# ANOPHELES (ANOPHELES) LESTERI BAISAS AND HU (DIPTERA: CULICIDAE): NEOTYPE DESIGNATION AND DESCRIPTION

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Abstract.—The Asian malaria vector, Anopheles (Anopheles) lesteri Baisas and Hu, 1936, is described with illustrations of the larval and pupal stages, adult female, and the male genitalia. Taxonomic and related literature records, diagnostic features, distribution, rDNA ITS2 sequence, bionomics, and medical importance of An. lesteri are included. A neotype female for the species from near the original type locality in Luzon, Philippines, is designated.

Key Words: Anopheles lesteri, Culicidae, taxonomy, description, neotype, mosquitoes, malaria

Because of the recent increase of human malaria cases in South Korea (Feighner et al. 1998, Lee et al. 1998), there is a serious need to clarify the identity of the possible mosquito vectors. Misidentifications of vector species often lead to inadequate understanding of the epidemiology of disease transmission and inadvertently affect control measures. Anopheles (Anopheles) lesteri Baisas and Hu, 1936, may be the most significant vector of malarial parasites in Korea, Taiwan, Japan (particularly Okinawa), and central and northern China (Harrison 1973). It is one of the 27 species of the Hyrcanus Group of Anopheles (Anopheles) having an Oriental or eastern Palearctic distribution (Ramsdale 2001, http:// www.mosquitocatalog.org). It may have a potential role in malarial and filarial parasite transmission and disease outbreaks in countries where it occurs. Recently, Wilkerson et al. (2003) demonstrated that An. anthropophagus Xu and Feng, the most important vector malaria vector in eastern China, is actually a junior synonym of An. lesteri.

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Anopheles lesteri was described by Baisas and Hu (1936: 214) as An. hyrcanus var. lesteri from 1 male (lot M1-8) and 1 female (lot M1-12), with corresponding larval and pupal exuviae, from Santa Mesa, Manila, Luzon, Philippines (Jose P. Ingal, coll. 2 March 1936). Many specimens (or "cotypes") were collected from Calauan, Laguna in 1935, but type specimens were selected from the Santa Mesa specimens collected in 1936. The syntypes or type specimens ("types" and "cotypes", collected from Santa Mesa and Calauan, Laguna, Luzon), were supposed to be deposited in the Philippine National Museum, Manila. Other specimens from the same batch were to be deposited in the National Museum of Natural History, Washington, DC, and Henry Lester Institute of Medical Research, Shanghai, China (Baisas and Hu 1936). Knight and Stone (1977: 22) noted that the type specimens could not be found. Our inquiries were also unsuccessful in search of these specimens in possible depositories in Manila and Shanghai. Specimens of An.

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Standard Form 298 (Rev. 8-98) Prescribed by ANSI Std Z39-18 lesteri found in the National Museum of Natural History include 2 slides with the following labels: slide # 1- right label light yellow, with reddish border: "M 1-13 An. hyr. var. lesteri Sta. Mesa, Manila Mar. 5, 1936", left label: "Anopheles (An.) lesteri lesteri Baisas & Hu det. B.A. Harrison"; slide # 2-right label light yellow, with reddish border: "F171-50 An. lesteri cotypemale Calauan, Laguna July 15, 1935." However, larval and pupal exuviae, particularly those mounted on slide # 2, are in bad conditions (i.e., dark unknown mounting media, cracked specimens, etc.), and most morphological characters are difficult to see under the compound microscope.

Also, the original adult description (Baisas and Hu 1936) is not sufficiently complete for accurate identification of the species, and no detailed descriptions of the larval and pupal stages or of the male terminalia of specimens from the type locality (Luzon) have been made. In view of this, it becomes imperative to provide detailed descriptions of various life stages and to designate a neotype for this important species.

In the present paper, a neotype female from near the original type locality is designated for *An. lesteri* and associated ribosomal DNA internal transcribed spacer 2 (rDNA ITS2) sequence provided. Descriptions and illustrations are provided for the adult female and male, pupa and larva of this species from the same type locality.

#### MATERIALS AND METHODS

Except for wing spot nomenclature, which is taken from Wilkerson and Peyton (1990), for wing venation terms from Belkin (1962), and for pupal abdominal dark marks, the terminology and abbreviations of Harbach and Knight (1980, 1982) are used for the morphological characters and illustrations. Abbreviations used are as follow: E, egg; G, genitalia; L, larva; Le, larval exuviae; NE, non-existent; P, pupa; Pe, pupal exuviae; var., variety. An asterisk following the abbreviation of a given life stage indicates that at least part of the life stage was illustrated in the publication cited. Collection codes of the most recent collections consist of a country code in capital letters followed by a collection number (e.g., PH 9–1 is an individual from collection 9 from the Philippines; a specimen number lower than 100 is used if there are associated larval and pupal exuviae, and equal or greater than 100 if there are no associated larval exuviae).

DNA isolation and sequencing. DNA was isolated by phenol-chloroform extraction, as described by Wilkerson et al. (1993), from a leg of the adult neotype female, a leg of a second female, and 2 entire males, minus genitalia, from new type locality in Calauan, Laguna. Direct sequencing was carried out as described in Wilkerson et al. (2004) using their primers. The beginning and end of the rDNA ITS2 was estimated as in Cornel et al. (1996).

## TAXONOMIC TREATMENT

# Anopheles (Anopheles) lesteri Baisas and Hu

(Figs. 1-4)

- Anopheles yesoensis Tsuzuki 1901: 717 (nomen dubium).
- Anopheles jesoensis Tsuzuki 1902: 286 (nomen dubium).
- Anopheles hyrcanus var. lesteri Baisas and Hu 1936: 229 (2, P\*, L\*, E\*). Type: Santa Mesa, Manila (Luzon), Philippines (NE), other specimens/"cotypes": Calauan, Laguna (Luzon); Ohmori 1957: 209 ( $\delta^*$ , E\*); Ohmori 1959: 222 (P\*).
- Anopheles (Anopheles) lesteri: Otsuru and Ohmori 1960: 47 (&\*, Q\*, P\*, L, E\*; taxonomy; bionomics; distribution, Japan: Honshu [Hyogo, Mie, Niigata, Yamaguchi], Kyushu [Kumamoto, Kagosima, Oita, Fukuoka, Saga, Nagasaki]); Whang 1962: 39 (distribution, Korea: Tansan, Wondang, Guidandong, Yongjueup); Reid 1968: 105 (type form); Cagampang-Ramos and Darsie 1970: 14 (identification key); Basio 1971: 36 (distribution, Philippines: widely found in

Luzon including Manila, Pampanga); Basio and Reisen 1971:60 (L, distribution, Guam); Tanaka 1971: 4 (distribution, Japan: Ryukyu Islands); Harrison 1973: 4 (taxonomy); Baisas 1974: 50 ( $\mathfrak{P}$ \*, P, L\*, E; taxonomy); Tanaka et al. 1979: 83 ( $\mathfrak{S}$ \*  $\mathfrak{P}$ \*, P\*, L\*, E; taxonomy, bionomics, distribution, Japan: Hokkaido, Honshu, Kyushu, Ryukyu Archipelago [Amami, Okinawa Gunto, Yaeyama Gunto]); Rueda et al. 2004 (distribution, China: Hong Kong).

- Anopheles (Anopheles) lesteri lesteri: Chau 1982 (distribution, China: Hong Kong).
- Anopheles (Anopheles) lesteri anthropophagus Xu and Feng 1975: 81, 97 (♀\*, ♂\*, P\*, L\*, E\*; taxonomy).
- Anopheles (Anopheles) anthropophagus: Ma 1981: 11 (key; distribution, China: Fukien, Kiangsi, Kiangsu, Kwangsi, Kweichow, Shanghai, provinces south of Yantze River); Wilkerson et al. 2003: 1 (new synonym of *lesteri*).

Other literature records.-Otsuru 1949: 139 (as possible malaria vector, Japan); Otsuru and Ohmori 1960: 33 (bionomics, Japan); Kamimura 1968: 15 (as possible malaria vector, Japan); Reisen et al. 1972; 319 (distribution, Guam); Zhang et al. 1980: 140 (as experimental vector of Vietnam monkey malarial parasite, near Plasmodium cynomolgi); Xu et al. 1981: 265 (scanning electron micrographs [SEMs] of adults, pupae, eggs, China); Takai et al. 1984: 251 (hybridization, Japan); Zhang et al. 1987: 191 (vectorial capacity for malayan filariasis, Sichuan, China); Xu et al. 1988: 247 (control using insecticide-treated bed net, Guangxi, China); Ma and Wang 1988: 65 (salivary gland chromosome, China); Wang and Zheng 1989: 175 (blood meals, Guizhou, China); Ye and Zhu 1989: 256 (enzyme electrophoresis, China); Dapeng et al. 1996: 100 (as vector of P. falciparum, and chemical vector control, Xinyang, China); Li et al. 1991: 8 (DNA-restriction fragment length differences, China); Liu et al. 1991: 147 (as vector of malayan filariasis, Fujian,

China); Niu et al. 1992: 267 (DNA probe); Chen et al. 1994: 27-30 (trace and macro elements in hemolymph); Cheng et al. 1995: 321, (control using insecticide-treated bed net, Henan, China); Gu et al. 1966: 34 (distribution, China); Shahjehan 1996: 205 (DNA probes, China); Song and Peng 1996: 161 (control using mermithid nematodes, Sichuan, China); Xu et al. 1997: 807 (as vector of filariasis, Henan, China); Sleigh et al. 1998: 265 (as P. vivax vector, Henan, China); Xu et al. 1998: 135-136 (as vector of P. vivax; control using insecticidetreated bed net and residual spraying, Hubei, China); Kim et al. 1999: 181 (seasonal prevalence, South Korea); Zizhao et al. 1999: 240-242 (as vector of P. falciparum malaria, China); Lee et al. 2000: 77 (PCR, presence of P. vivax circumsporozoite protein, South Korea); Ma et al. 2000a: 325 (PCR assay and rDNA-ITS2 sequencing, China); Ma et al. 2000b: 36 (rDNA-ITS2, Korea); Burkett et al. 2001: 196, 2002: 45 (trap attractants, South Korea); Huang et al. 2001: 340 (habitat and distribution, Hubei, China); Coleman et al. 2002: 244 (presence of P. vivax circumsporozoite protein, South Korea); Min et al. 2002: 198; Shin et al. 2002: 41 (vector competence to P. vivax, Korea); Toma 2002: 7 (distribution review, Ryukyu Archipelago, Japan); Wilkerson et al. 2003: 1 (species molecular confirmation, rDNA-ITS2, China, Philippines, South Korea; note on geographical range, China).

## ORIGINAL DESCRIPTION

In support of previous and present interpretations of the name *lesteri*, the original description given by Baisas and Hu (1936) is as follows. "Dark and pale scales of wings well contrasted. Costa—Jet black excepting for the preapical and subcostal pale spots. The subcostal spot is composed of from 10 to 22 pale scales. No scattered pale scales elsewhere on the dark portions of the costa. Subcosta—Invariably dark excepting for 1 to 4 pale scales at the apex, which form a part of the subcostal spot. Vein 1— Preapical pale spot distinct and complete. Subcostal spot usually incomplete, seldom complete, and more rarely absent. Mid pale spot usually small and incomplete, sometimes absent. Sector pale spot usually small and incomplete. A few scattered pale scales are sometimes present on the dark area between the preapical and subcostal spots but these are not as many as those found in nigerrimus. A few pale scales towards the base below the presector dark spot, which do not, however, form definite spots. Vein 2-Stem mainly pale with some greyish or dark scales on lateral borders. Anterior fork dark with complete preapical pale spot. Posterior fork dark with a pale spot at about, or a little below the middle. Vein 3-A definite dark area, at base, and another at apex; apical half of intervening area with median squames mainly pale; lateral squames mainly dark; basal half mainly pale with a few scattered dark scales. Vein 4-Stem dark or mainly dark towards base below cross viens, mainly pale towards apex. Forks dark at bases and apices, the intervening portion mainly pale but lateral squames with fair distribution of dark scales. Vein 5-Apical half or more of stem pale, sometimes with a few scattered dark scales. A definite dark area a little below middle, followed by a mixture of dark and pale scales, the pale ones sometimes predominating or occupying the whole area excepting the extreme base where a few dark scales are located. Anterior fork mainly dark with the usual dark spots ill-demarcated. Sometimes the basal and sub-basal dark areas are formed on the pale portion towards the apex. Posterior fork pale excepting for the apical dark spot. Vein 6-Pale with a dark area at middle, and another one at the apex. In some specimens, a few dark scales are sometimes scattered on the pale portion towards the base. Fringe-Pale spot at apex of wing involves variable portion of area opposite vein 1 and vein 4.1. Pale spot opposite vein 5.2 absent in all males and females examined. Humeral vein-Usually with 2 or 3 dark scales. Remigium—Mainly dark-scaled with a few pale scales on the anterior border."

## SUPPLEMENTAL DESCRIPTION FROM THIS STUDY

Female (Fig. 1).—Integument dark brown with silvery or gravish pollinosity. The following measurements and counts, n= 6, except when indicated. Head: Interocular space with 10-11 (n = 3) long, pale setae intermixed with long and small, narrow, appressed white scales; vertex, occiput and upper portion of postgena with numerous erect, truncate scales; patch of white scales on the middorsal portion of vertex; patch of dark brown to black scales on lateral portion of vertex, occiput and upper portion of postgena; long dark brown to black setae on ventral portion of postgena. Clypeus bare dorsally, with dark scales laterally. Pedicel of antenna with 6-11 (n =4) small, dorsolateral, narrow to broad, gravish white spatulate scales, and 3 short, dark ventrolateral setae; flagellomere 1 with numerous narrow to broad white scales; remaining flagellomeres with a few scattered narrow to broad white scales. Scales of maxillary palpus slender, spatulate, mostly dark brown to black with intermixed dark brown setae; narrow band of white scales at base of palpomeres 3 and 4, and at base and apex of palpomere 5; apical white band of palpomere 5 slightly shorter than other basal palpomere white bands; base of maxillary palpus dorsally with single long, erect dark seta; length of maxillary palpus 1.75-1.98 mm (mean = 1.86 mm); ratio of length of each of palpomeres 2-5 to total length of palpus, 2 = 0.33 - 0.34 (mean = 0.34), 3 = 0.32 - 0.37 (mean = 0.35), 4 = 0.17 - 0.23(mean = 0.20), 5 = 0.1-0.12 (mean = 0.1)0.11); ratio of combined palpomeres 2-5 to total length of palpus, 0.99-1.0 (mean = 0.99); ratio of combined palpomeres 4, 5 to total length of palpus, 0.27-0.35 (mean = 0.31); palpus 1.15-1.26 (mean = 1.22) forefemur length. Proboscis dark-scaled, except labellum light brown; base of proboscis with long, erect dark setae and scales;



Fig. 1. Anopheles lesteri, adult female, habitus.

proboscis length 1.75-1.95 mm (mean = 1.86 mm, n = 3); proboscis 0.99-1.01 (mean = 1.0, n = 3) palpus length. *Thorax:* Scutum dark brown, with gray pollinosity; 2 submedian longitudinal lines on anterior area; a pair of indistinct black spots near antealar area; median anterior promontory with patch of intermixed narrow, short and

long pale yellow scales. Darker lateral areas of scutum with longer dark setae. Scutal fossa, antealar area and supraalar area slightly pale pollinose. Scutellum dark, slightly pale dusted, with 16–22 shorter and 14–19 long setae, short setae intermixed pale yellow and dark brown, long setae dark brown. Antepronotum with 12–14

Table 1.	Anopheles lesteri:	descriptive sta	atistics for rati	os of veins	C and R-R <sub>1</sub>	wing spot 1	engths/length of
wing measu	red from humeral	crossvein*.					

Wing Spot	Range	Mean ± SD
Costa (C)		
Basal dark to sector dark (BD+PHD+HD+PD+SD)	0.69-0.74	0.72 ± 0.02 [0.73]
Subcostal pale (SCP)	0.03-0.07	0.05 ± 0.01 [0.05]
Preapical dark (PD)	0.27-0.31	0.29 ± 0.01 [0.29]
Preapical pale (PP)	0.03-0.05	0.04 ± 0.01 [0.03]
Apical dark (AD)	0.04-0,06	0.05 ± 0.01 [0.04]
Vein R-R <sub>1</sub>		
Basal dark to presector dark (BD+PHD+HD+PSD)	0.38-0.41	0.40 ± 0.01 [0.38]
Sector pale (SP)	0.04-0.13	0.08 ± 0.04 [0.12]
Sector dark (SD)	0.21-0.26	$0.24 \pm 0.02 [0.21]$
Subcostal pale (SCP)	0.05-0.06	0.05 ± 0.00 [0.06]
Preapical dark (PD)	0.24-0.29	0.26 ± 0.02 [0.25]
Preapical pale (PP)	0.04-0.06	0.05 ± 0.01 [0.06]
Apical dark (AD)	0.03-0.05	0.04 ± 0.01 [0.03]

\* Six wings, from the neotype and alloneotype, and 3 individuals; [ ], neotype female.

dark brown setae. Pleuron brown to dark brown: upper proepisternum with 3 or 4 setae, without scales; prespiracular area with 2 or 3 setae, without scales; prealar area with 4 or 5 setae, without scales; upper mesokatepisternum with 3 or 4 setae, without scales; lower mesokatepisternum with 4 or 5 setae, without scales; upper mesepimeron with 4 or 5 setae, without scales. Legs: Fore- and midlegs dark-scaled except white scales dorsally on apex of tibia; complete narrow apical pale bands on tarsomeres 1-3, and very narrow apical dorsal pale patch on tarsomere 4; apical bands on tarsomeres 2 and 3 longest, complete ventrally, about 0.1 length of tarsomere; pale scales on tarsomere 4 dorsally and laterally only, not connected ventrally; basal pale band on tarsomere 5 absent. Hindlegs dark-scaled as fore- and midlegs, except white scales on tarsomeres 1-3 dorsally and laterally only, not ventrally. Forefemur length 1.40-1.72 mm (mean = 1.53 mm, n = 6), ratio of forefemur length to proboscis length 0.79-0.88 (mean = 0.82). Midfemur length 1.74-2.12 mm (mean = 1.94 mm), ratio of midfemur length to proboscis length 0.93-1.12 (mean = 1.04). Hindfemur length 2.01-2.2 mm (mean = 2.11 mm), ratio of hindfemur length to proboscis length 1.07-1.21 (mean = 1.14).

Wing (Table 1): Length (measured from humeral cross vein to wing tip, excluding fringe) 2.8-3.18 mm (mean = 3.05 mm). Dark scales brown to black, pale wing scales white and pale yellow. Costa (C) dark-scaled with small subcostal pale spot (SCP, mean = 0.15) and preapical pale spot (PP, mean = 0.12); remigium dark scaled; humeral crossvein and arculus without scale patch. Subcosta (Sc) dark-scaled with few scattered spatulate white scales from base to sector dark (SD). Radius (R) to R1 darkscaled except 3 pale spots (SP, SCP and PP), scattered white spatulate scales from base to presector dark (PSD), and a stripe of white scales on SD before SCP; length of white stripe 0.3-0.38 mm (mean = 0.33); base of R, dark-scaled; bifurcation of R, and R<sub>1</sub> dark-scaled; tips of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4+5</sub> with pale fringe. Media (M) darkscaled with pale area before fork; bifurcation of M 1+2 and M3+4 dark scaled; tips of M<sub>1+2</sub> and M<sub>3+4</sub> with dark fringe. Cubitus (Cu) with basal dark spot, length 0.18-0.28 (mean = 0.24); Cu<sub>1</sub> with 4 dark spots, length of first basal spot 0.1-0.18 mm (mean = 0.14), second spot 0.23-0.25 mm (mean = 0.24), third spot 0.25-0.45 mm (mean = 0.33), distal fourth spot 0.13-0.3mm (mean = 0.21);  $Cu_2$  with distal dark spot only, length 0.18-0.25 mm (mean = 0.21); tips of Cu, and Cu, with dark fringe. Anal vein (1A) with 2 dark spots, basal spot length 0.23-0.25 mm (mean = 0.24), distal spot length 0.25-0.35 mm (mean = 0.31), tip of 1A with dark fringe. Halter: Scabellum, pedicel and capitellum dark brown with grayish pollinosity. Abdomen: Terga and sterna dark brown to black with grayish pollinosity, covered with pale brown to golden brown setae. For neotype female, descriptive statistics for ratios of costal and R-R, wing spot lengths/length of wing measured from humeral crossvein are shown in brackets in Table 1.

Male (Figs. 2C, D).-As in female except for following sexual differences. Maxillary palpus 0.94-0.98 length of proboscis (mean = 0.97; n = 4 for this and following measurements except where indicated), apex of palpomere 3 and all of palpomeres 4 and 5 enlarged. Maxillary palpus with dark brown and white scales, dorsal surface of all segments with white scales; palpomere 2 with slightly erect dark brown scales at base and light gray scales from middle to apex; palpomere 3 dark-scaled with long vellowish to light brown setae at apex; palpomere 4 pale yellow to dark brown-scaled with narrow basal white band, inner surface with long yellowish-brown to light brown setae; palpomere 5 pale brown-scaled with narrow basal white band, lateral surface with white scales and numerous dark brown short setae. Proboscis length 2.65-2.9 mm (mean = 2.69 mm), dark brown-scaled. Anal vein with single dark spot. Tergum IX (width, 2.92 mm) bare, with pair of elongate caudally directed capitate lobes; length of lobe from base to tip 0.82 distance between 2 lobes; middle of lobe narrower, 0.45 width of lobe tip and 0.42 width of lobe base. Gonocoxite 1.91-2.13× as long as wide at widest point, widest about 0.08 from base; dorsal (postrotational) surface with many long setae distally, slender fusiform and spatulate scales and numerous small spicules proximally; ventral surface as dorsal but with lateral scales and numerous longer spicules; mesal parabasal spine (parabasal 1) stout, borne on slightly raised base; parabasal 2 stout with slender tip; parabasal 1 base 0.07-0.15 from base of gonocoxite; parabasal 2 base 0.14-0.15 from base of gonocoxite; internal seta slender, about as long as parabasal 2, base 0.41-0.46 distance from base of gonocoxite. Gonostylus widened at base and narrowed toward middle and tip, base 2.27× wider than middle or tip; gonostylus 0.47 length of gonocoxite; gonostylus 8.89× longer than gonostylar claw. Claspette. Dorsal lobe of claspette with 3 closely appressed setae of about equal length; tips of 2 lateral setae curved and bluntly rounded; tip of middle seta slightly curved and round; tip of composite structure club-shaped. Ventral lobe of claspette with 2 long subapical setae, most apical much longer than other. Both ventral and dorsal lobes, and areas in between them, with numerous spicules. Aedeagal leaflets 4 per side; 2 most mesal leaflets broadest, with broad, thin, nearly transparent inner margins; other leaflets with narrow, thin, nearly transparent inner margins.

Pupa (Figs. 2A, B) .- Position and development of setae as figured; range and modal number of branches, and number of branches of neotype female as in Table 2. Integument darkly pigmented. Exuviae colorless to dark brown. Cephalothorax: Mesothoracic wing with checkered dark stripes; metathoracic wing pigmented on middle, ventral spiracular sensilla distinct. Trumpet with dark thickend areas bearing saw-toothed or serrate edge, meatus with simple cleft, and its subbasal part with numerous spinules; trumpet length 0.30-0.45 mm (mean = 0.35 mm, n = 10 for this and following measurements and counts except where indicated), width 0.16-0.23 mm (mean = 0.13 mm, measured at base of pinna), index 1.36-2.5 (mean = 1.73); tracheoid area 0.45 length of trumpet. Abdomen:



Fig. 2. Anopheles lesteri. (A) Pupa, ccphalothorax. (B) Pupa, metathorax and abdomen, left side dorsal, right side ventral. (C) Male genitalia. (D) Tergum IX. Abbreviations used include: AADM = anterior accessory dark mark, ADM = anterior dark mark, CT = cephalothorax, GL = genital lobe, Pa = paddle, PADM = posterior accessory dark mark, PDM = posterior dark mark.

Seta	Cephalothorax	Abdominal Segments						
No.	CT	1	11	10	IV	v		
0		_	2-4(3) [4]	2-4(3) [3]	3-4(4) [4]	2-4(3) [3]		
1	2-3(3) [2, 3]	15-23(21) [18, 16]	3-9(6) [8, 6]	13-19(13) [13, 14]	7-13(12) [12, 9]	3-6(6) [6, 3]		
2	2-3(2) [2]	4-7(5) [4, 5]	4-10(8) [5, 7]	5-9(8) [8, 9]	3-7(5) [7]	4-7(5) [5]		
3	1-3(1) [1, 2]	3-7(3) [6, 7]	1-3(2) [2, 3]	4-7(5) [7, 6]	3-6(5) [5, 4]	1-2(2) [2]		
4	1-3(2) [2]	2-7(5) [5]	1-4(3) [3]	1-4(1) [1]	1-4(2) [3]	1-3(3) [3]		
5	1-5(4) [4]	2-5(2) [2]	2-4(3) [3, 4]	10-16(13) [12, 13]	11-21(13) [12, 15]	13-24(13) [18, 19		
6	1-2(2) [2, 1]	1-3(2) [2]	1-2(1) [1]	1-4(2) [2]	1-2(2) [2]	1-2(1) [1, 2]		
7	1-2(1) [1]	2-3(3) [3]	2-4(3) [3]	I-4(1) [3]	2-3(2) [3]	2-3(2) [3]		
8	1-2(2) [2]	—	—	2-3(2) [2-3]	2-3(3) [3]	1-3(3) [3]		
9	1-2(2) [2]	1-2(2) [2]	1 [1]	1 [1]	1 [1]	1 [1]		
10	2 [2]	—		1-2(2) [2]	1-2(1) [1]	1 [1]		
11	1-4(4) [4, 3]		—	1-2(1) [1]	1-2(1) [1, 2]	1-2(1) [1]		
12	1-3(3) [3]	_	_		_	_		
13	_	-	-	_	_	_		
14	—	-	_	_	_	1 [-]		
Seta .			Abdominal Segments			Dudda		
No.	vi		VII	VIII	IX	Paddle Pa		
0	2-4(3) [4]	2	-4(3) [2-3]	1-2(1) [1]	-	_		
1	2-5(3) [4]		2-3(2) [2]		1 [1]	1 [1]		
2	3-7(5) [7, 6		2-4(4) [4]			1 [1]		
3	1-2(1) [1, 2	2]	1-3(2) [1]	_	_	_		
4	1-3(1) [1]	Ê l	-2(1) [1, 2]	1-2(2) [2]		_		
5	8-17(10) [8,	10] 3	-7(4) [6-7]		_	_		
6	1-2(1)[1]		1-2(1) [1]		—	_		
7	1-3(1) [1]		1-3(1) [1]		_	_		
8	1-3(2) [3]	2	-3(2) [2-3]	_	—			
9	I [I]		1 [1]	1 [1]	_	-		
10	1 [1]		1-4(1) [1]		-	-		
11	1-2(1) [1]	1	-3(1) [1-2]	_	-	_		
12				_				
13	_			—	-	_		
14	_		_	1 [1]		_		

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Table 2. Pupal setal branching for Anopheles lesteri: range (mode) based on counts made on 5–10 setae of the neotype, alloneotype, and 3 specimens collected with them; [ ], neotype female.

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Abdominal tergum I with 2 anterior dark marks (ADM), and 2 elongate posterior dark marks (PDM); each PDM narrows mesally at base, with maximum width (0.016-0.02 mm, mean = 0.019) towards distal tip, length 0.14-0.31 mm (mean = 0.26, n = 10), about 0.12-0.28 (mean = 0.24, n = 10) width of abdominal segment I, and longer than the longest branch of seta 1-I. Abdominal terga II-VII with 1 ADM, 2 anterior accessory dark marks (AADM), and 2 posterior accessory dark marks (PADM); VIII with 1 ADM and no AADM and PADM; cuticular reticulations distinct on II-IV; spinules scattered mostly on anterior 0.37 of dorsal and lateral sides of VII and VIII. Seta 1-I fan-like with 15-23 aciculate dendritic branches; 6-I with 1-3 branches; 7-I with 2 or 3 branches; 9-I with 1 or 2 branches. Setae 1, 5-II-VII well developed; 1-V 1.04-1.31 (mean = 1.16, n =4) length of 5-V; 1-VI 0.83-1.09 (mean = 0.97, n = 4; 1-VII 1.84-2.22 (mean = 1.09, n = 6; 3-VI aligned with and mesal of I-VI unlike on other segments; 8-I-II absent; 9-I simple, single or forked; 9-II very short, simple, spine-like; 9-III short, with slightly rounded tip, 1.50-4.00 (mean = 2.51) length of 9-II; 9-IV strong, lightly pigmented and slightly pointed, 01.00-2.80 (mean = 1.83) length of 9-III; 9-V-VIII long, lightly pigmented and slightly pointed; 9-V 1.00-1.50 (mean = 1.68) length of 9-IV; 9-VI 0.81-1.33 (mean = 1.02) length of 9-V; 9-VII 1.00-1.34 (mean = 1.11) length of 9-VI; 9-VIII 0.80-1.06 (mean = 0.96) length of 9-VII; 9-VI 1.11-1.39 (mean = 1.27, n = 5) length of segment VI; 9-VII 1.05–1.18 (mean = 1.13, n = 5) length of segment VII; 9-VIII 0.85-0.92 (mean = 0.90, n = 5) length of segment VIII. Seta 9-I positioned near anterolateral edge of tergum; 9-II on the posterolateral edge of tergum; 9-III-VIII near posterolateral edge of tergum. Segment VII 1.05-1.19 (mean = 1.12, n = 5) length of segment VI; segment VIII 1.11 - 1.39 (mean = 1.26, n = 5) length of segment VI; segment VIII 1.05-1.18 (mean = 1.13, n = 5) length of

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segment VII. Segment VII 0.85-0.92 (mean = 0.90, n = 5) width of segment VI (width at posterior margins); segment VIII 0.69-0.92 (mean = 0.84, n = 5) width of segment VI; segment VIII 0.75-1.01 (mean = 0.93, n = 5) width of segment VII. Width/ length of segment VI 2.29-2.88 (mean = 2.48, n = 5), VII 1.84–2.21 (mean = 1.99, n = 5), VIII 1.23-1.85 (mean = 1.65, n =5). Paddle length 0.70-0.75 mm (mean = 0.72 mm, n = 5), width 0.15-0.34 mm(mean = 0.22 mm, n = 5), length/width ratio 2.24-4.86 (mean = 3.73, n = 5); refractile index 0.34-0.67 (mean = 0.54, n =5); paddle seta 1-Pa simple or forked (2-4 apical branches), length 0.14-0.18 mm (mean = 0.16, n = 9); 2-Pa simple or forked (2 apical branches), length 0.40-0.14 mm (mean = 0.11); length of 1-Pa 1.11-3.80 (mean = 1.66, n = 9) length of 2-Pa. Width/length of genital lobe of female 1.33-1.43 (mean = 1.37, n = 3), male 0.87-0.89 (mean = 0.88, n = 2); numerous spicules present on subapical and apical margins of genital lobe of female, absent in male.

Larva (Fig. 3) .- Position and development of setae as figured; range and modal number of branches and number of branches of neotype female as shown in Table 3. Head: Length 0.68-0.71 mm (mean = 0.70, n = 4), width 0.66-0.76 mm (mean 0.71, n = 4). Antennal length 0.25-0.29mm (mean = 0.26, n = 8), slightly tapered toward apex, 4.57-6.00 (mean = 5.27, n =8) longer than wide; with spicules longer and more numerous ventrally and in vicinity of seta 1A; spicule length 0.01-0.02 mm (mean = 0.02, n = 12). Seta 1-A with 9-13 (mode = 10, n = 8) branches, length 0.16-0.24 mm (mean = 0.19, n = 8), inserted 0.11–0.22 mm (mean = 0.14, n = 8) from base of antenna; 2-A single, pointed, length 0.10–0.20 mm (mean = 0.13, n =8); 3-A single, pointed, length 0.05-0.17 mm (mean = 0.08, n = 7); 4-A with 6-8 branches (mode = 8, n = 7); 5-A short, spine-like, 0.06-0.17 (mean = 0.13, n = 8) length of seta 1-A; 6-A spine-like about



Fig. 3. Anopheles lesteri, larva. (A) Head, left side dorsal, right side ventral. (B) Dorsomentum (Dm). (C) Thorax and abdominal segments I-VI, left side dorsal, right side ventral. (D) Pecten plate (PP) and pecten spines. (E) Abdominal segments VIII-X, side view.

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	Head	2 2 320032003	Thorax	II	Abdominal Segments			
Seta No.	C*	P	м	Т	I	11	111	
0				-	-	2-4(3) [3, 4]	2-4(3) [4, 3]	
I	1 [1]	1-3(1) [2, 3]	21-29(24) [25, 27]	[1] 1	9-14(12) [13, 14]	10-15(13) [13]	18-19(19) [18, 19	
2	1 [1]	8-12(9) [8, 9]	1-4(1) [1]	1 [1]	4-7(6) [6]	4-8(6) [6]	3-6(5) [6, 5]	
3	32-68(42) [51, 57]	1 [1]	1 [1]	9-13(11) [13, 12]	2-3(2) [2]	111	1 [1]	
4	3-5(3) [3]	12-15(15) [12]	3-5(3) [3, 5]	3-6(4) [4]	5-10(8) [8, 9]	4-8(5) [5]	3-5(5) [5, 4]	
5	11-16(15) [14, 15]	21-29(26) [26, 22]	1 [1]	25-34(29) [28, 29]	3-5(5) [5]	8-15(9) [8, 9]	5-8(7) [6, 8]	
6	11-15(15) [15]	1 [1]	3-5(3) [3, 4]	3-4(3) [3]	14-21(18) [14]	22-25(24) [24]	18-20(19) [19, 2	
7	13-18(15) [15]	23-27(25) [27, 25]	3 [3]	22-29(25) [27]	15-20(18) [18, 16]	21-24(22) [22]	3-4(4) [4]	
8	5-13(9) [9]	15-27(22) [22, 21]	10-15(13) [10, 12]	27-30(28) [28]		3 [3]	3-5(3) [3]	
9	7-9(9) [7, 9]	1 [1]	1 [1]	1 [1]	6-9(6) [6, 7]	7-9(8) [7]	6-11(6) [6]	
10	1-3(2) [2]	1 [1]	1 [1]	1 [1]	1 [1]	2-3(2) [2]	1 [1]	
11	32-56(48) [56, 48]	3-4(4) [4]	1 [1]	1 [1]	3-4(3) [3, 4]	1 [1]	2 [2]	
12	2-4(3) [4, 3]	1 [1]	1 juj	1-2(1)[1]	1-2(1)[1]	101	1-3(2) [2]	
13	3-8(3) [4, 3]	9-14(11) [11]	6-10(6) [6]	1-3(2) [2]	7-11(8) [8]	7-11(10) [7]	8-11(9) [11]	
14	1-5(5) [5]	5-9(6) [6]	8-17(10) [12, 10]			<u> </u>		
15	6-8(8) [8, 7]	<u> </u>	—	-	_		_	
				Abdominal Segments				
Seta No.	IV	V	VI		VII	VIII	x	
0	2-4(3) [4, 3]	2-3(3) [3]	2-3(2)	3] 2-3(	2) [3, 2]	1-2(1) [1]		
1	16-21(18) [18]	16-19(19) [19]	16-19(18) [	19, 18] 14-17	7(17) [17]	1-2(1)[1]	1 [1]	
2	2-3(2) [2, 3]	2-3(3) [3]	3-8(5) [3	, 5] 3-7(	5) [4, 3]	-12(11)[11]	16-51 (18) [19]	
3	3-4(3)[3, 4]	1 [1]	1 [1]	3-5(	4) [4, 5]	-8(7) [7, 8]	6-22(6) [12]	
4	4-6(4) [5, 4]	3-5(4) [4]	TÍ			1 [1]	9 [9]	
5	3-5(4) [5, 4]	4-6(5) [5, 4]	6-7(6)			6(4) [4, 6]	_	
6	2-4(2) [2]	2 [2]	5-6(5)	6] 4-7(	5) [7, 5]	1-S	4-5(4) [5]	
7	3-4(3) [3, 4]	3-4(3) [3, 4]	3-5(3) [3	. 5] 3-0	6) [6]	2-S	3-5(4) [3, 4]	
8	2-3(3)[3, 2]	3-6(4) [5, 4]	3-4(4)	4  3-5	5(4) [5]	6-S	2 [2]	
9	7-11(10) [10, 8]	5-9(6) [6, 5]	6-8(7)		6(5) [6]	7-S	Τμ	
10	1 [1]	111	2-5(3) [3		5(3) [3]	8-S	4-5(4) [4]	
11	2-3(2) [2]	2-5(3) [3]	2 [2]		2(2) [2]	9-S	3-5(5) [5]	
12	2-3(3) [3, 2]	1-4(1) [2]	ī (ī)					
13	3-5(4) [4]	3-5(5) [5]	6-12(6)		5(4) [4]		_	
14	1-2 []	2 [2]	2 [2]	1-4	—	1-2(1) [2]	<del></del>	
15		- (-)	- [-]					

Table 3. Larval setal branching for Anopheles lesteri: range (mode) based on counts made on 5-8 setae of the neotype and alloneotype, and 2 specimens collected with them; [], neotype female.

\* C, head; P, prothorax; M, mesothorax; T, metathorax.

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1.6× longer than seta 5-A. Seta 2-C single 1.69-2.37 (mean = 1.92, n = 3) length of 3-C; seta 2-C close to mate of opposite side 0.002-0.006 mm (mean = 0.004, n = 4);3-C densely dendritic with 32-68 main branches (mode = 42), 0.10–0.14 (mean = 0.12, n = 8) length of 2-C, clypeal index (distance between bases 2-C and 3-C on 1 side/distance between bases of 2-C) 11.33-40.00 (mean = 27.83, n = 4). Thorax: Seta 1-P with 1-3 branches (mode = 1, n = 8); 9-10,12-P single; 9-12-P setal support plate spine length 0.04 mm. Setae 9-12-M single; 9-M 3.12–12.67 (mean = 9.48, n =3) length 10-M; 9-12-M setal support plate spine length 0.01-0.02 mm (mean = 0.02, n = 4). Setae 9–10-T single; 9-T 1.18–1.31 (mean = 1.24, n = 4) length of 10-T; seta 12-T with 1-3 branches; 9-12-T setal support plate spine length 0.02 mm (n = 2); 13-T with 3 branches. Abdomen. Seta 1-I with 9-14 branches (mode = 12, n = 8); 1-II 10-15 branches (mode = 13, n = 8). Seta 1-III-VII palmate with well-developed leaflets, each leaflet with short filament; 0-II-VIII and 14-III-VIII weakly developed; 0,8,14-I, 14-II absent or rare; 3-II-III, V-VI single; 3-I, IV, VII branched. Seta 1-X single, 1.30-2.40 (mean = 1.81, n = 5) length of saddle; 1-X inserted on saddle. Saddle with minute, sparse spicules on lateral surface. Integument of posterior margin of segment X with strongly developed dark brown to black spicules. Spiracular apparatus. Pecten plate with 12-18 spines; arrangement of spines alternating long and short, with 7 or 8 (mode = 7, n = 6) long spines and 5–11 (mode = 9, n = 6) short spines; long spines 1.27-11.67 (mean = 3.86, n = 37) length of short spines. Two posterolateral spiracular lobe plates present, each plate with elongate, slender, sclerotized projection from inner caudal margin.

Type material.—Neotype female with associated slide-mounted larval and pupal exuviae and DNA of a midleg of female; reared from a larva collected from a ditch on hill with slow flowing clear water, 28.8°C, pH 6.79, salinity 0.07 ppt, conductivity 0.15 mS, data as follows: "Tanque", Calauan, Laguna, Luzon, Philippines, L. M. Rueda Coll. 29 July 2002, 14°08'44"N, 121°18'54"E, collection and specimen no. PH 9-7. Deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC. (WRBU ACC No. 1729). Alloneotype male with slide-mounted genitalia, extracted DNA of combined head, thorax and abdomen, and associated slidemounted pupal exuviae with collection no. PH 9-6 and same collection data as neotype female. The morphological descriptions of the head, thorax and abdomen of the alloneotype male were recorded before being processed for DNA. We were unable to collect any specimens from the original type locality of Santa Mesa, Manila, Luzon, because it is now a highly urban area totally lacking typical larval habitats. Baisas and Hu (1936) noted that many cotypes of An. lesteri were collected from Calauan, Laguna, Luzon, about 50 km south of Santa Mesa. This locality remains rural and we were able to collect specimens from Calauan for the present study. Morphological data in Tables 1-3 are based on measurements of the neotype, alloneotype and associated specimens collected from Calauan in 2002.

Other material examined .--- 247 specimens in the National Museum of Natural History, Smithsonian Institution, Washington DC, consisting of 54 9, 29 8, 76 pupal exuviae, 85 larval exuviae, and 2 & genitalia. PHILIPPINES, LUZON: Province of LAGUNA, Calauan, same collection data as female neotype, PH 9-3, 1 F PeLe; PH 9-8, 1 9 PeLe; PH 9-11, 1 & G PeLe; 17 April 1930, Lot 77-19, 1 &; 20 Apr. 1930, Lot 77-19, 1 &; 19 Jan. 1931, Lot 122, 2 δ; 9 July 1931, Lot 247, 3 ♀; 17 Sept. 1931, 9 9, 3 d; ; 1 Sept. 1932, W. V. King coll., Lot K317i, 1 &. Province of MIN-DORO ORIENTAL, Ordovilla, 0.5 km W. Victoria, seepage spring, B. Harrison and Kol coll., 17 July 1969, P58-127, 1 Pe; B. Harrison coll., 19 July 1969, P61-37, 1 LePe; 1969, B. Harrison and Kol coll.,

1	aattagaagt	ttggcaaaca	gaaaactacg	cagtgattgg	tgctggtcac	cacgtcacgg
61	tcgtgcataa	aggtgtaaga	gagatctcgt	cgatcgcttg	catctcggaa	cttgtgttga
121	aaggccgcga	agacagacaa	gtagtaaaca	gcagcagatg	tgttcccgcg	attggcggaa
181	gttctaggca	ggcgcgccct	gacgctgtgt	gtagatggag	caggtgtctt	cctcatctat
241	ttttatttta	aaaattgagg	taagacttcc	aacgtttctt	cgagatagtg	gaatgggctg
301	caagagactg	gaatcggaag	ttgaacaacg	gaacactcta	ttagcaaaca	ctacccagaa
361	tccgtgcaga	acgactggaa	gatgcaagtt	ctacctgaga	atcattatca	cttacgagtg
421	aggccactcg	gtggtcaa				

Fig. 4. Internal transcribed spacer 2 (ITS2) sequence of the ribosomal DNA for An. lesteri from the type locality, Calauan, Laguna, Philippines. GenBank accession number AY375469.

SEAMP Acc. 233, P44-1, 1 9; P58-36, 1 ♂; P58-37, 1 ♀; P58-111, 1 ♂; P58-116L, 1 δ; P58-127, 1 δ; Caminawit Pt., 30 Dec. 1944, G. H. Pena coll., No. C-41, 1 M. **MOUNTAIN PROVINCE, Baguio, 12 June** 1945, 32MSU#140, 1 9; S. E. Sheilds coll., 10 Aug. 1945, 24MSU#432, 12 9, 4 8; Trinidad, May 1945, J. G. Franclemont, 4 9, 1 3. Province of NUEVA ECIJA, Munoz, Rozeboom, Knight and Laffoon coll., 8 Aug. 1945, #1153, 3 LePe. Province of PAMPANGA, Olongapo (Santa Rita), Rozeboom, Knight and Laffoon coll., 10 Aug. 1945, #1115, 4 PeLe; #1116, 1 PeLe. Province of RIZAL, Camp Nichols, PVT Carraway coll., 14 Dec. 1924, 1 9; 18 Dec. 1945, P469, salty fishpond with algae, 1  $\delta$ ; Mandaluyong, 17 Dec. 1945, P468, rice field, 1 9. VISAYAS: Province of LEYTE, Tacloban, Rozeboom, Knight and Laffoon coll., 16 Aug. 1945, No. 1713, 1 LePe, 1 9, 1 ♂; Southern Leyte, 2 Dec. 1944, O. H. Graham, 4 , 1  $\delta$ ; Palo Alto, 1945, 1 ♀; 8 Jan. 1945, 32MSU#384a, 1 ♀; 1 Feb. 1945, 32MSU#P430, 1 8; 11 Mar. 1945, 32MSU#474, 1 9. Province of NEGROS OCCIDENTAL, La Carlota, 31 Jan. 1930, W. V. King coll., Lot 124-16, 1 d.; Silay, 3 Feb. 1930, Lot 137, 1 d. Province of SA-MAR. Osmena. Rozeboom. Knight and Laffoon coll., 8 Sept. 1945, #1334.30, progeny brood, 9 Le, 29 PeLe; #1334.34, 2 Le, 12 PeLe; #1348.3, 13 PeLe; #1348.6, 4 PeLe; 1945, Rozeboon, Knight, Laffoon coll., No. 1348.5, 3 M; San Antonio, 29 Nov. 1944, J. H. Paullos coll., No. 506, 1 9; 1 Dec. 1944, J. H. Paullos coll., No. 507, 1 9; Dec. 1944, 2 9, 3 d.

Molecular characterization.-DNA was extracted from a midleg of the neotype female (PH9-7), the whole body (excluding genitalia) of the alloneotype male (PH9-6), a midleg of a female (PH9-3), and the whole body (excluding genitalia) of a male (PH9-11). Ribosomal DNA ITS2 sequences are the same for all 4 (GenBank accession number AY375469 (PH9-6; Fig. 4). Other sequences in GenBank that match these sequences are as follow: under the name An. anthropophagus, Acc. Nos. AF384172, AJ004941, AF543860; An. lesteri Korea, Acc. No. AY375468); An. lesteri China (locally identified as An. anthropophagus), Acc. No. AY375467.

Distribution.—China (Hong Kong, south and central areas of the mainland, extending west to 105°E longitude and north to 43°N), Guam, Japan (including Ryukyu Islands), Korea, Philippines (Luzon: Laguna, Manila, Mindoro Oriental, Mountain Province, Nueva Ecija, Pampanga, Rizal; Visayas: Leyte, Negros Occidental, Samar).

Medical importance.—Anopheles lesteri is a human biter and is considered a principal vector of malaria in southern China (Beales 1984, Chow 1991, Ho et al. 1962, Ma 1981) and other areas of the country (as anthrophophagus, Tang et al. 1991). It is suspected of being a primary vector in Japan and Korea (Kamimura 1968, Otsuru 1949, Tanaka et al. 1979). Natural infection rates of An. lesteri in the 1960s were 1.9 to 14.4 times greater than An. sinensis in China (Gu et al. 1966). In the Philippines and Guam, its biting habits are unknown, and it is not known to transmit malarial parasites.

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It (as anthropophagus) has a strong preference for human blood, and plays an important role in the transmission of filariasis and malaria in central and south China (Xu and Feng 1975). Harrison (1973) suggested a need to determine the distribution, behavior, and malarial and filarial parasite susceptibilities of An. lesteri throughout its range. This species, instead of An. sinensis, may be the more significant vector in Taiwan, Okinawa, Japan, Korea, and central and northern China.

Bionomics.-The larvae of An. lesteri are found in a variety of habitats including freshwater pools, margins of ponds, lakes, areas preferably not affected by salt tides (Baisas 1974), and ditches with slowly flowing clear water in hilly areas in the Philippines. In Japan, the larvae occur in marshes, sluggish streams, ground pools, ponds, rice fields, and other impounded waters (Tanaka et al. 1979). Unlike An. sinensis, An. lesteri prefers places that are cool and shaded. Adult populations of An. lesteri reach their peaks during the summer season in Hokkaido (Kamimura 1976), and during June and October in Honshu and Kyushu, Japan (Otsuru and Ohmori 1960). The species is more frequently found in coastal areas than inland. In Hong Kong, it commonly occurs in hilly areas and grassy fields (Chau 1982). In Guam, An. lesteri larvae were found in a carabao wallow (Basio and Reisen 1971). Adults of An. lesteri, An. sinensis and other anophelines were collected in cow sheds and living rooms of houses in villages during malaria surveys in Korea (Whang 1962). Anopheles lesteri has been confused with An. sinensis and other members of the Hyrcanus Group, and some published records of its distribution and bionomics are not accurate, particularly in Japan, Korea and China.

## DISCUSSION

Although Baisas and Hu (1936) provided the original description of *An. lesteri*, it was not sufficient for accurate identification of the species. This resulted in misidentifications of the species in many parts of its geographical range in Asia, particularly Korea, Japan and China. The morphological information in this paper, coupled with rDNA ITS2 sequence (Wilkerson et al. 2003), will help in solving those problems.

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Anophleles lesteri has the following diagnostic features. Adult female. Maxillary palpus with palpomere 3 having narrow basal pale band about as wide as pale bands of other palpomeres; vein Cu2 without apical pale fringe spot (unlike sinensis, sineroides, pullus); subcostal pale (SCP) spot narrow; humeral crossveins without scales (unlike *pseudosinensis*); midcoxa without upper patch of pale scales (unlike sinensis); hindtarsomeres 2-4 with narrow apical pale bands, hindtarsomere 4 without basal pale band (unlike peditaeniatus). Adult male. Male genitalia with dorsal lobe of claspette having 3 closely appressed setae of about equal length. Aedeagus with 4 leaflets on each side; 2 most mesal leaflets with broader transparent inner margins than other leaflets. Tergum IX bare, with pair of caudally directed elongate capitate lobes. Pupa. Trumpet with thick and serrate rim. Wing with checkered dark stripes. Setae 9-III-VII single, with narrowly rounded apex; seta 5-V with 13-24 branches. Larva. Setae 2-C, 3-P, 3, 5-M single; 3-C with 32-68 branches; 4-M with 3-5 erect branches; 9-M more than 3 times the length of 10-M; 9-M about 1.5 times longer than 10-T; 5-III with 5-8 branches; 9-III with 6-11 branches; 13-IV with 3-5 branches; 1-X strong, single about 2 times or more length of saddle; pecten with 7 or 8 long spines and 5-11 short spines. Reid (1953) and Harrison (1973) provided useful diagnostic pupal and adult morphological characters to separate An. lesteri from An. sinensis. Harrison and Scanlon (1975) also listed several characters of all life stages of 10 species of the Hyrcanus Group found in Thailand. They also discussed extensively the morphological taxonomy of the Lesteri Subgroup. Comparisons of pupal and larval characters of An. lesteri and related species of the Hyr•

canus Group from China and other areas of Asia are needed to further clarify species differences. Other morphological features described in this paper for larvae, pupae and adults may be helpful for separating *An. lesteri* from related species.

Morphological similarities in all stages, along with intraspecific variation of many species in the Hyrcanus Group, have lead to much confusion in Korea, Japan and China (Tanaka et al. 1979), and possibly in other areas of Asia where they occur. Wilkerson et al. (2003) suggested that the best way to infer conspecificity of populations across large geographic areas is to compare specimens from type localities. Based on the combination of published and their newly generated rDNA ITS2 sequences, Wilkerson et al. (2003) found that An. lesteri from South Korea and An. anthropophagus from Jiangsu Province, China, are the same as An. lesteri from near its type locality in the Philippines (Calauan, Laguna, Luzon). Anopheles anthropohagus, considered a major malaria vector in central and north China, is actually An. lesteri, not a separate species. With that finding, they placed An. anthropophagus in synonymy with its senior synonym, An. lesteri. Any morphological features previously thought to differentiate An. anthropophagus and An. lesteri are evidently variable characters of a single species. What is called An. lesteri in China (as reported by Gao et al. 2004: 7, 9) is actually an unknown species when compared with the work of Wilkerson et al. (2003). Several molecular studies (e.g., Li et al. 1991; Ma et al. 2000a, b; Gao et al. 2004) were conducted but were unable to clarify the taxonomic identity of An. lesteri found in China and Korea. With the collection of An. lesteri specimens from near the type locality in Luzon, Philippines, Wilkerson et al. (2003) were able to compare the specimens with those from Korea and China, and they concluded that the so called An. anthropophagus from China, and the so called An. lesteri from Korea are conspecific with An. lesteri from the Philippines.

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With the designation of the neotype and detailed descriptions of various life stages of An. lesteri based on specimens collected near the type locality, future systematic studies may be conducted using various methods, including morphological, molecular or biochemical. With the identity of An. lesteri resolved, the effectiveness of malaria vector control practices could be further improved. As suggested by Harrison (1973), additional information is needed on the distribution, behavior, and malarial and filarial parasite susceptibilities of vector species throughout their ranges. A vector species may be a more significant parasite vector in one geographical area than in others. For example, An. lesteri is considered an important malaria vector in China and Korea, but not in the Philippines. Furthermore, because several species of the Hyrcanus Group are involved in the transmission of malaria and filariasis in Asia, there is a need to revise the taxonomy of the whole group and to further clarify the identities of the cryptic species, particularly the vectors, in the group.

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- Baisas, F. E. 1974. Mosquito fauna of Subic Bay Naval Reservation, Republic of the Philippines. Headquarters, First Medical Service Wing (PACAF) Technical Report 72-2: 1–170.
- Baisas, F. E. and S. M. K. Hu. 1936. Anopheles hyrcanus var. sinensis of the Philippines and certain parts of China, with some comments on Anopheles hyrcanus var. nigerrimus of the Philippines. Monthly Bulletin of the Bureau of Health, Manila, Philippines 16: 205–241.
- Basio, R. G. 1971. The mosquito fauna of the Philippines. National Museum of the Philippines Monograph No. 4 and Headquarters, First Medical Service Wing (PACAF) Technical Report 71-1: 1– 198.
- Basio, R. G. and W. K. Reisen. 1971. On some mosquitoes of Guam, Marianas Islands (Diptera: Culicidae). Philippine Entomologist 2: 57-61.
- Beales, P. F. 1984. A review of the taxonomic status of Anopheles sinensis and its bionomics in relation to malaria transmission (WHO/MAL/84.1007) (WHO/VBC/ 84.898). World Health Organization, Geneva, Switzerland.
- Belkin, J. N. 1962. The mosquitoes of the South Pacific (Diptera: Culicidae). University of California Press, Berkeley and Los Angeles, 2 vols., 608 and 412 pp.
- Burkett, D. A., W. J. Lee, K. W. Lee, H. C. Kim, H. I. Lee, J. S. Lee, E. H. Shin, R. A. Wirtz, H. W. Cho, D. M. Claborn, R. E. Coleman, W. Y. Kim, and T. A. Klein. 2001. Light, carbon dioxide, and octenol-baited mosquito trap and host-seeking activity evaluations for mosquitoes in a malarious area of the Republic of Korea. Journal of the American Mosquito Control Association 17: 196-205.
  - ——. 2002. Late season commercial mosquito trap and host seeking activity evaluation against mosquitoes in a malarious area of the Republic of Korea. Korean Journal of Parasitology 40: 45–54.
- Cagampang-Ramos, A. and R. F. Darsie. 1970. Illustrated keys to the Anopheles mosquitoes of the Philippine Islands. USAF Fifth Epidemiological Flight (PACAF) Technical Report 70-1: 1–49.
- Chau, G. W. 1982. An illustrated guide to the identification of the mosquitoes of Hong Kong. Urban Council, Hong Kong, 53 pp.
- Chen, P. H., Y. H. Bian, F. W. Li, Z. H. Zeng, and Q. Tian. 1994. Studies on the components of trace elements and macro elements in hemolymph of *Anopheles anthropophagus*. Chinese Journal of Parasitology and Parasitic Diseases 12: 27–30.
- Cheng, H., W. Yang, W. Kang, and C. Liu. 1995. Large scale spraying of bednets to control mosquito vectors and malaria in Sichuan, China. Bulletin of the World Health Organization 73: 321–328.
- Chow, C. Y. 1991. Malaria vectors in China, Proceed-

ing of the Fourth National Vector Control Symposium. Chinese Journal of Entomology: 67-79

- Coleman, R. E., C. Kiattibut, J. Sattabongkot, J. Ryan, D. A. Burkett, H. C. Kim, W. J. Lee, and T. A. Klein. 2002. Evaluation of anopheline mosquitoes (Diptera: Culicidae) from the Republic of Korea for *Plasmodium vivax* circumsporozoite protein. Journal of Medical Entomology 39: 244-247.
- 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. Journal of Medical Entomology 33: 109-116.
- Dapeng, L., S. Leyuan, L. Xili, and Y. Xiance. 1996. A successful control program for *falciparum* malaria in Xinyang, China. Transactions of the Royal Society of Tropical Medicine and Hygiene 90: 100–102.
- Feighner, B. H., S. I. Pak, W. L. Novakoski, L. L. Kesley, and D. Strickman. 1998. Reemergence of *Plasmodium vivax* malaria in the Republic of Korea. Emerging Infectious Diseases 4: 295-297.
- Gao, Q., N. W. Beebe, and R. D. Cooper. 2004. Molecular identification of the malaria vectors Anopheles anthropophagus and Anopheles sinensis (Diptera: Culicidae) in Central China using polymerase chain reaction and appraisal of their position within the hyrcanus group. Journal of Medical Entomology 41: 5-11.
- Gu, Z.C., H. L. Quian, and X. Zheng. 1996. Distribution, ecologic behavior, potential of malaria transmission and control of *Anopheles anthropophagus* in China, pp. 33–34. *In Tan*, L. H., ed. Malariology (Part II). Qing Dao Ocean University Press, Qing Dao.
- Harbach, R. E. and K. L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Plexus, Marlton, New Jersey, 415 pp.
- —. 1982. Corrections and additions to taxonomists' glossary of mosquito taxonomy. Mosquito Systematics 13: 201–217.
- Harrison, B. A. 1973. A lectotype designation and description for Anopheles sinensis Wiedemann 1828, with discussion of the classification and vector status of this and some other oriental Anopheles. Mosquito Systematics 5: 1-13.
- Harrison, B. A. and J. E. Scanlon. 1975. Medical Entomology Studies—II. The subgenus Anopheles in Thailand (Diptera: Culicidae). Contributions of the American Entomological Institute (Ann Arbor) 12: 1–307.
- Ho, C., T. C. Chou, T. H. Chen, and A. T. Hsueh. 1962. The Anopheles hyrcanus group and its relation to malaria in East China. Chinese Medical Journal 81: 71–78.
- Huang, G. Q., H. F. Li, H. X. Zhang, J. Y. Liu, J. B. Zhang, G. Y. Chen, P. H. Yu, and G. Z. Ming.

2001. Environmental investigation in areas where *Anopheles anthropophagus* is distributed in Hubei Province. Chinese Journal of Parasitology and Parasitic Diseases 19: 340–343.

- Kamimura, K. 1968. The distribution and habits of medically important mosquitoes of Japan (in Japanese). Japanese Journal of Sanitary Zoology 19: 15-34.
- ———. 1976. On Anopheles yesoensis Tsuzuki, 1901 reported from Hokkaido (abstract, in Japanese). Japanese Journal of Sanitary Zoology 27: 2.
- Kim, H.C., K. W. Lee, T. A. Klein, and D. A. Strickman. 1999. Seasonal prevalence of mosquitoes collected from light traps in Korea (1995–1996). Korean Journal of Entomology 29: 181–187.
- Knight, K. L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae). The Thomas Say Foundation, Entomological Society of America, College Park, Maryland, 611 pp.
- Lee, J. S., W. G. Kho, H. W. Lee, M. Seo, and W. J. Lee. 1998. Current status of vivax malaria among civilians in Korea. Korean Journal of Parasitology 36: 241–248.
- Lee, W. J., H. W. Lee, E. H. Shin, Y. C. Yang, N. R. Kim, S. T. Hong, and J. S. Lee. 2000. Vector determination of tertian malaria (*Plasmodium vivax*) by polymerase chain reaction. Korean Journal of Entomology 30: 77–83.
- Li, B. W., H. L. Lu, K.T. Yao, and D. Liu. 1991. Restriction fragment length differences of genomic repetitive DNA from five sibling species of *Anopheles hyrcanus* group. Chinese Journal of Parasitology and Parasitic Diseases 9: 8-11.
- Liu, J. Y., X. J. Liu, Z. Chen, Z. P. Tu, G. B. Zheng, Y. N. Chen, Y. Z. Zhang, S. P. Weng, X. H. Huang, and F. Z. Yang. 1991. Filariasis and its control in Fujian, China. Southeast Asian Journal of Tropical Medicine and Public Health 22: 147–154.
- Ma, S. F. 1981. Studies on the Anopheles (A.) sinensis group of mosquitoes in China, including four new sibling species. Sinozoologica 1: 59–70.
- Ma, Y. J. and J. Wang. 1988. Salivary gland chromosome study of Anopheles lesteri anthropophagus (Diptera: Culicidae). Journal of Shaanxi Normal University Natural Science Edition, Supplement 16: 65-68.
- Ma, Y. J., F. Y. Qu, Y.C. Cao, and B. J. Yang. 2000a. On molecular identification and taxonomic status of Anopheles lesteri and Anopheles anthropophagus in China (Diptera: Culicidae). Chinese Journal of Parasitology and Parasitic Diseases 18: 325-328.
- Ma, Y., F. Qu., J. Xu, X. Li, and G. Song. 2000b. Differences in sequences of ribosomal DNA second internal transcribed spacer among 3 members of the *Anopheles hyrcanus* complex from the Republic of Korea. Entomologica Sinica 7: 36-40.
- Min, G. S., W. Choochote, A. Jitpakdi, S. J. Kim, W.

Kim, J. Jung, and A. Junkum. 2002. Intraspecific hybridization of *Anopheles sinensis* (Diptera: Culicidae) strains from Thailand and Korea. Molecular Cells 14: 198–2004.

- Niu, L. L., Z.Yuan, G. Y. Coi, and C. A. Molcolm. 1992. DNA probe for the identification of sibling species of Anopheles sinensis and Anopheles anthropophagus. Chinese Journal of Parasitology and Parasitic Diseases 10: 267–270.
- Ohmori, Y. 1957. On the male hypopygium of the sinensis-group of anopheline mosquitoes in Japan. Acta Medica et Biologica, Niigata 5: 209-217.
- ——. 1959. The pupae of Japanese Anopheles. Japanese Journal of Sanitary Zoology 10: 210–225.
- Otsuru, M. 1949. A new species of Anopheles hyrcanus in Japan (in Japanese). Fukuoka Igaku Zassi 40: 139–148.
- Otsuru, M. and Y. Ohmori. 1960. Malaria studies in Japan after World War II. Part II. The research for Anopheles sinensis sibling species group. Japanese Journal of Experimental Medicine 30: 33– 65.
- Ramsdale, C. D. 2001. Internal taxonomy of the Hyrcanus Group of Anopheles (Diptera: Culicidae) and its bearing on the incrimination of vectors of malaria in the west of the Palearctic Region. European Mosquito Bulletin 10: 1–8.
- Reid, J. A. 1953. The Anopheles hyrcanus group in southeast Asia (Diptera: Culicidae). Bulletin of Entomological Research 44: 5-76.
- ———, 1968. Anopheline mosquitoes of Malaya and Borneo. Studies from the Institute for Medical Research Malaysia, No. 31: 1–520.
- Reisen, W. K., J. P. Burns, and R. G. Basio. 1972. A mosquito survey of Guam Marianas Islands with notes on the vector -borne disease potential. Journal of Medical Entomology 9: 319–324.
- Rueda, L. M., J. E. Pecor, M. C. Yuen, and M. W. Lee. 2004. New record, habitats and updated checklist of the mosquitoes of Hong Kong. Journal of the American Mosquito Control Association 20: 204– 207.
- Shahjehan, I. A. 1996. DNA probes to identify Anopheles sinensis and An. lesteri anthropophagus, Chinese species. Pakistan Journal of Zoology 28: 205-212.
- Shin, E. H., T. S. Kim, H. W. Lee, J. S. Lee, and W. J. Lee. 2002. Vector competence of Anopheles lesteri Baisas and Hu (Diptera: Culicidae) to Plasmodium vivax in Korea. Korean Journal of Parasitology 40: 41-44.
- Sleigh, A. C., X. L. Liu, S. Jackson, P. Li, and L. Y. Shang. 1998. Resurgence of vivax malaria in Henan Province, China. Bulletin of the World Health Organization 76: 265–270.
- Song, J. and Y. Peng. 1996. Field trials of combined use of two species of mermithid nematodes to

control Anopheles and Culex breeding in China. Indian Journal of Malariology 33: 161-165.

- Takai, K., T. Kanda, Y. Ogama, W. H. Cheong, A. M. Joesoef, and S. Sucharit. 1984. Postmating reproductive isolation between 7 members of the *Anopheles hyrcanus* species group in East Asia. Medical Entomology and Zoology 35: 251–260.
- Tanaka, K. 1971. Mosquitoes of the Ryukyu Islands. A list of the family Culicidae from the Ryukyu Islands (abstract). Abstracts of speeches of the 23<sup>rd</sup> annual meeting of the Japanese Society of Sanitary Zoology, p. 4.
- Tanaka, K., K. Mizusawa, and E. S. Saugstad. 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). Contributions of the American Entomological Institute (Ann Arbor) 16: 1–987.
- Tang, L. H., H. L. Quian, and S. H. Xu. 1991. Malaria and its control in the People's Republic of China. Southeast Asian Journal of Tropical Medicine and Public Health 22: 467–476.
- Toma, T. 2002. Studies on the Anopheles mosquitoes in the Ryukyu Archipelago, Japan. Medical Entomology and Zoology 53: 7–19.
- Tsuzuki, J. 1901. Results of an investigation on malaria in Hokkaido. Saikingaku Zasshi No. 71: 717–724 (in Japanese).
- ———, 1902. Malaria und ihre Vermiitler in Japan. Archiv Schiffs- und Tropen-Hygiene 6: 285–295.
- Wang, S. H. and H. J. Zheng. 1989. Blood meals of mosquito vectors of filariasis in Guizhou Province, China. Southeast Asian Journal of Tropical Medicine and Public Health 2: 175-176.
- Whang, C. H. 1962. Biological observations on anopheline mosquitoes in Korea, with special reference to Anopheles (Anopheles) sinensis Weidemann. Yonsei Medical Journal (South Korea) 3: 39-50.
- Wilkerson, R. C. and E. L. Peyton. 1990. Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 27: 207-224.
- Wilkerson, R. C., C. Li, L. M. Rueda, H. C. Kim, T. A. Klein, H. H. Song, and D. Strickman. 2003. Molecular confirmation of *Anopheles (Anopheles) lesteri* from the Republic of South Korea and its genetic identity with *An. (Ano.) antrhopophagus* from China (Diptera: Culicidae). Zootaxa 378: 1– 14.
- Wilkerson, R. C., T. J. Parsons, T. A. Albright, T. A. Klein, and M. J. Braun. 1993. Random amplified

polymorphic DNA (RAPD) markers readily distinguish cryptic mosquito species (Diptera: Culicidae: *Anopheles*). Insect Molecular Biology 1: 205-211.

- Wilkerson, R. C., J. F. Reinert, and C. Li. 2004. Ribosomal DNA ITS2 sequences differentiate six species in the Anopheles (Anopheles) crucians complex (Diptera: Culicidae). Journal of Medical Entomology 41: 392-401.
- Xu, B., X. Xiao, R. H. Webber, and J. D. Lines. 1998. Comparison of the effect of insecticide-treated bed nets and DDT residual spraying on the prevalence of malaria transmitted by Anopheles anthropophagus in China. Transactions of the Royal Society of Tropical Medicine and Hygiene 92: 135– 136.
- Xu, B., Z. Cui, J. Chang, Q. Zhao, Q. Huang, and X. Lin. 1997. Studies on the transmission potential of filariasis in controlled areas of Henan Province. China Medical Journal 110: 807–810.
- Xu, J., M. Zhao, and J. Ma. 1981. Comparative studies on the scanning electron micrographs of Anopheles sinensis and Anopheles lesteri anthropophagus. Entomotaxonomia 3: 265-272.
- Xu, J. J. and L. C. Feng. 1975. Studies on the Anopheles hyrcanus group of mosquitoes in China. Acta Entomologica Sinica 77: 77–104.
- Xu, J. J., M. L. Zao, X. F. Luo, R. G. Geng, S. X. Pan, and S. Y. Liu. 1988. Evaluation of permethrinimpregnated mosquito-nets against mosquitoes in China. Medical and Veterinary Entomology 2: 247-251.
- Ye, B. H. and C. L. Zhu. 1989. Comparative studies on several biochemical indices of Anopheles anthropophagus and Anopheles sinensis. Chinese Journal of Parasitology and Parasitic Diseases 7: 256-258.
- Zhang, X. M., L. Y. Bai, F. Zhang, C. L. Wen, M. S. Wang, S. H. Lu, X. H. Qian, F. Wu, and W. C. Wang. 1987. Vectorial capacity of Anopheles lesteri anthropophagus in transmitting filariasis malayi in Leshan Prefecture, Schuan Province. Chinese Journal of Parasitology and Parasitic Diseases 5: 191-193.
- Zhang, J., Z. Huang, S. Zheng, B. Yang, and X. Ye. 1980. On identification of a species of malaria parasite from monkeys. Acta Zoologica Sinica 26: 140-145.
- Zizhao, L., S. Luoyuan, Z. Lian, L. Dongfang, and S. Yunpu. 1999. Control strategies of malaria in Henan Province, China. Southeast Asian Journal of Tropical Medicine and Public Health 30: 240– 242.