching of setae on pupae of *Anopheles smaragdinus* (20 specimens).

Seta no.	Cephalothorax	Abdominal segment I	II	III	IV	V	VI	VII	VIII	IX	Paddle
0			1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (1)	1–3 (1)		
1	2–5 (3)	16–47 (22)	3–8 (6)	5–11 (8)	3–8 (5)	1–3 (2)	1–3 (1)	1, 2 (1)		1–3 (2)	1, 2 (1)
2	2–5 (3)	3–8 (6)	5–8 (7)	4–8 (5)	3–6 (4)	2–8 (4)	2–6 (3)	3–5 (4)			1–3 (2)
3	2–4 (3)	1–4 (2)	1–3 (1)	2–6 (3)	2–8 (5)	2, 3 (3)	1–4 (2)	2–5 (3)			
4	2–5 (3)	4–10 (6)	2–7 (4)	1–6 (4)	1–6 (4)	2–6 (4)	1–3 (2)	1–3 (2)	2–4 (3)		
5	2–7 (5)	2–6 (4)	2–4 (3)	4–10 (7)	3–9 (6)	2–5 (3)	1–4 (2)	1–4 (2)			
6	2–4 (2)	1–5 (2)	1–4 (2)	2–6 (3)	1–3 (2)	1–3 (2)	1, 2 (1)	1–3 (1)			
7	1, 2 (1)	2–7 (4)	2–6 (3)	2–8 (4)	1–6 (3)	1–5 (3)	1	1			
8	1–4 (2)			2–4 (3)	2–5 (3)	2–5 (3)	2–4 (3)	1–5 (3)			
9	2–5 (3)	1–4 (2)	1	1	1	1	1	1	5–14 (9)		
10	1–9 (2)		0–3 (0)	2–4 (3)	1–3 (2)	1, 2 (1)	1, 2 (1)	1–4 (2)			
11	3–5 (4)			1, 2 (1)	1, 2 (1)	1, 2 (1)	1–4 (1)	1–5 (3)			
12	2–7 (4)										
13											
14				1	1	1	1	1	1		

Table 11. Sum of both pupal seta 10-CT[1] branches listed by percent.

Species	No. examined	No. of branches 2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
diluvialis	350	20.0	22.9	34.0	14.9	5.1	2.0	1.1										
inundatus	415			5.8	11.6	31.3	23.6	16.9	5.1	3.9	1.0	1.0						
maverlius	350					1.7	5.7	11.4	16.6	18.0	10.9	17.7	7.1	5.4	2.6	1.4	1.1	0.3
quadrimaculatus	350			7.7	24.9	23.7	16.9	12.6	6.3	2.9	4.3	0.6	0.3					
smaragdinus	350	13.7	16.0	27.7	12.3	13.4	5.1	4.6	2.0	1.4	2.3	0.9	0.3	0.3				

[1] 10-CT, seta 10 on cephalothorax.

Table 12. Sum of both pupal seta 1-Pa[1] branches listed by percent.

Species	No. examined	No. of branches										
		2	3	4	5	6	7	8	9	10	11	12
diluvialis	457	97.2	2.8									
inundatus	457	98.7	1.3									
maverlius	457	82.1	11.4	4.2	2.2	0.2						
quadrimaculatus	837	0.2	2.0	24.0	26.8	22.9	12.9	6.8	2.6	1.2	0.4	0.2
smaragdinus	543	85.3	12.9	1.9								

[1] 1-Pa, seta 1 on paddle.

Table 13. Sum of both pupal seta 5-I[1] branches listed by percent.

Species	No. Examined	No. of branches														
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
diluvialis	246				0.8	4.1	8.1	29.3	17.5	25.2	8.1	4.9	0.8	0.4	0.8		
inundatus	246						1.6	10.6	13.0	22.4	13.8	14.2	11.8	7.3	3.3	1.6	0.4
maverlius	246	0.8	6.9	40.2	28.5	18.7	4.1	0.8									
quadrimaculatus	246			2.4	8.5	35.8	29.3	17.5	5.3	0.8	0.4						
smaragdinus	246	0.9	7.7	22.0	34.1	27.6	6.1	1.2		0.4							

[1] 5-I, seta 5 on abdominal segment I.

Table 14. Sum of both pupal seta 6-III[1] branches listed by percent.

Species	No. examined	No. of branches																	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
diluvialis	225			0.9		2.7	6.7	10.7	14.7	17.3	16.4	18.2	7.1	0.9	3.1	1.3			
inundatus	225					1.3	6.2	12.9	12.9	21.3	17.3	12.9	6.7	4.0	2.2	1.3		0.4	0.4
maverlius	242		1.2	9.9	11.6	23.6	22.7	17.4	7.9	4.3	0.8	0.4							
quadrimaculatus	225		3.1	19.1	27.1	19.6	15.6	12.0	3.1	0.4									
smaragdinus	225	16.9	23.1	34.7	17.8	5.3	1.3	0.9											

[1] 6-III, seta 6 on abdominal segment III.

Table 15. Sum of both pupal seta 6-I[1] branches listed by percent.

Species	No. examined	No. of branches														
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
diluvialis	241				1.2	4.1	4.6	13.7	15.4	27.4	13.7	11.2	3.7	4.1	0.8	
inundatus	241					2.5	2.9	20.3	16.2	21.6	10.8	15.8	3.7	4.1	1.7	0.4
maverlius	241		0.8	4.1	6.6	23.2	25.3	23.7	10.4	4.6	0.8	0.4				
quadrimaculatus	241			19.9	14.5	40.7	17.4	6.6	0.4	0.4						
smaragdinus	241	1.7	2.5	28.2	26.1	27.8	10.0	3.3	0.4							

[1] 6-I, seta 6 on abdominal segment I.

Table 16. Sum of both pupal seta 7-I[1] branches listed by percent.

Species	No. examined	No. of branches																			
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
diluvialis	225					0.9	0.9	5.3	11.1	20.2	20.5	13.3	8.0	8.4	3.1	4.4	0.4	1.3	0.4	0.9	0.4	0.4
inundatus	225					0.4		5.3	5.8	14.7	15.1	18.7	10.7	11.6	11.6	3.1	1.8		0.4	0.9		
maverlius	250			0.8	2.0	4.8	8.8	23.6	21.2	22.4	8.8	4.4	1.6	1.6								
quadrimaculatus	225			0.9	1.3	6.2	11.1	34.7	17.8	15.6	8.4	1.8	1.8	0.4								
smaragdinus	225	0.9		8.4	16.9	27.6	22.2	16.4	6.7	0.9												

[1] 7-I, seta 7 on abdominal segment I.

Table 17. Sum of both pupal seta 9-VIII[1] branches listed by percent.

Species	No. examined	No. of branches															
		9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
diluvialis	375											0.3	0.3	0.3	0.8	6.1	3.2
inundatus	375						0.3			0.5	1.1	1.9	1.3	1.6	1.6	2.4	6.4
maverlius	375	0.3	1.6	0.8	1.3	2.1	4.5	3.5	9.3	6.9	9.1	9.9	11.7	10.4	8.8	8.5	4.3
quadrimaculatus	375													0.3	1.1	0.8	3.7
smaragdinus	392				1.0	1.8	4.3	4.8	7.7	9.9	12.8	15.6	15.3	11.2	7.4	5.4	1.5

[1] 9-VIII, seta 9 on abdominal segment VIII.

Table 18. Record of branching of setae on larvae of *Anopheles diluvialis* (15 specimens).

Seta no.	Cranium	Prothorax	Mesothorax	Metathorax	Abdominal segment		
					I	II	III
0		1				1, 2 (1)	1
1	1	2–5 (3)	18–27 (24)	1–4 (2)	4–10 (7)	6–11 (9)	15–22 (17)
2	1	6–12 (9)	2–5 (3)	1	3–8 (5)	6–10 (7)	3–9 (5)
3	19–44 (29)	1	1	8–13 (10)	1, 2 (1)	1	1
4	1–4 (2)	12–20 (15)	2–5 (3)	3–5 (4)	4–9 (6)	4–8 (5)	2–5 (3)
5	11–18 (15)	21–28 (26)	1	18–32 (23)	4–10 (7)	7–16 (8)	5–11 (8)
6	11–21 (16)	1	3–7 (4)	2–4 (3)	16–25 (18)	16–28 (20)	13–21 (18)
7	14–24 (18)	21–29 (24)	3, 4 (3)	18–26 (21)	11–19 (14)	16–27 (19)	2–5 (4)
8	5–9 (6)	20–28 (27)	10–19 (15)	18–29 (23)		3–8 (4)	2–6 (4)
9	4–11 (7)	1, 2 (1)	1–4 (1)	1	3–8 (5)	4–12 (7)	6–11 (10)
10	2–4 (2)	1	1	1	1, 2 (1)	2–4 (3)	1
11	27–45 (42)	1, 2 (1)	1, 2 (1)	1, 2 (1)	3–8 (4)	1	1–4 (3)
12	2–5 (4)	1	1	1–4 (2)	1–3 (2)	1	2, 3 (2)
13	4–8 (5)	7–18 (10)	5–10 (8)	2–4 (3)	4–9 (5)	5–11 (8)	5–12 (8)
14	2–5 (3)	6–13 (9)	6–13 (10)				1
15	3–7 (5)						

Table 19. Record of branching of setae on larvae of *Anopheles inundatus* (15 specimens).

Seta no.	Cranium	Prothorax	Mesothorax	Metathorax	Abdominal segment		
					I	II	III
0		1				1, 2 (1)	1, 2 (1)
1	1	2–4 (3)	17–27 (23)	1–4 (2)	6–12 (8)	7–12 (12)	16–22 (17)
2	1, 2 (1)	5–10 (7)	2–5 (3)	1	4–10 (6)	5–10 (8)	3–9 (5)
3	22–31 (28)	1, 2 (1)	1	7–13 (12)	1, 2 (1)	1	1
4	2–4 (3)	11–18 (14)	2–5 (4)	3–6 (4)	4–8 (5)	4–7 (5)	2–4 (3)
5	10–18 (13)	18–28 (22)	1	17–27 (21)	5–9 (7)	7–14 (10)	6–11 (8)
6	11–17 (15)	1	3–6 (4)	2, 3 (3)	15–26 (17)	17–29 (22)	12–23 (16)
7	10–21 (17)	20–32 (24)	2–4 (3)	15–26 (22)	10–19 (15)	15–25 (20)	3–5 (4)
8	4–8 (7)	18–28 (19)	11–24 (18)	15–31 (21)		3–9 (6)	3–7 (4)
9	5–9 (7)	1, 2 (1)	1	1, 2 (1)	4–11 (6)	6–14 (9)	8–14 (9)
10	2–4 (3)	1	1	1	1	2–4 (3)	1, 2 (1)
11	38–53 (38)	1–3 (1)	1	1, 2 (1)	2–6 (4)	1	1–4 (2)
12	2–5 (4)	1	1, 2 (1)	2–4 (3)	1–5 (2)	1	2, 3 (2)
13	4–8 (6)	7–14 (11)	6–13 (8)	2–5 (3)	3–8 (5)	6–12 (8)	5–10 (7)
14	1–5 (2)	4–12 (7)	7–15 (10)				1, 2 (1)
15	4–7 (5)						

Table 17. Extended.

No. of branches																		
25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
10.4	12.0	10.9	15.7	11.7	9.9	7.5	5.6	2.4	1.9	0.3	0.5	0.3						
5.6	12.0	10.9	8.0	11.5	12.0	6.9	6.4	2.7	2.9	1.3	1.3	0.5	0.5			0.3		
2.9	2.4	0.8	0.3	0.3	0.3													
13.3	12.3	13.6	10.7	9.3	6.9	6.9	6.4	5.3	1.6	2.9	0.8	1.3	0.8	0.3	0.3	0.5		0.5
0.8	0.5																	

Table 18. Extended.

Abdominal segment						
IV	V	VI	VII	VIII	X	Siphon
1, 2 (1)	1, 2 (1)	1	1, 2 (1)	2–5 (2)		
14–23 (20)	12–23 (18)	14–21 (18)	13–21 (17)	1	1	3–7 (4)
1, 2 (1)	1, 2 (1)	3–6 (4)	3–7 (6)	5–9 (7)	14–21 (17)	4–8 (5)
2–4 (3)	1	1	2–6 (3)	5–11 (7)	7–12 (9)	
2, 3 (3)	3–9 (5)	1	1	1		1
5–9 (7)	5–9 (7)	5–10 (7)	5–11 (6)	3–5 (4)		1
2–6 (3)	2–4 (3)	2–5 (3)	2–5 (4)			1–3 (2)
2–5 (3)	2–5 (3)	2–5 (3)	3–6 (4)			1, 2 (2)
2–4 (3)	2–5 (3)	2–5 (3)	3–7 (4)			2–5 (3)
7–11 (7)	6–10 (9)	5–9 (8)	2–5 (4)			3–7 (4)
1	1	1–4 (2)	3–6 (4)			
2, 3 (2)	2, 3 (3)	1–3 (2)	1–4 (1)			1
2, 3 (3)	1–3 (3)	1	1			1, 2 (1)
3–6 (4)	3–5 (4)	6–13 (9)	2–4 (3)			1
1	1	1	1	1		

Table 19. Extended.

Abdominal segment						
IV	V	VI	VII	VIII	X	Siphon
1, 2 (1)	1, 2 (1)	1, 2 (1)	1–3 (1)	1–3 (2)		
15–23 (22)	8–23 (16)	13–21 (17)	12–23 (16)	1	1	3–5 (4)
1, 2 (1)	1, 2 (1)	2–5 (4)	4–8 (5)	5–10 (7)	14–19 (17)	4–8 (6)
2–4 (3)	1	1	2–4 (3)	5–12 (7)	7–10 (9)	
2, 3 (2)	3–6 (5)	1	1	1		1
5–8 (6)	6–10 (6)	5–10 (8)	4–9 (7)	4, 5 (4)		1
2–5 (3)	2–4 (3)	3–6 (3)	3–7 (4)			1–3 (2)
2–4 (3)	2–5 (4)	2–4 (3)	3–7 (5)			1, 2 (2)
3–5 (3)	2–4 (3)	2–5 (3)	3–6 (5)			3–5 (4)
6–14 (8)	7–11 (9)	7–9 (7)	3–6 (4)			3–6 (4)
1, 2 (1)	1	2–5 (2)	3–7 (4)			
1–4 (2)	1–3 (3)	1–3 (1)	1–3 (1)			1
1–3 (3)	2–4 (3)	1	1			1
3–6 (4)	3–5 (4)	6–15 (7)	3–7 (3)			1
1	1	1	1, 2 (1)	1		

Table 20. Record of branching of setae on larvae of *Anopheles maverlius* (15 specimens).

					Abdominal segment		
Seta no.	Cranium	Prothorax	Mesothorax	Metathorax	I	II	III
0		1				1, 2 (1)	1, 2 (1)
1	1	2–5 (3)	17–26 (21)	1–3 (2)	6–12 (8)	5–12 (9)	12–20 (16)
2	1, 2 (1)	5–11 (6)	1–4 (2)	1, 2 (1)	3–7 (4)	4–10 (6)	1–5 (3)
3	28–49 (34)	1	1	9–14 (12)	1–3 (1)	1	1
4	1–4 (2)	10–16 (13)	3, 4 (3)	3–6 (3)	5–10 (5)	4–7 (5)	2–5 (3)
5	9–15 (12)	18–31 (21)	1	19–32 (25)	5–8 (7)	8–14 (9)	5–8 (7)
6	11–18 (15)	1	3–5 (4)	2–4 (3)	14–26 (19)	14–23 (20)	12–20 (16)
7	12–21 (15)	14–29 (24)	2–4 (3)	19–28 (23)	13–23 (15)	11–22 (19)	2–5 (4)
8	3–8 (6)	17–27 (21)	10–19 (14)	14–24 (23)		8–12 (7)	2–9 (6)
9	3–7 (5)	1, 2 (1)	1	1, 2 (1)	5–12 (8)	8–17 (11)	8–16 (9)
10	1–4 (3)	1	1	1	1	2–4 (3)	1
11	31–46 (44)	1	1	1	5–8 (5)	1	1–4 (3)
12	2–4 (3)	1	1	2–4 (2)	1, 2 (2)	1	1–4 (2)
13	4–8 (6)	6–12 (10)	5–11 (9)	3–5 (4)	3–6 (4)	4–9 (6)	4–9 (6)
14	1–4 (3)	4–10 (6)	9–15 (12)				1
15	3–8 (5)						

Table 21. Record of branching of setae on larvae of *Anopheles quadrimaculatus* (15 specimens).

					Abdominal segment		
Seta no.	Cranium	Prothorax	Mesothorax	Metathorax	I	II	III
0		1				1	1, 2 (1)
1	1	1–4 (1)	20–32 (24)	1–4 (1)	6–9 (6)	8–18 (11)	11–23 (20)
2	1	6–13 (9)	1–4 (2)	1, 2 (1)	3–6 (4)	5–9 (7)	1–6 (3)
3	11–35 (22)	1	1	7–14 (9)	1, 2 (1)	1	1
4	1–4 (2)	13–25 (18)	3–5 (4)	3–6 (4)	5–9 (7)	5–9 (6)	2–6 (4)
5	12–19 (16)	18–27 (24)	1	14–35 (29)	4–7 (5)	7–13 (9)	5–8 (7)
6	12–20 (17)	1	2–7 (4)	2–5 (3)	18–29 (24)	23–30 (28)	18–33 (26)
7	14–23 (19)	20–32 (27)	2–5 (3)	21–28 (24)	16–23 (23)	21–29 (25)	3–6 (4)
8	7–12 (7)	23–35 (24)	11–23 (19)	25–30 (29)		2–6 (4)	2–6 (4)
9	6–10 (7)	1–4 (1)	1, 2 (1)	1	5–9 (7)	5–12 (8)	7–13 (9)
10	2, 3 (3)	1, 2 (2)	1	1	1	2–4 (3)	1
11	29–49 (43)	1, 2 (1)	1, 2 (1)	1	4–6 (5)	1	2–4 (3)
12	3–5 (4)	1	1, 2 (1)	2–4 (3)	2, 3 (2)	1	2–4 (3)
13	3–7 (5)	8–15 (11)	5–12 (8)	3–5 (4)	3–7 (5)	7–11 (8)	5–11 (8)
14	1–5 (2)	6–13 (9)	8–14 (10)				1
15	4–8 (6)						

Table 22. Record of branching of setae on larvae of *Anopheles smaragdinus* (15 specimens).

					Abdominal segment		
Seta no.	Cranium	Prothorax	Mesothorax	Metathorax	I	II	III
0		1				1	1, 2 (1)
1	1	1–5 (3)	19–30 (25)	1–3 (1)	4–10 (6)	6–12 (8)	13–23 (20)
2	1	6–11 (7)	1–4 (2)	1	2–5 (3)	4–8 (6)	1–5 (3)
3	30–47 (41)	1	1	8–14 (8)	1	1, 2 (1)	1
4	2–4 (3)	10–21 (16)	2–4 (3)	3–5 (3)	4–8 (6)	4–7 (5)	2–5 (3)
5	13–19 (16)	23–35 (25)	1	22–34 (30)	3–6 (4)	6–11 (7)	6–10 (7)
6	11–19 (17)	1	3–5 (5)	2–4 (3)	20–28 (22)	20–31 (28)	20–27 (25)
7	14–25 (19)	21–32 (28)	2–5 (3)	20–27 (24)	14–21 (19)	21–29 (23)	3–5 (4)
8	6–13 (8)	20–32 (26)	12–24 (16)	24–33 (24)		2–4 (3)	2–5 (3)
9	4–10 (7)	1, 2 (1)	1	1	4–8 (6)	5–12 (7)	6–11 (9)
10	2–4 (3)	1	1	1, 2 (1)	1, 2 (1)	2–4 (2)	1
11	31–48 (39)	1, 2 (1)	1–3 (1)	1	3–6 (4)	1	1–4 (2)
12	3–6 (4)	1	1	1–4 (2)	1–3 (2)	1	2, 3 (2)
13	4–7 (5)	8–18 (11)	7–12 (8)	3–5 (4)	3–5 (4)	4–10 (6)	3–8 (7)
14	2–4 (2)	7–15 (10)	8–15 (10)				1
15	3–9 (5)						

Table 20. Extended.

Abdominal segment						
IV	V	VI	VII	VIII	X	Siphon
1–3 (2)	1–3 (1)	1–3 (2)	1–3 (2)	2–5 (3)		
14–23 (16)	14–22 (17)	12–19 (16)	11–18 (15)	1	1	3–6 (5)
1–3 (1)	1–3 (1)	3–5 (3)	3–6 (5)	7–12 (9)	14–19 (16)	3–7 (5)
2–5 (3)	1	1, 2 (1)	3–6 (4)	6–8 (6)	8–12 (9)	
2–4 (3)	3–9 (5)	1	1	1		1
5–8 (6)	5–9 (6)	5–8 (6)	4–8 (6)	3–5 (4)		1
2–4 (3)	2–4 (3)	3–6 (4)	2–4 (3)			2, 3 (2)
2–5 (3)	2–4 (3)	2–5 (3)	4–6 (5)			1, 2 (2)
2–6 (4)	3–5 (4)	3–8 (5)	3–6 (4)			2–5 (4)
9–15 (10)	7–13 (8)	4–9 (7)	3–6 (4)			3–7 (5)
1	1	1–3 (1)	2–4 (3)			
1–4 (2)	2–4 (3)	1–4 (3)	1–3 (2)			1
2, 3 (2)	2–6 (4)	1–3 (1)	1			1
3–5 (4)	3–5 (4)	5–9 (6)	3, 4 (3)			1
1	1	1, 2 (1)	1	1, 2 (1)		

Table 21. Extended.

Abdominal segment						
IV	V	VI	VII	VIII	X	Siphon
1, 2 (1)	1, 2 (1)	1, 2 (1)	1, 2 (2)	2, 3 (2)		
13–22 (20)	14–23 (19)	13–22 (19)	10–23 (14)	1, 2 (1)	1	4–7 (6)
1, 2 (1)	1, 2 (1)	2–4 (3)	3–5 (5)	5–11 (8)	16–22 (20)	3–7 (5)
2–6 (3)	1–3 (1)	1	2–5 (3)	6–12 (8)	8–13 (8)	
3–5 (3)	3–7 (5)	1	1	1		1
5–9 (6)	4–8 (7)	5–9 (7)	5–9 (7)	4–6 (5)		1
2–7 (4)	2–5 (3)	2–5 (4)	2–6 (4)			2–4 (2)
3–6 (4)	3–6 (4)	2–6 (4)	4–9 (6)			1–3 (2)
3–5 (4)	3–6 (4)	2–5 (4)	3–8 (4)			3–6 (4)
7–15 (8)	7–13 (10)	7–11 (8)	3–5 (5)			4–8 (5)
1	1	1–4 (3)	3–6 (4)			
1–5 (3)	2, 3 (3)	2, 3 (2)	1–3 (2)			1
2–4 (3)	2–4 (3)	1	1			1, 2 (1)
3–6 (4)	3–5 (4)	6–13 (9)	2–5 (3)			1
1	1, 2 (1)	1, 2 (1)	1	1		

Table 22. Extended.

Abdominal segment						
IV	V	VI	VII	VIII	X	Siphon
1	1	1, 2 (1)	1	2–4 (2)		
13–24 (19)	14–26 (21)	12–23 (18)	13–21 (19)	1	1	4–6 (5)
1, 2 (1)	1, 2 (1)	2–4 (3)	2–5 (4)	6–10 (8)	15–23 (17)	3–6 (4)
2–4 (3)	1, 2 (1)	1	1–5 (3)	4–11 (9)	7–13 (9)	
2–5 (3)	3–6 (4)	1	1	1		1
5–8 (6)	5–8 (6)	5–8 (6)	4–9 (6)	4–6 (4)		1
3–7 (4)	2–5 (3)	2–5 (3)	2–4 (2)			1–3 (2)
3–5 (4)	2–4 (3)	2–4 (2)	3–8 (5)			1, 2 (1)
2–4 (3)	2–4 (3)	2–4 (3)	2–5 (3)			3–6 (4)
6–13 (11)	6–12 (9)	6–10 (8)	2–4 (3)			4–6 (5)
1	1	1–3 (3)	2–6 (4)			
1–3 (2)	1–3 (2)	1–3 (2)	1, 2 (1)			1
2–4 (2)	2–4 (3)	1	1, 2 (1)			1
3–5 (4)	3–5 (4)	5–9 (6)	2–4 (3)			1
1	1	1, 2 (1)	1	1		

Table 23. Sum of both larval seta 2-I[1] branches listed by percent.

Species	No. examined	No. of branches														
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
diluvialis	241				1.2	8.7	14.9	24.9	23.7	14.9	5.8	4.6	0.8	0.4		
inundatus	241							2.9	9.5	18.7	25.3	21.2	12.9	7.5	1.7	0.4
maverlius	241			0.8	9.1	27.0	34.4	24.1	3.3	0.8		0.4				
quadrimaculatus	283			3.9	11.7	32.5	28.3	18.0	4.6	1.1						
smaragdinus	241	3.3	14.5	44.8	24.9	10.8	0.8	0.8								

[1] 2-I, seta 2 on abdominal segment I.

Table 24. Sum of both larval seta 2-I[1] plus both seta 2-III branches listed by percent.

Species	No. examined	No. of branches												
		8	9	10	11	12	13	14	15	16	17	18	19	20
diluvialis	242									0.8	1.7	4.1	8.7	9.1
inundatus	242											0.4	0.4	2.5
maverlius	242			1.2	3.7	9.9	17.8	21.1	21.1	14.5	7.0	2.1	1.2	
quadrimaculatus	242					2.5	11.6	15.3	22.3	23.1	11.6	8.7	3.3	0.8
smaragdinus	242	0.4	2.1	7.4	17.8	20.7	23.1	11.2	8.7	4.5	1.7	2.1	0.4	

[1] 2-I, seta 2 on abdominal segment I, 2-III, seta 2 on abdominal segment III.

Table 25. Sum of both larval seta 2-III[1] branches listed by percent.

Species	No. examined	No. of branches											
		2	3	4	5	6	7	8	9	10	11	12	13
diluvialis	245							2.0	7.3	20.8	22.5	26.5	10.2
inundatus	245						0.4	3.7	5.3	23.3	24.1	22.4	12.2
maverlius	245	0.8	2.9	20.4	24.1	31.0	16.7	3.7		0.4			
quadrimaculatus	286		0.3	0.7	7.3	35.0	28.0	15.7	8.7	3.8	0.3		
smaragdinus	245			6.5	19.6	41.2	19.2	7.8	2.9	2.9			

[1] 2-III, seta 2 on abdominal segment III.

Table 26. Sum of both larval seta 8-II[1] plus both seta 9-II branches listed by percent.

Species	No. examined	No. of branches														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
diluvialis	317			0.9	1.9	4.1	10.1	13.2	21.1	20.8	17.4	4.4	3.2	0.6	0.9	0.3
inundatus	317								0.3	0.3	2.8	9.5	13.2	10.4	14.2	15.8
maverlius	217												0.5		1.4	
quadrimaculatus	217		0.9	1.4	5.1	3.2	8.8	11.5	15.2	14.3	18.0	10.1	4.1	4.6	1.8	0.9
smaragdinus	217	3.2	2.8	9.2	11.1	13.8	16.6	16.1	9.7	6.0	5.5	4.6	0.5	0.9		

[1] 8-II, seta 8 on abdominal segment II; 9-II, seta 9 on abdominal segment II.

Table 24. Extended.

No. of branches													
21	22	23	24	25	26	27	28	29	30	31	32	33	34
17.4	15.3	16.9	11.6	6.6	3.3	2.5	0.8	0.4	0.4	0.4			
5.8	11.6	13.6	15.3	15.7	14.5	9.1	5.4	4.1	0.4	0.4	0.4		0.4
0.4													
0.8													

Table 25. Extended.

No. of branches				
14	15	16	17	18
7.3	1.6	0.8	0.8	
4.9	2.0	1.2		0.4

Table 26. Extended.

No. of branches																		
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
0.9																		
8.2	7.6	5.4	3.5	4.4	1.9	1.3	0.9		0.3									
1.4	4.1	7.8	6.0	7.4	9.2	9.7	9.7	11.0	8.8	9.2	5.1	2.8	3.7	0.9	0.5		0.5	0.5

Table 27. Sum of both larval seta 8-II[1] branches listed by percent.

Species	No. examined	No. of branches										
		4	5	6	7	8	9	10	11	12	13	14
diluvialis	300			1.0	7.0	29.3	35.7	15.0	7.7	2.7	1.0	0.3
inundatus	300					4.3	3.0	21.0	19.0	20.7	13.3	11.7
maverlius	300							0.7	3.6	10.9	16.0	25.5
quadrimaculatus	275	0.4	0.7	23.6	18.9	36.0	13.5	5.8	1.1			
smaragdinus	275	6.2	14.5	56.7	16.4	5.5	0.7					

[1] 8-II, seta 8 on abdominal segment II.

Table 28. Sum of both larval seta 8-II[1] plus both seta 8-III branches listed by percent.

Species	No. examined	No. of branches														
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
diluvialis	205						3.4	8.8	14.1	24.9	22.0	17.6	5.4	1.0	2.0	0.5
inundatus	209								1.0	2.9	3.3	5.3	16.3	18.2	19.1	12.9
maverlius	209														0.4	1.4
quadrimaculatus	209				2.4	11.5	11.5	20.1	11.5	20.6	11.0	7.2	2.9	1.4		
smaragdinus	205	0.5	2.4	6.3	17.1	41.0	19.0	9.3	2.9	0.5	1.0					

[1] 8-II, seta 8 on abdominal segment II; 8-III, seta 8 on abdominal segment III.

Table 29. Sum of both larval seta 8-III[1] branches listed by percent.

Species	No. examined	No. of branches												
		4	5	6	7	8	9	10	11	12	13	14	15	16	17
diluvialis	250		0.4	11.6	27.6	38.8	15.2	5.6	0.8						
inundatus	250			2.8	16.8	34.8	27.2	13.2	3.2	1.2	0.4	0.4			
maverlius	286						0.3	2.8	12.9	32.2	25.9	16.1	7.3	1.4	1.0
quadrimaculatus	250		1.6	26.8	20.8	37.2	12.4	1.2							
smaragdinus	250	6.0	19.2	59.2	10.8	4.8									

[1] 8-III, seta 8 on abdominal segment III.

Table 30. Sum of both larval seta 8-III[1] plus both seta 8-VI branches listed by percent.

Species	No. examined	No. of branches									
		8	9	10	11	12	13	14	15	16	17
diluvialis	244				1.6	9.0	19.3	32.0	20.5	9.0	5.3
inundatus	244					4.1	7.8	23.0	25.0	16.8	12.7
maverlius	244										
quadrimaculatus	246				0.8	13.0	16.3	18.7	22.0	18.3	6.9
smaragdinus	244	0.4	5.7	16.0	25.0	36.5	8.6	5.3	2.0	0.4	

[1] 8-III, seta 8 on abdominal segment III; 8-VI, seta 8 on abdominal segment VI.

Table 31. Sum of both larval seta 1-A[1] branches listed by percent.

Species	No. examined	No. of branches										
		5	6	7	8	9	10	11	12	13	14	15	16
diluvialis	254	0.8	2.4	6.3	10.6	11.0	20.5	14.6	10.6	8.7	3.9	3.9	4.7
inundatus	254		0.8	1.2	10.2	14.6	22.4	17.3	17.3	5.5	5.1	3.1	2.0
maverlius	254												
quadrimaculatus	354	2.5	10.2	14.1	18.1	15.0	17.5	7.9	5.6	4.8	2.8	0.6	0.8
smaragdinus	254		0.6		1.6	4.3	7.1	9.4	13.4	12.2	14.2	18.5	10.2

[1] 1-A, seta 1 on antenna.

Table 27. Extended.

No. of branches						
15	16	17	18	19	20	21
0.3						
5.3	1.0	0.7				
19.6	13.1	9.5	0.7			0.4

Table 28. Extended.

No. of branches											
23	24	25	26	27	28	29	30	31	32	33	34
0.5											
6.2	4.8	5.3	3.8	1.0							
2.4	4.8	9.6	20.1	18.7	13.4	12.9	8.6	3.3	2.4	1.0	1.0

Table 30. Extended.

No. of branches										
18	19	20	21	22	23	24	25	26	27	28
2.9	0.4									
8.2	1.2	1.2								
0.4	1.6	16.4	16.4	20.5	18.4	13.1	6.6	4.5	1.6	0.4
3.7	0.4									

Table 31. Extended.

No. of branches												
17	18	19	20	21	22	23	24	25	26	27	28	29
0.8	0.8	0.4										
0.4												
1.1	2.8	16.9	18.1	16.1	13.0	7.9	11.0	2.4	5.9	2.8	1.6	0.4
6.7	1.6				0.4							

Table 32. Mobility values (*Rf*) for electromorphs of corresponding loci for type progeny broods[1] of 5 sibling species calculated relative to the standard reference band (Q_2).

Locus	Species				
	A	B	C	D	E
Acon-1	100	91/100	118	109	100
Ao-1	105	96/105	91	96	96
Est-2	100	84	84	81/84	103
Est-5	91/106	75	100	94/100	83
Est-6	94	—	94	100	103
Got-1	100	100	100	89	89/100
Got-2	38/100	100	38	38/138	38
Had-3	100	100	45	45	100
Idh-1	100	86	100	100/115	115
Idh-2	132	162	162	162/224	162/242
Me-1	100	100	100	92	108
Mpi-1	87	95	106	106	62
Pep-2	100	100/110	110	110	110
Pep-4	100	100	100	100	135/165
Pgi-1	100	100	95	95	105

[1] Isofemale progeny broods: MN95.10 = *Anopheles quadrimaculatus* (A); FL95.57 = *Anopheles smaragdinus* (B); FL95.51 = *Anopheles diluvialis* (C): FL95.25 = *Anopheles inundatus* (D); and FL95.14 = *Anopheles maverlius* (E). Full names of enzyme loci are included in the text in the electrophoresis studies section.

APPENDIX 1. DETAILED PROCEDURES FOR REARING AND PRESERVATION OF MOSQUITOES USED FOR STUDIES IN SYSTEMATICS

BY JOHN F. REINERT

The following procedures for individually rearing and preserving mosquitoes were developed, improved and refined over the last 30 years while conducting systematics studies in various parts of the world. During the development of these procedures, a number of useful techniques were adapted or modified from those suggested by E. L. Peyton and Bruce A. Harrison and published articles of other investigators (e.g., Fairchild and Hertig 1948, Foote 1952, Burton 1953, Belkin 1962, Belkin et al. 1965, Rattanarithikul 1983). Nomenclature used follows Harbach and Knight (1980). Care should be taken by the user to avoid contact with skin or inhalation of all chemicals used in the following procedures.

Rearing of Adults with Associated Immature Exuviae

Late 4th-instar larvae of field-collected or laboratory-reared (e.g., isofemale progeny) mosquitoes are individually isolated in rearing vials, and the emerged adult is linked with its associated pupal and 4th-instar larval exuviae by a common identification number. Each larva is placed in a separate 3.5-dram plastic vial (modified by filing 3 shallow vertical grooves evenly spaced in the upper lip) containing *ca.* 5 ml of tap water. Fresh rainwater, if available, is used if rearing is done in the field. Each vial is initially washed with clean water without detergent or soap. Vials containing larvae from the same field collection or isofemale brood are grouped together on a laboratory or field table with a label indicating the identification number, and they are separated from other collections by strips of tape on the table. Acid-free cardstock paper containing sequential identification numbers for the individual specimens of each collection or progeny brood are generated by a computer, printed with a laser printer, cut into strips, and placed with the corresponding group of larvae being reared; a duplicate set of identification numbers is printed and saved for use with the emerged pinned adults.

Upon pupation, the 4th-instar larval exuviae is gently removed from the 3.5-dram vial with a small (10 cm long), smooth, fine-pointed forceps (MC 32-B, Fine Science Tools Inc., 373-G Vintage Park Drive, Foster City, CA) and then submerged in 80% ethanol inside a 0.25-dram shell vial. The specimen identification number (cut from the strip of sequenced numbers) is taped, with clear tape, to the exterior of the shell vial thus preventing damage to the exuviae that could be caused by the movement of the label if it is placed inside the vial. Extreme care is taken not to crush the exuviae with the forceps during the transfer. An alternate method of transfer is to allow the exuviae in the 3.5-dram vial to adhere to the distal portion of a smooth, cylindrical wooden applicator stick (2 mm diam × *ca.* 15 cm long) and then submerge it in the alcohol in the 0.25-dram shell vial, where the exuviae floats off without any damage. The vial is completely filled with alcohol because the movement of an air bubble inside the vial could damage the exuviae during transit. The shell vial is stoppered with a diaphragm (no. 5, red rubber) and secured to the side of a 9-dram plastic vial with a rubber band (no. 10). A hypodermic needle (25 gauge, ⅝ in. long) is inserted through the top center of the diaphragm to release pressure inside the alcohol-filled shell vial prior to removal; the needle is not removed until after the diaphragm is replaced in the top of the 0.25-dram shell vial. The 9-dram plastic vial, with the attached 0.25-dram vial containing the larval exuviae, is slipped onto the top portion of the 3.5-dram vial and thus provides an emergence chamber for the adult. The grooves in the lip of the smaller vial provide release points for pressure caused by placement of the larger vial over it. Nine- and 3.5-dram plastic vials (crystal clear, with snap-on caps that are not used) were obtained from Thornton Plastics, 745 Pacific Avenue, Salt Lake City, UT, and the no. 5 red rubber diaphragms were obtained from West Company, 101 Gordon Drive, Lionville, PA. Use of 80% ethanol prevents decay of the exuviae and larvae, even when stored for extended periods of time.

Approximately 30 min after eclosion, the adult is coaxed, by a gentle tap of a finger on the side of

the rearing chamber, into the upper portion of the 9-dram vial, which is quickly removed and transferred to another 3.5-dram vial containing *ca.* 5 ml of water. The rearing chamber is held, side by side, with the second 3.5-dram vial in one hand during the transfer of the 9-dram vial with the other hand, thus providing efficient transfer without loss of the adult. The pupal exuviae is gently removed with the fine-pointed forceps (or applicator stick) and added to the vial containing the associated larval exuviae, and the vial is reattached with the rubber band to the 9-dram vial containing the emerged adult. The 4th-instar larval and pupal exuviae were each normally removed from the water in the rearing chamber as soon as possible, usually within 30 min to 4 h because exuviae left for long periods in the rearing water decayed and became worthless, especially in hot environments. Adults of both sexes were held in the emergence chambers for approximately 24 h to fully harden and, in the case of males, to allow the genitalia to completely rotate 180°. During this period, the water in the bottom vial maintained sufficient humidity within the rearing chamber to allow survival of the adult. Using this procedure, thousands of adults, with their associated pupal and 4th-instar larval exuviae, have been reared efficiently and with a minimal chance of mixups.

Adult Preservation Procedures

After the *ca.* 24-h-posteclosion period, the adult is transferred to a killing tube which has a no. 10 rubber band encircling it *ca.* ¼ from the top (the rubber band prevented the 9-dram vial from sliding down the killing tube). After the adult has succumbed to the vapors and fallen into the killing tube, the tube is stoppered, and the vial containing the associated exuviae is attached with the rubber band to the killing tube. Adults held in the rearing chamber for periods longer than *ca.* 28 h could become denuded by their movement. The dead adult is gently shaken from the killing tube onto a white, glazed ceramic tile (*ca.* 11 cm^2) and oriented with its left side facing down. The tile allows easy movement and orientation of the adult with minimal handling and damage. A 10-mm-long paper triangle, punched from no. 2 thickness, white, 100% rag Bristol board, is inserted on a no. 3 stainless steel insect pin to a height 10 mm from the head. Numerous pins with points are prepared using a 4-step pinning block, prior to beginning the procedure. A very tiny droplet of ambroid® cement is placed dorsoapically on the paper triangle, and the cement is very gently touched to the right mesokatepisternum of the adult thorax. Final orientation of the adult on the top of the paper triangle is with the left side up, head facing left, and extended legs directed toward the pin. This orientation provided maximum protection to the specimen from breakage and corresponded to the orientation of illustrations in many taxonomic publications. Bristol board was obtained from an art supply store. Ambroid® cement, obtained from Ambroid Company, P. O. Box 164, Lynfield, MA, or from local hobby stores, can be thinned with amyl acetate.

Labels are placed on a sheet of cardboard (*ca.* 11 cm^2) which provides for their easy penetration by the point of the insect pin with the attached adult. The labels are then positioned at specific heights on the pin using a 4-stepped pinning block and initially include a duplicate specimen identification label corresponding to the specimen identification number of the vial containing the associated immature exuviae. At a later time, 2 additional labels are attached to the pin, one with collection data (country, state, county/parish/secondary administrative district, locality, date of collection, and collectors names), and the other label containing the species identification with the identifier's name. Adults are pinned in airtight wooden insect boxes containing a sheet of polyethelene foam glued in the bottom and stored for later study.

A killing tube for adults is constructed in a well-ventilated area from a large (*ca.* 36 ml) glass shell vial (25 mm outside diam × 95 mm high). Natural rubber tubing with an outside diam of 13 mm is cut into small pieces, and these are placed into the bottom of the vial forming a *ca.* 35-mm-deep layer to which several drops of chloroform are added. A *ca.* 10-mm layer of cotton is inserted over the chloroform-impregnated rubber, a disc fitting tightly inside the vial is cut from white poster board and placed firmly on top of the cotton, the vial is capped with a cork stopper, and a label with "contains chloroform" is attached to the lower exterior of the killing tube. The outside diam of the killing tube is of a size that allows the 9-dram vial containing the adult to fit snugly over the top of the killing tube, thus preventing the chloroform vapors from escaping. Adults are rapidly killed with chloroform, and they tend to spread their wings, which is an advantage when wing patterns need to be examined. An alternate method of preparing the killing tube (similar to that given by Chamberlain 1956) consists of pouring a mixture of plaster of paris and water into the bottom of a 36-ml shell vial to a depth of 2.5 cm, completely drying the plaster in an oven, and then adding *ca.* 3 ml of ethyl acetate to the top of the plaster layer, capping the vial with a cork stopper, and labeling the vial to indicate the killing agent. After the ethyl acetate has been absorbed by the plaster, any moisture remaining inside the vial is removed with a paper towel. Adults are killed more slowly with ethyl acetate and tend to extend their legs so that they are parallel to the point when mounted.

Preparation and Preservation of 4th-Instar Larval and Pupal Exuviae

Fourth-instar larval and pupal exuviae are stored in the alcohol-filled 0.25-dram shell vials until slide

mounts can be prepared. In preparation for permanent slide mounts, the pupal and larval exuviae are very gently removed, along with the specimen identification number, from the storage vials with a small, smooth, fine-pointed forceps and transferred to 0.5 ml of cellosolve (ethylene glycol monethyl ether, purified grade) in 1 well of a 24-well plastic cell culture dish. Extreme care is taken not to crush the exuviae with the forceps during the transfer. The hollow depression in the bottom of the diaphragm is checked if both the larval and pupal exuviae are not found in the vial. The dish is covered, and the specimens are allowed to soak in the cellosolve for *ca.* 30–60 min. The 24 wells of the dish allow multiple specimens to be processed. A clean microscope slide is etched on the lower left-hand corner with the specimen identification number using a diamond-pointed stylus. In the slide's center, a *ca.* 12-mm circular layer of thinly diluted mounting medium is placed in which the larval and pupal exuviae are positioned side by side. Canada balsam (neutral, filtered), diluted with xylene ($C_6H_4(CH_3)_2$), is used as a medium for mounting specimens. Euparal (thinned with euparal essence) is an alternate mounting medium.

While viewing through a stereomicroscope, the cephalothorax of the pupal exuviae is detached from the metanotum with fine-pin dissecting tools and opened along the dorsal ecdysial line so that both halves are extended laterally with the external surface up and the dorsal apotome facing toward the bottom margin of the slide. The abdomen, with the metanotum attached, is positioned with the dorsal surface up behind the cephalothorax longitudinally. The 4th-instar larval exuviae, placed to the left of the pupal exuviae, is gently extended to its normal length and width with fine-pin dissecting tools and oriented with the head toward the bottom margin of the slide. Next, the slide is placed in a drying oven set at *ca.* 47°C for *ca.* 2–3 h, which evaporates any excess solvent, hardens the mounting medium, and anchors the specimens. Additional thinned mounting medium is applied drop by drop (4–6 drops for average-sized specimens) in a thin layer over the specimens with an applicator tool and then a circular 15-mm no. 1 thickness glass coverslip is placed over the mounting medium with a 45° bent-tipped coverslip forceps. Care is taken not to touch the specimens with the applicator tool when applying the mounting medium. Trapped air bubbles underneath the coverslip are prevented by using a thinned mounting medium and by initially touching part of the edge of the coverslip to the margin of the circle of mounting medium at an angle and slowly allowing the coverslip to descend. The slide, placed on an aluminum slide tray (with a 20-slide capacity), is dried for *ca.* 30 days in a drying oven set at *ca.* 47°C. When dry, 2 labels are affixed to the slide, one on each end, containing the same information as the associated adult labels. Finished slides are stored horizontally, with the coverslip side up, in plastic slide boxes with a 100-slide capacity.

A fine-pin dissecting tool is constructed by first soaking one end of a wooden applicator stick (2 mm diam × *ca.* 15 cm long) in water for *ca.* 10 min to soften it. The base of a 0.2-mm diam stainless steel minuten pin is then pushed (with the blunt end in) *ca.* 2 mm into the water-soaked end of the stick with a smooth-tipped pliers. A mounting medium application tool is fabricated by pushing the sharpened end of a 40-mm section of a standard-sized steel paper clip, which has been straightened, into the end of a wooden dowel rod (4-mm diam × 11 cm long).

Preparation and Preservation of Larvae

Fourth-instar larvae, from a single collection or from part of a collection from which some larvae were individually reared, are killed by transferring them with a pipette to a stender dish (38 mm diam × 20 mm high) containing hot water (*ca.* 85°C) for *ca.* 45 sec. The larvae, with the specimen collection number, are then transferred with a glass pipette to a 25-ml glass storage vial. Any water transferred with the specimens is carefully pipetted from the vial, which is then completely filled with 80% ethanol very slowly, taking care not to agitate the specimens, and then stoppered for storage. Slide-mounted preparations of the larvae are prepared similarly to those mentioned above for the exuviae except that specimens are soaked in cellosolve for 24 h and slightly thicker mounting medium is used in preparing the slides because of the greater thickness of the specimens. Larvae are mounted, one to a slide, with the dorsal surface up, and the abdomen is cut through the intersegmental area between segments VI and VII with a sharp, fine-pointed scalpel (no. 11 blade). The resulting terminal section is positioned with the lateral surface up and the spiracular apparatus facing to the left. Two or 3 whole larvae from each collection of 12 or more are mounted with the ventral surface up to provide easy observation of ventral setae and structures; this orientation is especially useful if the ventral setae are to be illustrated. After the slide-mounted larvae have dried in the oven for *ca.* 7 days they are examined for shrinkage of the mounting medium. If shrinkage has occurred, a drop of thin mounting medium is applied with the applicator stick next to the coverslip at the point of shrinkage to fill the void.

Preparation and Preservation of Male Genitalia

A pinned adult is placed in a relaxing chamber for *ca.* 0.5–1.0 h, removed, pinned onto an adjustable examination stage with biaxes rotation capability, and then placed on top of a sheet of white paper towel *ca.* 27.5 cm^2 that has been spread over the stage of a stereomicroscope. Segment VII of the

male abdomen is severed transversely at midlength with sharp, fine-pointed dissecting spring scissors while observing through the microscope. The towel provided a background for easy observation of the clipped portion of the abdomen and also reduced the risk of its loss. The relaxing chamber consists of a Pyrex sleeve top desiccator (200 mm inside diam × 315 mm high), with a lid having a vacuum hookup (in open position) and a shelf near the bottom supporting a perforated ceramic plate (Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA) into which *ca.* 500 ml of boiling water is added to the bottom. The adult, pinned to a small sheet of polyethelene foam and placed on the ceramic plate, is relaxed by steam inside the chamber resulting from the boiling water.

The severed terminal portion of the abdomen is transferred to a glass test tube (12 × 75 mm) by moistening the tip of a wooden applicator stick with water, gently touching the tip to the severed abdominal segment lying on the paper towel where it had fallen, and then gently submerging it into 1 ml of preheated 10% potassium hydroxide (KOH) within the test tube. Ten percent sodium hydroxide can be used as an alternate for the KOH. The specimen identification number is transferred with the specimen throughout the preparation process. The test tube is then returned to the hot water bath, in which it has been preheated, and held for *ca.* 5–10 min; the amount of time is dependent on the amount of sclerotization and pigmentation of the specimen. The specimen is pipetted with a small glass dropper to 1 well of a 12-well white porcelain spot plate, and then gently transferred by small, smooth, fine-tipped forceps to a different well containing 5% acetic acid and held for 5 min to neutralize any remaining KOH. Next the specimen is gently transferred with the forceps to a different depression plate containing liquefied phenol (C_6H_5OH) and held for 30–60 min. A cover is placed over the depression plate to contain the phenol vapors. Extra care should be taken by the user to avoid getting phenol on the skin or inhaling the vapors. A hot water bath was fabricated by placing *ca.* 45 ml of water in a 100-ml glass beaker and heating to *ca.* 98°C on an electric hot plate.

A permanent preparation is prepared by gently removing the specimen from the phenol with a fine-tipped forceps and transferring it to a small drop of copal on a microscope slide that has been marked with the specimen identification number. While viewing through a stereomicroscope, the specimen is first partially dissected by teasing the genitalia from segment VIII with 2 fine-pin dissecting tools. During this step any remaining phenol from the specimen is absorbed by the drop of copal during the dissection. Copal drys slowly, thus allowing considerable time to perform delicate dissections without producing a stringing effect when the dissecting tools are removed. It is compatible with Canada balsam or euparal and has a similar refraction index. Even though the refraction of copal is similar to that of the other mounting media, it may be slightly darker; therefore, special care needs to be taken concerning the size of drops used in the preparations. If copal is not available, the dissections can be done in a drop of Canada balsam or euparal diluted to a thin consistency; however, these media dry much more quickly, and rapidly become tacky, allowing a shorter time for conducting the procedure. Copal, as used here, consists of high-grade, finely pulverized, crystalline copal gum dissolved in liquefied phenol and filtered with a vacuum pump system. Copal gum was purchased from Edward Gurr Ltd., London, United Kingdom.

In preparation for final dissection and positioning, the 2 parts of the specimen are next transferred, using a fine-pin dissecting tool, to a small drop of copal, positioned slightly off-center, on a different slide, which has been etched with the specimen identification number. Using 2 fine-pin dissecting tools, segment VIII is separated from the posterior half of segment VII, and then tergum VIII is separated from sternum VIII, by gently tearing along the intersegmental membranes. Resulting structures are positioned with external surfaces up in a minute drop of copal located to the lower right of the first drop. If additional dissection is not warranted, the remaining genitalia are then positioned with the dorsal surface up (genitalia prerotation aspect) in a 2nd minute drop of copal located in the center of the slide. However, in most cases, additional dissection or positioning of component parts in a flattened aspect is desirable. In the latter case, each structure of the genitalia is gently separated from the others with 2 fine-pin dissecting tools and positioned in the central area of the slide in minute drops of copal. The suggested sequence of structure removal follows: segment IX; separation of tergum IX from sternum IX, accomplished with a scissoring motion of 2 fine-pin dissecting tools at the lateral connecting points of the 2 structures; proctiger; phallosome; gonostylus; and basal mesal lobes/claspettes. The slide is placed in a drying oven set at *ca.* 47°C for *ca.* 6–8 h (or overnight) to evaporate the solvent and securely anchor the specimen parts. The preparation is then finalized, labeled, dried, and stored as noted above for pupal and 4th-instar larval exuviae preparations. A wooden applicator stick with a headless no. 2 stainless steel, insect pin attached to one end provides a tool for transferring minute drops of copal.

Preparation and Preservation of Female Genitalia

A detailed description for preparation and dissection procedures and preservation of female genitalia was given by Reinert (1974). Even though these techniques were initially described for use with aedine mosquitoes, they have been found to

be equally applicable to all other groups of mosquitoes.

APPENDIX 2. LIST OF COLLECTORS

BBB = Bonnie B. Broussard
BJS = Burrel J. Smittle
BKB = Brian K. Birky
BRM = Bobby R. McDuff
CLM = C. Lamar Meek
CRR = C. Roxanne Rutledge
CSA = Charles S. Apperson
EDW = Edward D. Walker
EO = E. Ostheimer
GCL = Gregory C. Lanzaro
HMS = Heather M. Scheer
JFR = John F. Reinert
JKN = J. K. Nesheim
JKO = Jimmy K. Olson
JN = Jai Nayar
JSB = John S. Billodux
JT = J. Tessmer
KJT = Ken J. Tennessen
KRK = Karl R. Kangas
KS = K. Samui
MQB = Mark Q. Benedict
ORW = Osborne R. Willis
PEK = Paul E. Kaiser
RHD = Richard H. Daggy
SEM = Sharon E. Mitchell
SEW = Susan E. White
SGS = Scott G. Straub
SKN = Sudhir K. Narang
SPR = Shawn P. Robertson
TF = Tokuo Fukuda
TJ = Truls Jensen
VW = V. Wright