

## Two new species of *Anopheles* (*Anopheles*) Hyrcanus Group (Diptera: Culicidae) from the Republic of South Korea

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### Abstract

Two new mosquito species belonging to the Hyrcanus Group, *Anopheles* (*Anopheles*) *belenrae* Rueda and *An.* (*Ano.*) *kleini* Rueda, are described from the Republic of South Korea, with illustrations of the larvae, pupae, adults, and male genitalia.

**Key words:** *Anopheles belenrae*, *kleini*, Culicidae, taxonomy, description, mosquitoes, Hyrcanus Group

### Introduction

The recent increase of human malaria cases in South Korea (Feighner *et al.* 1998, Lee *et al.* 1998, Wilkerson *et al.* 2003) necessitates a serious urgency to clarify the identity of the possible mosquito vectors. Misidentifications of vector species often lead to poor understanding of the epidemiology of disease transmission and inadvertently affect control measures. Integrated systematic research, combining both morphology and molecular biology, for the *Anopheles* (*Anopheles*) Hyrcanus Group is underway in the Walter Reed Biosystematics Unit, in collaboration with other laboratories, to clearly define the relationships among the species and to revise the taxonomy of the group.

The known and potential vectors of malarial parasites in South Korea belong to the *An.* (*Ano.*) Hyrcanus Group (Wilkerson *et al.* 2003). Previously, there were 27 species listed under the group (Harbach 2004), with a wide distribution from the Oriental Region to eastern and western Palearctic Regions (Ramsdale 2001, Water Reed Biosystematics Unit 2001, A. Lee, L. M. Rueda, R. C. Wilkerson and Y. M. Litton, unpublished data). Recently, Wilkerson *et al.* (2003) and Li *et al.* (2005) recognized two unnamed species closely related to *An. sinensis*, using rDNA ITS2 sequence.

Based on the above molecular data, these new species of the *An. (Ano.)* Hyrcanus Group are described, and illustrations are provided for the adult female and male, pupa, and larva of these species from South Korea. Types are deposited in the collections of the National Museum of Natural History, Smithsonian Institution, Washington, D.C., (NMNH) as indicated below.

### Materials and methods

The terminology and abbreviations of Harbach and Knight (1980, 1982) are used for the morphological characters and illustrations, except for wing spot nomenclature (Wilkerson and Peyton 1990), wing venation (Belkin 1962), and pupal abdominal dark marks (Rueda *et al.* 2005). Abbreviations for specimens examined are as follow: F, female; G, genitalia; Le, larval exuviae; M, male; Pe, pupal exuviae. Collection codes of the most recent collections consist of a country code in capital letters followed by a collection number (*e.g.*, KS 7(27)-1 is an individual or progeny from female 27 of collection 7 from South Korea; and KS 02-6(2)-11 is an individual or progeny from female 2 of collection 6 from South Korea in year 2002).

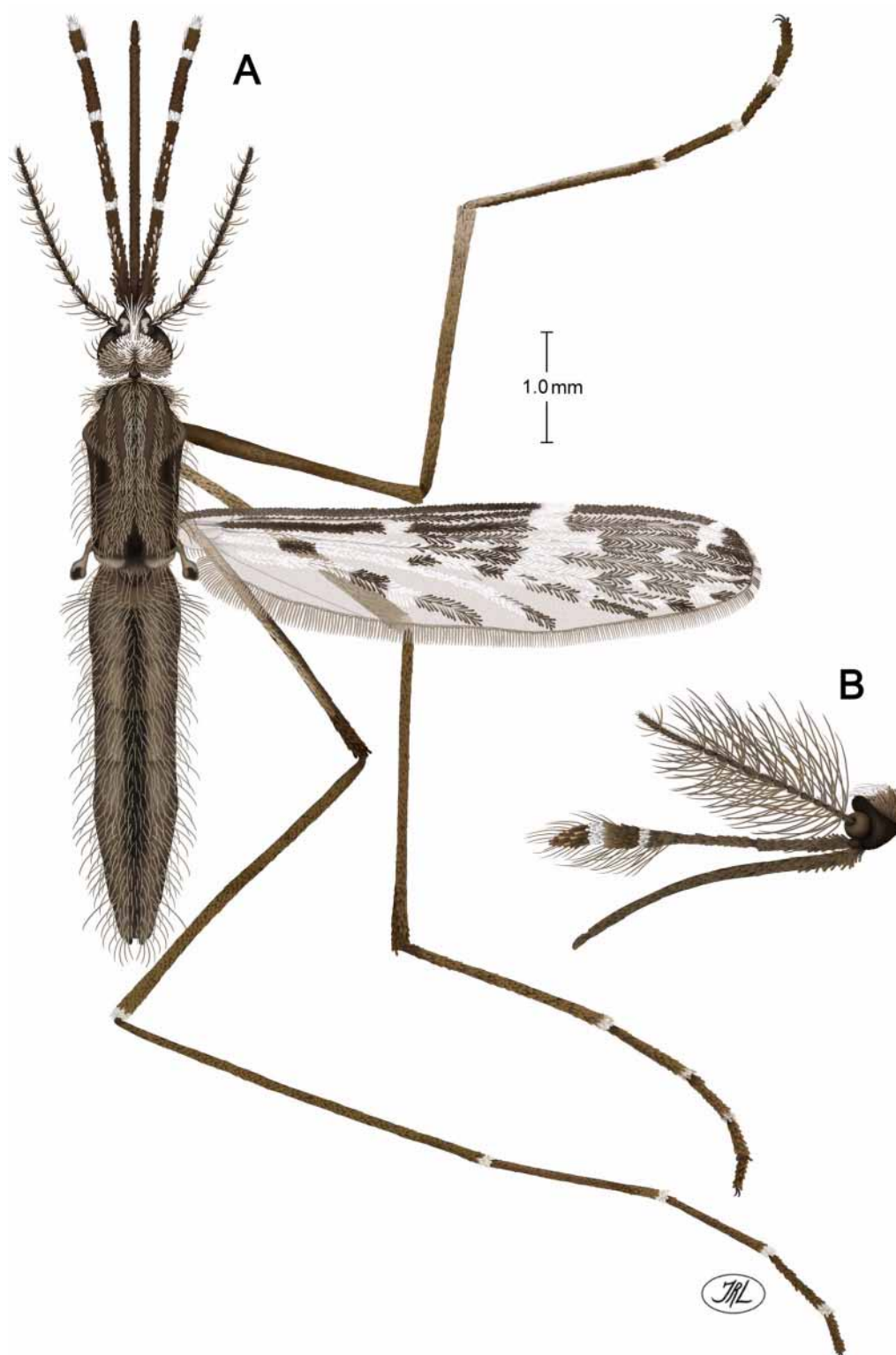
### *Anopheles (Anopheles) belenrae* Rueda, new species

(Figs. 1–4)

*Anopheles* “unknown sp. Korea” of Wilkerson *et al.*, 2003: 9 (rDNA ITS2 sequence).

*Anopheles* “unknown 1” of Li *et al.*, 2005 (rDNA ITS2 sequence).

**Description.** — **Female** (Fig. 1). Integument dark brown with silvery or grayish pollinosity. For the following measurements and counts,  $n = 10$ , except where indicated. **Head.** Interocular space with 9–14 long, pale setae intermixed with long and small, narrow, appressed white scales; vertex, occiput, and upper portion of postgena with numerous erect, truncate scales; middorsal portion of vertex with patch of white scales; lateral portion of vertex, occiput and upper portion of postgena with patch of dark brown to black scales; ventral portion of postgena with long dark brown to black setae. Clypeus bare dorsally, with dark brown scales laterally. Pedicel of antenna with 8–18 ( $n = 16$ ) small dorso-lateral, narrow to broad, grayish white spatulate scales; flagellomere 1 with numerous narrow to broad white scales; flagellomeres 2–8 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 longer than other basal palpomere white bands; base of maxillary palpus dorsally with single long, erect dark seta; length of maxillary palpus 1.65–2.40 mm (mean = 2.10 mm); ratio of length of each of palpomeres 2–5 to total length of palpus,  $2 = 0.26–0.35$  (mean =



**FIGURE 1.** *Anopheles belenrae*, adult. (A) Female, habitus. (B) Male head, side view.

0.32), 3 = 0.33–0.39 (mean = 0.36), 4 = 0.10–0.12 (mean = 0.014), 5 = 0.10–0.15 (mean = 0.12); ratio of combined palpomeres 4, 5 to total length of palpus, 0.30–0.36 (mean = 0.32); palpus 0.88–1.18 (mean = 1.04) forefemur length. Proboscis dark-scaled, labellum light brown; base of proboscis with long, erect dark setae; proboscis length 1.05–2.35 mm (mean = 2.04 mm); proboscis 0.91–1.16 (mean = 1.10) palpus length. *Thorax*. Scutum dark brown, with gray pollinosity, usually covered with intermixed narrow, short and long pale yellow to light brown scales; median anterior promontory with patch of intermixed narrow, short and long white scales. Darker lateral areas of scutum with longer dark setae. Scutal fossa, antealar area and supraalar area slightly pale pollinose. Scutellum dark brown, slightly pale dusted, with 14–21 shorter and 12–16 long setae, short setae intermixed pale yellow and dark brown, long setae dark brown. Anteprepronotum with 14–24 dark brown setae, and patch of dark scales anteriorly. Pleuron brown to dark brown; upper proepisternum with 3–7 setae, without scales; prespiracular area with 3–6 setae, without scales; prealar area with 4–13 setae, without scales; upper mesokatepisternum with 3–9 setae, without scales; lower mesokatepisternum with 1–9 setae, without scales; upper mesepimeron with 5–14 setae, without scales. *Legs*. Fore- and midlegs dark-scaled except white scales dorsally on apex of femora and tibiae, and pale scales ventrally on parts of femora, tibiae and tarsomeres 1; complete narrow apical pale bands on tarsomeres 1–3; apical pale band on tarsomere 4 absent. Hindlegs dark-scaled as on fore- and midlegs, except apical white scales on tarsomeres 4. Midcoxa with upper and lower patches of pale scales; upper patch 5–11 scales, lower patch 6–14 scales. Forefemur length 1.65–2.30 mm (mean = 2.02 mm,  $n = 8$ ), ratio of forefemur length to proboscis length 0.85–1.04 (mean = 0.94,  $n = 8$ ). Midfemur length 1.85–2.50 mm (mean = 2.30 mm,  $n = 9$ ), ratio of midfemur length to proboscis length 0.93–1.12 (mean = 1.04,  $n = 9$ ). Hindfemur length 1.95–2.75 mm (mean = 2.39 mm,  $n = 9$ ), ratio of hindfemur length to proboscis length 1.00–2.23 (mean = 1.24,  $n = 9$ ).

*Wing* (Table 1). Length (measured from humeral crossvein to wing tip, excluding fringe) 3.40–4.65 mm (mean = 3.94 mm). Dark scales brown to black, pale scales white and pale yellow. Costa (C) dark-scaled with small subcostal pale spot (SCP, mean = 0.25 mm) and preapical pale spot (PP, mean = 0.15 mm); remigium with dark and pale scales; 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  $R_1$  dark-scaled except 3 pale spots (SP, SCP and PP), scattered white spatulate scales from base to presector dark (PSD), and stripe of white scales on SD before SCP; base of  $R_s$  dark-scaled; bifurcation of  $R_2$  and  $R_3$  dark-scaled; base of  $R_{4+5}$  dark-scaled; tips of  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_{4+5}$  usually with pale fringe. Media (M) mostly pale-scaled; bifurcation of  $M_{1+2}$  and  $M_{3+4}$  dark-scaled; tips of  $M_{1+2}$  and  $M_{3+4}$  with dark fringe. Cubitus (Cu) with basal dark spot, length 0.2–0.4 (mean = 0.25);  $Cu_1$  with 3 or 4 dark spots, length of first basal spot 0.15–0.30 mm (mean = 0.19 mm), second spot 0.20–0.35 mm (mean = 0.25 mm), third spot 0.50–0.65 mm (mean = 0.58 mm), distal fourth spot 0.15–0.25 mm (mean = 0.18 mm);  $Cu_2$  with dis-

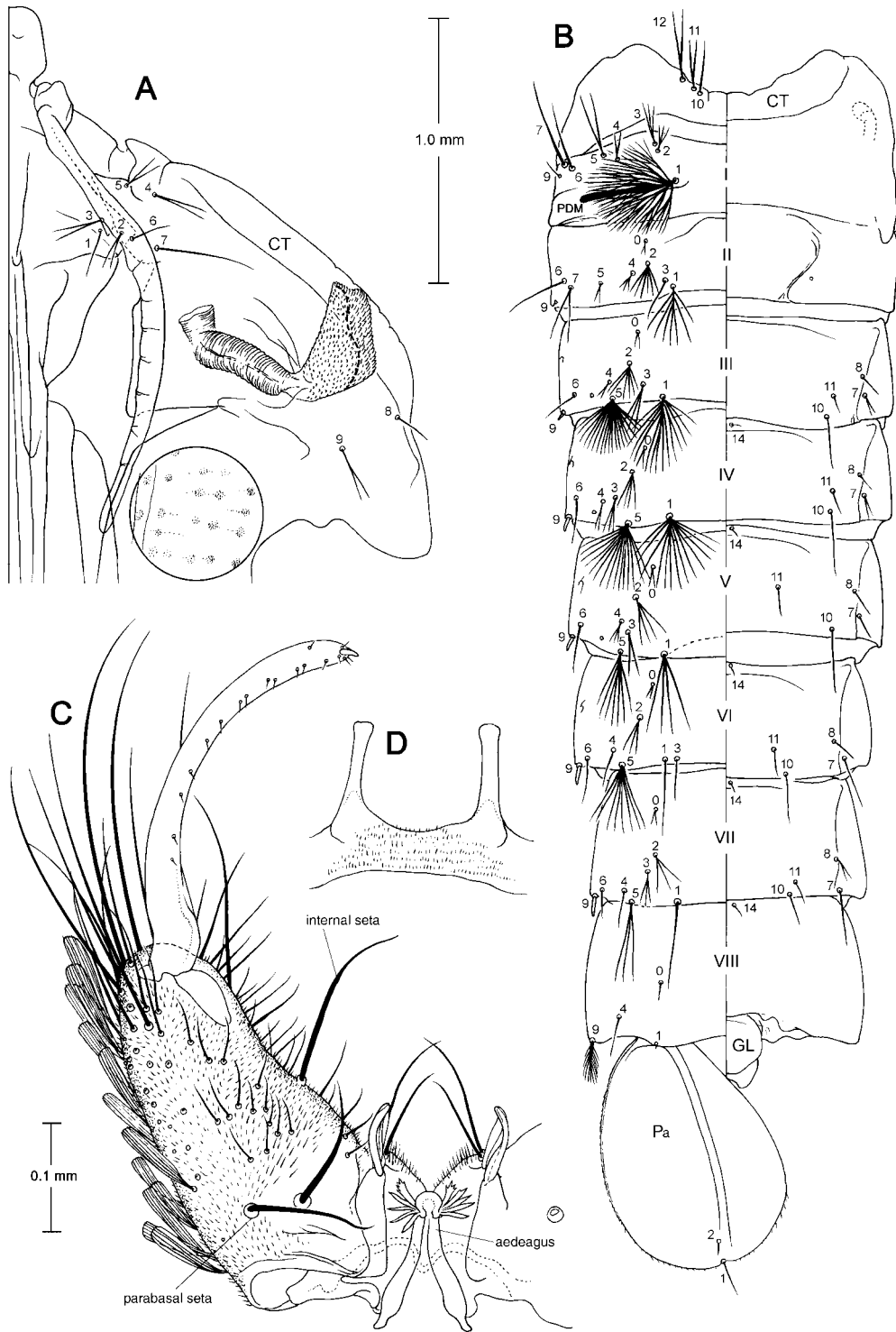
tal dark spot only, length 0.10–0.30 mm (mean = 0.22 mm); tips of  $Cu_1$  and  $Cu_2$  with dark fringe. Anal vein (1A) with 1 or 2 dark spots, basal spot length 0.20–0.40 mm (mean = 0.27 mm), distal spot length 0.35–1.10 mm (mean = 0.55 mm), tip of 1A with dark fringe. *Halter*. Scabellum, pedicel and capitellum pale to light brown with grayish pollinosity. Descriptive statistics for ratios of costal and  $R-R_1$  wing spot lengths/length of wing measured from humeral crossvein are shown in Table 1, with holotype male wing measurements in brackets. *Abdomen*. Terga and sterna dark brown to black with grayish pollinosity, covered with pale brown to golden brown setae.

**TABLE 1.** *An. belenrae*: descriptive statistics for ratios of veins C and  $R-R_1$  wing spot lengths/length of wing measured from humeral crossvein\*.

Wing spot	Range	Mean $\pm$ SD	
<b>Costa (C)</b>			
Basal dark to sector dark (BD+PHD+HD+PD+SD)	0.56 - 0.69	0.59 $\pm$ 0.04	[0.60]
Subcostal pale (SCP)	0.05 - 0.08	0.07 $\pm$ 0.01	[0.07]
Preapical dark (PD)	0.11 - 0.27	0.24 $\pm$ 0.04	[0.21]
Preapical pale (PP)	0.03 - 0.05	0.04 $\pm$ 0.01	[0.04]
Apical dark (AD)	0.001 - 0.06	0.05 $\pm$ 0.02	[0.001]
<b>Vein <math>R-R_1</math></b>			
Basal dark to presector dark (BD+PHD+HD+PSD)	0.25 - 0.37	0.27 $\pm$ 0.043	[0.27]
Sector pale (SP)	0.08 - 0.11	0.10 $\pm$ 0.01	[0.10]
Sector dark (SD)	0.21 - 0.39	0.24 $\pm$ 0.05	[0.22]
Subcostal pale (SCP)	0.06 - 0.08	0.07 $\pm$ 0.01	[0.07]
Preapical dark (PD)	0.19 - 0.26	0.23 $\pm$ 0.02	[0.21]
Preapical pale (PP)	0.04 - 0.08	0.06 $\pm$ 0.01	[0.06]
Apical dark (AD)	0.02 - 0.06	0.05 $\pm$ 0.02	[0.06]

\*Eleven wings from the holotype and paratypes; [ ], holotype male.

**Male** (Figs. 2C, D). As in female except for following sexual differences. Maxillary palpus 0.88–1.11 length of proboscis (mean = 0.97;  $n = 18$  for this and  $n = 6$  for 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 pale scales from middle to apex; palpomere 3 dark-scaled with long yellowish 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 2.48–3.48 mm (mean = 2.81 mm,  $n = 9$ ),



**FIGURE 2.** *Anopheles belenrae*. (A) Pupa, cephalothorax. (B) Pupa, metathorax and abdomen, left side dorsal, right side ventral. (C) Male genitalia. (D) Tergum IX. Abbreviations used include CT = cephalothorax, GL = genital lobe, Pa = paddle, PDM = posterior dark mark.

dark brown-scaled. Anal vein with single dark spot. Tergum IX bare, with pair of elongate caudally directed capitate tergal lobes; length of lobe from base to tip 0.94 distance between 2 lobes; middle of lobe narrower, 0.50 width of lobe tip and 0.06 width of lobe base. Gonocoxite 1.87–2.16x as long as wide at widest point; 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 0.06 from base of gonocoxite; parabasal 2 base 0.04–0.06 from base of gonocoxite; internal seta slender, base 0.15–0.22 distance from base of gonocoxite. Gonostylus widened at base and narrowed toward middle and tip; gonostylus 1.09 length of gonocoxite; gonostylus 26.44x 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 4–6 per side; 3 mesal leaflets broadest, with broad, thin, nearly transparent inner margins; other leaflets with narrow, thin, nearly transparent inner margins; most mesal leaflet with 0–3 aciculae.

**Pupa** (Figs. 2 A–B). Position and development of setae as figured; range and modal number of branches, and number of branches of holotype male as in Table 2. Integument with light to moderate dark pigmentation. Exuviae colorless to dark brown. *Cephalothorax*. Mesothoracic wing with distinct rows of round dark spots. Trumpet with thin, lightly serrated pale brown rim; meatus with simple cleft, and its subbasal part with numerous spinules; trumpet length 0.35–0.52 mm (mean = 0.41 mm,  $n = 20$  for this and following measurements and counts except where indicated), width 0.32–0.46 mm (mean = 0.39 mm, measured at base of pinna), index 0.83–1.31 (mean = 1.07); tracheoid area 1.27 length of trumpet. *Abdomen*. Abdominal tergum I with 2 elongate posterior dark marks (PDM); each PDM narrows mesally at base, with maximum width (0.02–0.05 mm, mean = 0.02 mm) towards distal tip, length 0.26–0.38 mm (mean = 0.31 mm), about 0.24–0.27 (mean 0.25,  $n = 9$ ) width of abdominal segment I, and longer than the longest branch of seta 1-I. Seta 1-I fan-like with 14–28 aciculate dendritic branches; 6-I with 1–4 branches; 7-I with 1–5 branches; 9-I with 1 or 2 branches. Setae 1,5-III-VII well developed; 1-V 0.92–1.55 (mean = 1.12,  $n = 18$ ) length of 5-V; 1-VI 0.71–1.64 (mean = 1.09,  $n = 15$ ); 1-VII 0.82–1.27 (mean = 0.99,  $n = 17$ ); 3-VI aligned with and mesal of 1-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, 1.14–6.00 (mean = 2.28) length of 9-II; 9-IV strong, lightly pigmented and slightly pointed, 1.00–3.11 (mean = 1.64) length of 9-III; 9-V-VIII long, lightly pigmented and slightly pointed; 9-V 0.96–1.33 (mean = 1.16) length of 9-IV; 9-VI 0.83–1.43 (mean = 1.11) length of 9-V; 9-VII 0.53–1.41 (mean = 1.01) length of 9-VI; 9-VIII 0.81–2.94 (mean = 1.45) length of 9-VII; 9-VI 0.10–0.18

(mean = 0.15,  $n = 10$ ) length of segment VI; 9-VII 0.08–0.16 (mean = 0.13,  $n = 10$ ) length of segment VII; 9-VIII 0.10–0.26 (mean = 0.17,  $n = 10$ ) length of segment VIII. Seta 9-I positioned near anterolateral edge of tergum; 9-II on posterolateral edge of tergum; 9-III–VIII near posterolateral edge of tergum. Segment VII 0.98–1.19 (mean = 1.09,  $n = 10$ ) length of segment VI; segment VIII 0.60–1.33 (mean = 1.16,  $n = 10$ ) length of segment VI; segment VIII 0.56–1.23 (mean = 1.06,  $n = 10$ ) length of segment VII. Segment VII 0.81–0.93 (mean = 0.89,  $n = 10$ ) width of segment VI (width at posterior margins); segment VIII 0.79–0.96 (mean = 0.89,  $n = 10$ ) width of segment VI; segment VIII 0.94–1.06 (mean = 0.99,  $n = 10$ ) width of segment VII. Width/length of segment VI 2.269–2.77 (mean = 2.55,  $n = 10$ ), VII 1.89–2.19 (mean = 2.06,  $n = 10$ ), VIII 1.74–3.83 (mean = 2.05,  $n = 10$ ). Paddle length 0.78–0.98 mm (mean = 0.85 mm,  $n = 17$ ), width 0.50–0.69 mm (mean = 0.62 mm,  $n = 18$ ), length/width ratio 1.42–1.54 (mean = 1.38,  $n = 18$ ); refractile index 0.59–0.82 (mean = 0.69,  $n = 17$ ); seta 1-Pa simple or forked (2–3 apical branches); 2-Pa simple or forked (2–3 apical branches); 1-Pa thicker and 1.45x longer than 2-Pa. Width/length of genital lobe of female 1.06–1.65 (mean = 1.39,  $n = 7$ ), male 0.83–1.41 (mean = 1.01,  $n = 3$ ); numerous spicules present on subapical and apical margins of genital lobe of female, absent in male.

**TABLE 2.** Pupal setal branching for *An. belenrae*: range mode ( ) based on counts made on 9–20 setae of the holotype and paratypes; [ ], male holotype.

Seta no.	Cephalothorax CT	Abdominal segments			
		I	II	III	IV
0	–	–	1–5(2) [2]	1–3(2) [2]	1–2(2) [2]
1	1–4 (2) [1, 2]	14–28(24) [16, 27]	4–15(9) [6, 8]	8–26(10) [26, 15]	5–16(13) [16, 5]
2	1–3(2) [3, 2]	2–4(3) [2, 4]	1–8(6) [6, 7]	3–8(6) [7, 3]	3–6(4) [4, 5]
3	1–4(1) [1]	1–7(3) [3]	1–5(1) [1]	1–4(3) [1, 3]	2–4(3) [3]
4	1–3(2) [1, 2]	2–5(2) [2, 4]	1–4(3) [3, 4]	1–3(2) [1]	1–2(2) [2, 1]
5	1–5(2) [2, 4]	2–7(6) [6, 2]	1–3(2) [2]	8–24(20) [20, 23]	8–25(12) [19, 11]
6	1–3(1) [1]	1–4(1) [1]	1–2(1) [1]	1–3(1) [1]	1–2(1) [1]
7	1–2(1) [2]	1–5(1) [1]	1–3(2) [1, 2]	1–3(2) [2, 3]	1–3(2) [3]
8	1–2(1) [1]	–	–	1–2(1) [2, 1]	1–3(1) [1, 2]
9	1–3(1) [2]	1–2(1) [1]	1 [1]	1 [1]	1 [1]
10	1–3(1) [2]	–	–	1–2(1) [2, 1]	1–2(1) [1]
11	1–3(2) [2]	–	–	1–2(1) [1]	1 [1]
12	1–4(2) [2]	–	–	–	–
13	–	–	–	–	–
14	–	–	–	1 [1]	1 [1]

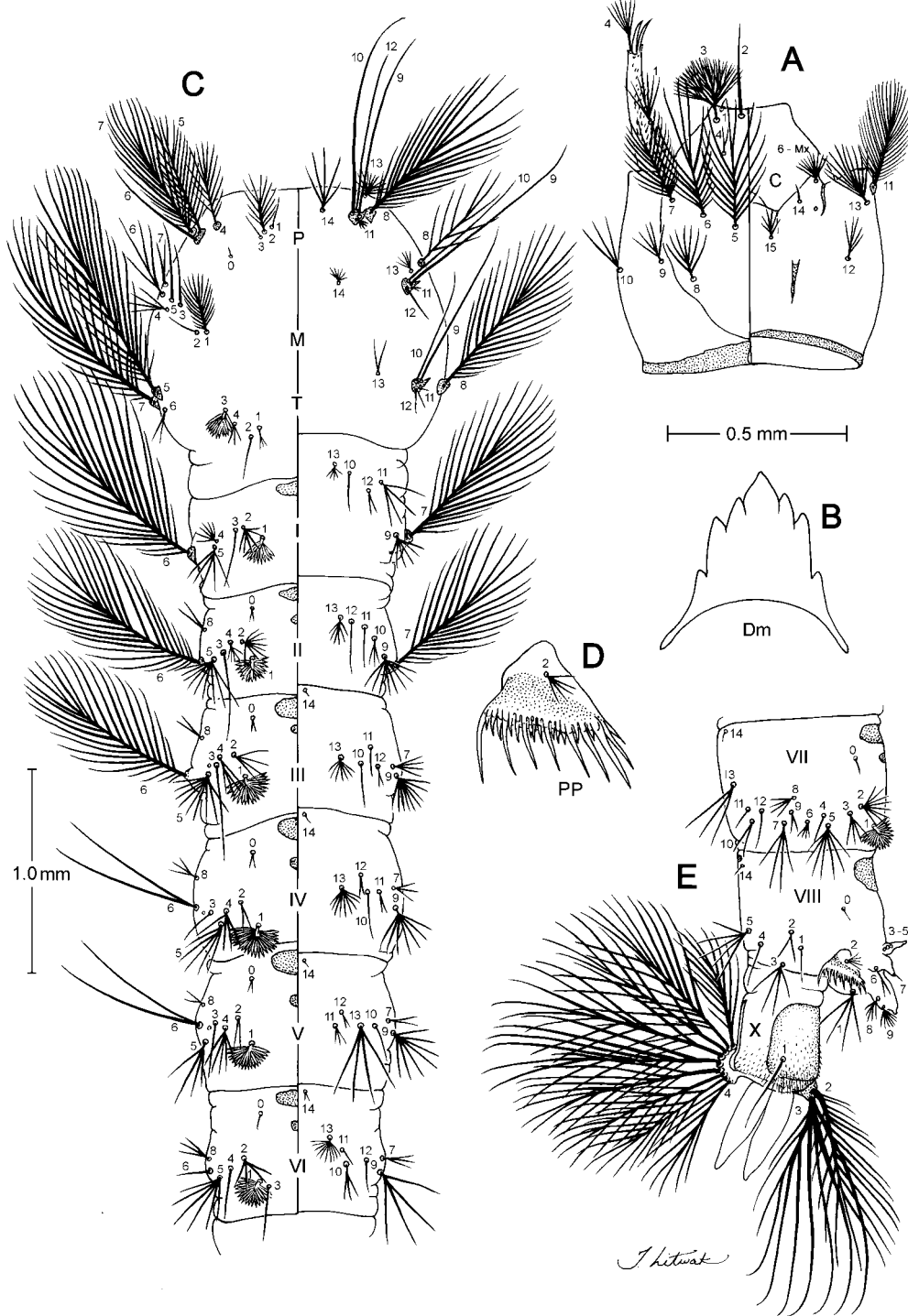
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TABLE 2 continued.

Seta no.	Abdominal segments					Paddle
	V	VI	VII	VIII	IX	Pa
0	1-2(2) [2]	1-3(2) [2]	1-3(2) [2, 3]	1-2(1) [2, 1]	-	-
1	2-7(4) [4]	1-3(2) [2]	1-2(1) [1, 2]	-	1 [1]	1 [1]
2	3-5(4) [4]	2-5(3) [3, 4]	2-3(3) [3]	-	-	1 [1]
3	1-3(2) [1, 3]	1-4(1) [1]	1-3(2) [2]	-	-	-
4	1-4(3) [3, 2]	1-2(1) [1-2]	1-2(1) [1]	1-2(2) [2]	-	-
5	2-14(7) [8, 7]	3-10(8) [5, 8]	1-4(3) [3, 2]	-	-	-
6	1 [1]	1 [1]	1-2(1) [1]	-	-	-
7	1-3(1) [3]	1-2(1) [2]	1-2(1) [1]	-	-	-
8	1-3(1) [2]	1-2(1) [2, 1]	1-3(2) [2]	-	-	-
9	1 [1]	1 [1]	1 [1]	1 [1]	-	-
10	1-2(1) [1]	1-2(1) [1]	1-2(1) [2]	-	-	-
11	1-2(1) [1]	1 [1]	1-3(2) [2]	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	1 [1]	1 [1]	1 [1]	1 [1]	-	-

**Larva** (Fig. 3). Position and development of setae as figured; range and modal number of branches and number of branches of holotype male as shown in Table 3. *Head*. Length 0.83–0.90 mm (mean = 0.87 mm,  $n = 9$ ), width 0.71–0.83 mm (mean = 0.78 mm,  $n = 9$ ). Antennal length 0.31–0.38 mm (mean = 0.35 mm,  $n = 20$ ), slightly tapered toward apex, 4.86–6.00 (mean = 5.51,  $n = 20$ ) 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 mm,  $n = 12$ ). Seta 1-A with 2–7 (mode = 5,  $n = 18$ ) branches, length 0.08–0.15 mm (mean = 0.12 mm,  $n = 18$ ), inserted 0.14–0.19 mm (mean = 0.17 mm,  $n = 19$ ) from base of antenna; 2-A single, pointed, length 0.09–0.14 mm (mean = 0.12 mm,  $n = 9$ ); 3-A single, pointed, length 0.10–0.22 mm (mean = 0.15 mm,  $n = 7$ ); 4-A with 4–7 branches (mode = 5,  $n = 11$ ); 5-A short, spine-like, 0.13–0.30 (mean = 0.21,  $n = 13$ ) length of seta 1-A; 6-A spine-like about as long as seta 5-A. Seta 2-C single 1.30–1.79 (mean = 1.53,  $n = 12$ ) length of 3-C; seta 2-C close to mate of opposite side 0.002–0.008 mm (mean = 0.004 mm,  $n = 9$ ); 3-C densely dendritic with 16–30 main branches (mode = 29), longest branch 0.10–0.14 mm (mean = 0.12 mm,  $n = 8$ ), clypeal index (distance between bases 2-C and 3-C on 1 side/distance between bases of 2-C) 7.75–32.00 (mean = 18.66,  $n = 8$ ). *Thorax*. Seta 1-P with 1–3 branches (mode = 1,  $n = 14$ ); 9, 10, 12-P single; 9-12-P setal support plate spine length 0.03–0.08 mm (mean = 0.05 mm,  $n = 15$ ). Setae 9, 10, 12-M single; 9-M 1.03–1.56 (mean



**FIGURE 3.** *Anopheles belenrae*, 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.

= 1.21,  $n = 8$ ) length 10-M; 9-12-M setal support plate spine length 0.02–0.04 mm (mean = 0.03 mm,  $n = 10$ ). Setae 9, 10-T single; 9-T 1.19–2.09 (mean = 1.64,  $n = 7$ ) length of 10-T; seta 12-T with 1–3 branches (mode = 2,  $n = 14$ ); 9-12-T setal support plate spine length 0.01–0.04 mm ( $n = 14$ ); 13-T with 2–6 branches (mode = 2,  $n = 15$ ). *Abdomen*. Seta 1-I with 8–14 branches (mode = 8,  $n = 19$ ); 1-II with 10–19 branches (mode = 12,  $n = 17$ ). Seta 1-I-VII palmate with well-developed leaflets, each leaflet with short filament; 0-II-VIII and 14-III-VIII weakly developed; 0,8,14-I and 14-II absent; 3-II,V,VI single; 3-I, III,IV single or branched. Seta 1-X single, 0.61–1.00 (mean = 0.83,  $n = 9$ ) 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 16–22 spines (mode = 17,  $n = 20$ ); arrangement of spines alternating long and short, with 7–10 (mode = 8,  $n = 20$ ) long spines and 7–14 (mode = 9,  $n = 20$ ) short spines; long spines 2.75–5.00 (mean = 3.87,  $n = 20$ ) length of short spines. Two posterolateral spiracular lobe plates present, each plate with elongate, slender, sclerotized projection from inner caudal margin.

**TABLE 3.** Larval setal branching for *An. belenrae*: range mode ( ) based on counts made on 6-20 setae of the holotype and paratypes; [ ], male holotype.

Seta no.	Head	Thorax			Abdominal segments		
	C <sup>a</sup>	P	M	T	I	II	III
0	–	1 [1]	–	–	–	1–3(2) [2]	1–3(2) [1]
1	1 [1]	1–3(1)[3]	15–26(18) [23]	1 [1]	8–14(8) [8]	10–19(12) [10]	14–21(17) [17]
2	1 [1]	6–12(6) [9]	1 [1]	1–2 [1]	4–7(6) [6]	4–8(8) [8, 6]	4–6(4) [5]
3	16–30(29) [29]	1 [1]	1 [1]	7–14(11) [11]	1–2(1) [2]	1 [1]	1–2(1) [1]
4	1–4(2) [1]	5–15(5) [–]	2–6(4) [–]	1–5(3) [3]	3–8(6) [5]	1–7(5) [6, 1]	2–5(4) [–]
5	12–19(15) [–]	11–25(23) [–]	1 [1]	22–28(26) [–]	3–8(5) [4]	4–12(9) [9, 4]	6–12(8) [8]
6	12–19(17) [–]	1 [–]	2–6(4) [–]	2–3(2) [–]	12–29(22) [–]	18–33(24) [–]	17–28(25) [–]
7	14–23(18) [–]	18–28(23) [–]	2–4(2) [–]	21–25(25) [–]	15–22(21) [–]	19–29(23) [–]	3–7(4) [4]
8	4–10(7) [6]	15–26(21) [–]	7–14(8) [–]	22–32(26) [–]	–	2–3(2) [3]	2–4(3) [2]
9	4–9(5) [5]	1 [–]	1 [1]	1 [–]	4–10(6) [5]	2–13(8) [13]	5–10(9) [6, 9]
10	2–4(3) [–]	1 [–]	1 [1]	1 [–]	1 [1]	2–3(3) [2, 3]	1–3(1) [2]
11	20–38(32) [–]	2–5(4) [5]	1–2 [2]	1–2 [–]	3–5(4) [4]	1 [1]	1–4(2) [2]
12	2–6(5) [2]	1 [–]	1 [1]	1–3(2) [2]	1–5(2) [2]	1 [1]	1–4(2) [2, 3]
13	5–11(8) [–]	6–12(11) [12, 11]	3–9(5) [–]	2–6(2) [3]	5–13(5) [9, 11]	5–13(6) [6, 7]	5–10(7) [9]
14	1–2(1) [–]	4–8(5) [7]	4–13(7) [11]	–	–	–	1 [1]
15	5–12(6) [–]	–	–	–	–	–	–

.....continued on the next page

TABLE 3 continued.

Seta no.	Abdominal segments					
	IV	V	VI	VII	VIII	X
0	2-3 (2) [-]	2 [-]	1-4(1) [3, 4]	1-2(1) [2]	1-2(1) [1]	-
1	15-26(17) [17, 18]	14-23(19) [17]	16-23(17) [18, 17]	3-8(4) [18]	3-8(4) [6]	1 [1]
2	2-4(2) [3]	2-4(2) [2]	2-6(5) [4]	2-6(5) [6]	2-4(2) [3]	16-32(22) [-]
3	1-3(1) [-]	1 [1]	1 [1]	2-6(4) [3]	4-8(4) [4]	12-24(24) [-]
4	2-6(4) [-]	2-5(5) [5]	1 [1]	1 [1]	1-2(1) [1]	9-10(9, 10) [10]
5	3-8(5) [5]	4-8(4) [5]	6-8(6) [7]	5-9(7) [7]	3-8(5) [7, 5]	-
6	2-4(2) [3, 4]	1-5(2) [2]	1-3(1) [1]	3-5(4) [4]	1-S	1 [1]
7	3-5(3) [5, 3]	1-4(4) [4]	2-5(3) [3]	3-7(4) [4]	2-S	1 [1]
8	2-4(3) [2, 3]	2-5(2) [2]	2-5(3) [3, 2]	2-6(4) [-]	6-S	1-2(2) [-]
9	3-11(7) [8, 6]	3-9(7) [9, 7]	3-9(4) [9, 7]	1-4(2) [2]	7-S	1-2(1) [-]
10	1 [1]	1 [1]	1-2(1) [1]	2-4(2) [3, 2]	8-S	2-8(5) [-]
11	1-3(2) [2]	2-7(3) [7, 6]	1-3(1) [2, 1]	1 [1]	9-S	2-9(6) [-]
12	1-3(2) [2]	2-5(2) [2]	1-3(1) [1]	1 [1]	-	-
13	3-10(9) [5]	2-6(4) [2]	6-11(10) [10]	3-12(3) [4, 3]	-	-
14	1-3 [-]	1-2 [1]	2 [2]	1-2(1) [1]	1 [1]	-
15	-	-	-	-	-	-

<sup>a</sup>C, head; P, prothorax; M, mesothorax; T, metathorax.

**Type material.** Holotype male with associated slide-mounted larval and pupal exuviae, and male genitalia; T. Klein, A. Schuster and Park, coll.; reared from a female collected at dairy farm, between 2100 and 2130 hr, 12 July 2001, in Tonggilchon (37° 51'N 126° 47'E), Gyeonggi-do, South Korea. Deposited in the NMNH, Smithsonian Institution, Washington, D.C. (WRBU ACC No. 1684, KS 7(27)-12). Paratypes. SOUTH KOREA: GYEONGGI-DO, Tonggilchon, same data as holotype male, 11 F PeLe, KS 7(27)-1,5-9,11,13-15,17; 1 F, KS 7(27); 1 PeLe, KS 7(27)-10; Ogum-ri, Paju (37° 49'N 126° 43'E), H.C. Kim coll., reared from a female collected at cow shed between 2030 and 2130 hr, 29 July 2001, 9 F PeLe, KS 8(12)-5,7-9,11,13-16; 2 M G PeLe, KS 8(12)-6,10; 1 M G PeLe, KS 8(12)-12; 4 PeLe, KS 8(12)-1-4; Gangwha (37° 45'N 126° 29'E), H.C. Kim coll., progeny of adult collected at cow shed between 2000 and 2230 hr, 1 September 2001, 8 F PeLe, KS 9(8)-9-11,18-21,23; M G PeLe, KS 9 (8)-13,1; 1 F, KS 9(8); 10 PeLe, KS 9 (8)-1,2,4,6,7,12,14-17. DNA vouchers are deposited in the NMNH, Smithsonian Institution, Washington, D.C.

**Diagnosis.** *Anopheles belenrae* 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 (like *An. lesteri* Bai-

sas and Hu, but unlike *An. sinensis* Wiedemann, *An. sineroides* Yamada, and *An. pullus* Yamada); subcostal pale (SCP) spot narrow; humeral crossveins without scales (unlike *An. pseudosinensis* Baisas); midcoxa with upper patch of pale scales (like *An. sinensis* but unlike *An. lesteri*); hindtarsomeres 1–4 with narrow apical pale bands, hindtarsomere 4 without basal pale band [unlike *An. peditaeniatus* (Leicester)]. *Adult male*. Male genitalia with dorsal lobe of claspette having 3 closely appressed setae of about equal length. Aedeagus with 4–6 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 thin, slightly serrated pale brown rim (like *sinensis*, and unlike *lesteri* with thickly serrated rim). Wing sheath with distinct rows of round dark spots (like *An. sinensis*, unlike *An. lesteri* which has checkered dark stripes). Setae 9-III-VII single, with narrowly rounded apex (like *An. lesteri*); seta 5-V with 8–25 branches. *Larva*. Setae 2-C, 3-P, 3,5-M single; 3-C with 16–30 branches; 4-M with 2–6 erect branches; 9-M less than twice the length of 10-M,T; 5-III with 6–12 branches; 9-III with 5–10 branches; 13-IV with 3–10 branches; 1-X strong, single about as long as the saddle (unlike *An. lesteri* which is about 2x or more longer than the saddle); pecten with 7–10 long spines and 7–14 short spines.

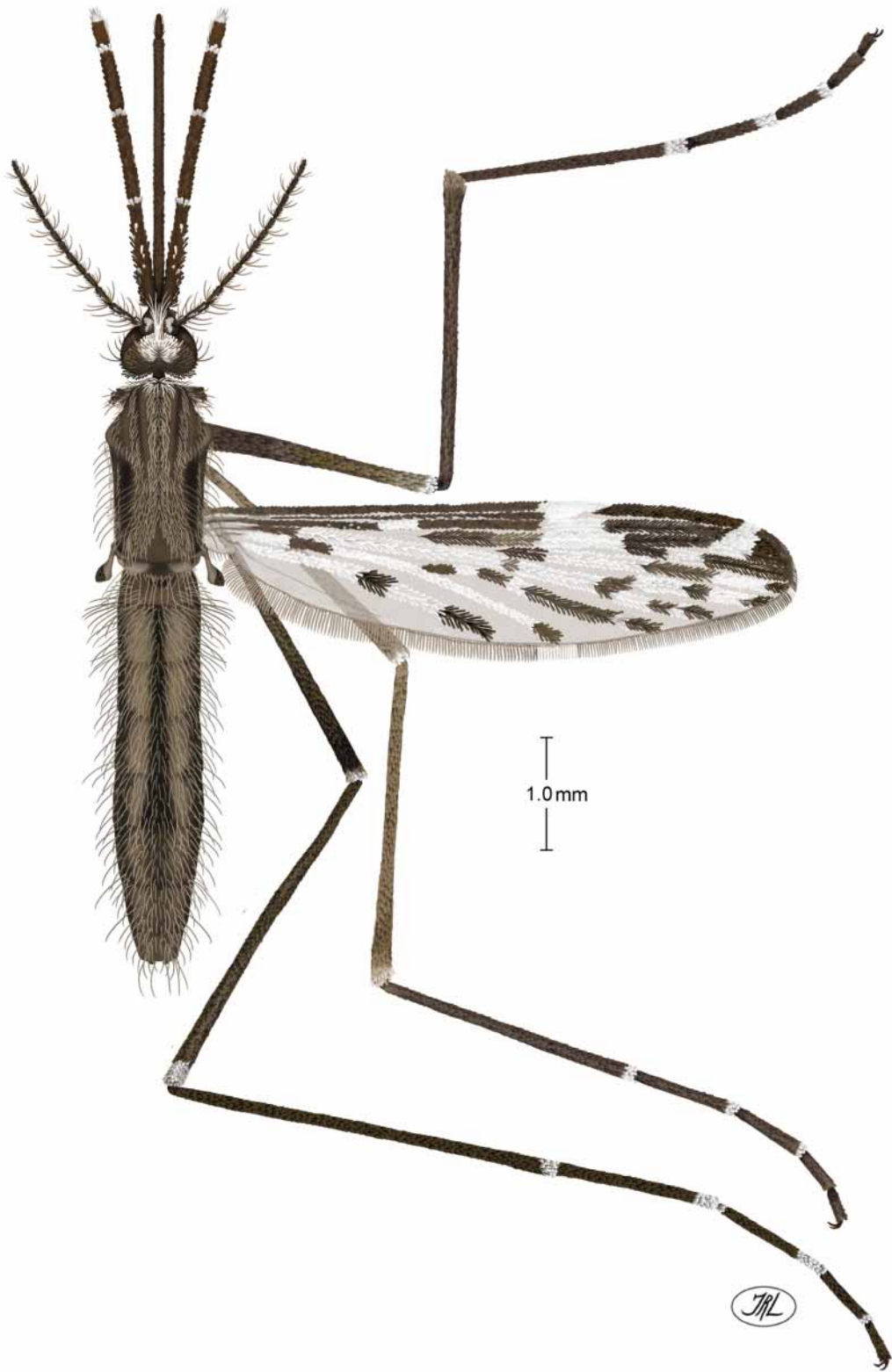
**Etymology.** The species is named with gratitude and affection for Belen P. Rueda, for her encouragement and support.

**Remarks.** The specimens used in this study were progeny reared from adults that were collected in cow sheds at 2 villages with rice paddies, and at a dairy farm in an urban area. Nothing is known about the natural habitats of the immature stages. The ribosomal DNA ITS2 sequence of *An. belenrae* is provided by Wilkerson *et al.* (2003, page 9, Figure 1, no. 4, “unknown sp. Korea”; GenBank Accession no. AY375466), and Li *et al.* (2005, *Anopheles* unknown 1).

***Anopheles (Anopheles) kleini* Rueda, new species**  
(Figs. 4–6)

*Anopheles* “unknown 2” of Li *et al.* 2005 (rDNA ITS2 sequence).

**Description.** — **Female** (Fig. 4). Integument dark brown with silvery or grayish pollinosity. For, the following measurements and counts,  $n = 8$ , except when indicated. *Head*. Interocular space with 10–18 long, pale setae intermixed with both long and small, narrow, appressed white scales; vertex, occiput, and upper portion of postgena with numerous erect, truncate scales; middorsal portion of vertex with patch of white scales; lateral portion of vertex, occiput and upper portion of postgena with patch of dark brown to black scales; ventral portion of postgena with long dark brown to black setae. Clypeus bare dorsally, with dark brown scales laterally. Pedicel of antenna with 8–19 ( $n = 13$ ) small, dorso-lateral, narrow to broad, grayish white spatulate scales; flagellomere 1 with numerous



**FIGUER 4.** *Anopheles kleini*, adult female, habitus.

narrow to broad white scales; flagellomeres 2–8 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 longer than other white bands; base of maxillary palpus dorsally with single long, erect dark seta; length of maxillary palpus 2.00–2.60 mm (mean = 2.38 mm); ratio of length of each of palpomeres 2–5 to total length of palpus, 2 = 0.32–0.35 (mean = 0.33), 3 = 0.31–0.36 (mean = 0.34), 4 = 0.15–0.22 (mean = 0.19), 5 = 0.09–0.18 (mean = 0.12); palpus 0.88–1.18 (mean = 1.07) forefemur length. Proboscis dark-scaled, labellum light brown; base of proboscis with long, erect dark setae; proboscis length 2.10–2.55 mm (mean = 2.36 mm); proboscis 0.93–1.13 (mean = 0.99) palpus length. *Thorax*. Scutum dark brown, gray pollinose, with 2 submedian longitudinal lines on anterior portion; a pair of black round spots slightly distinct; median anterior promontory with patch of intermixed narrow, short and long white scales. Darker lateral areas of scutum with longer dark setae. Scutal fossa, antear area and supraalar area slightly pale pollinose. Scutellum dark, slightly pale pollinose, with 17–26 shorter and 13–18 long setae, short setae intermixed pale yellow and dark brown, long setae dark brown. Anteprepronotum with 13–18 dark brown setae, and patch of dark scales anteriorly. Pleuron brown to dark brown; upper proepisternum with 4–8 setae, without scales; prespiracular area with 2–5 setae, without scales; prealar area with 5–18 setae, without scales; upper mesokatepisternum with 1–5 setae, without scales; lower mesokatepisternum with 2–8 setae, without scales; upper mesepimeron with 5–13 setae, without scales. *Legs*. Fore- and midlegs dark-scaled except white scales dorsally on apex of femora and tibiae, and pale scales ventrally on parts of femora, tibiae and tarsomeres 1; complete narrow apical pale bands on tarsomeres 1–3; very narrow or indistinct apical pale band on tarsomere 3; apical pale band on tarsomere 4 absent. Hindlegs dark-scaled as fore- and midlegs, except apical white scales on tarsomere 4. Midcoxae with upper and lower patches of pale scales; upper patch 7–13 scales, lower patch 5–9 scales. Forefemur length 2.15–2.40 mm (mean = 2.23 mm), ratio of forefemur length to proboscis length 0.88–1.02 (mean = 0.95). Midfemur length 2.30–2.65–2.65 mm (mean = 2.46 mm), ratio of midfemur length to proboscis length 0.98–1.12 (mean = 1.04). Hindfemur length 2.35–2.80 mm (mean = 2.58 mm), ratio of hindfemur length to proboscis length 1.00–1.67 (mean = 1.09).

*Wing* (Table 4). Length (measured from humeral crossvein to wing tip, excluding fringe) 4.15–4.55 mm (mean = 4.37 mm,  $n = 9$ ). Dark scales brown to black, pale wing scales white and pale yellow. Costa (C) dark-scaled with small subcostal pale spot (SCP, mean = 0.47 mm) and preapical pale spot (PP, mean = 0.27 mm); remigium covered with numerous white to pale scales and few dark scales; 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  $R_1$  dark-scaled except 3 pale spots (SP, SCP and PP), scattered white spatulate scales from base to presector dark (PSD), and stripe of white scales on SD before SCP; base of  $R_s$  dark-scaled; bifurcation of  $R_2$  and  $R_3$  dark-scaled;

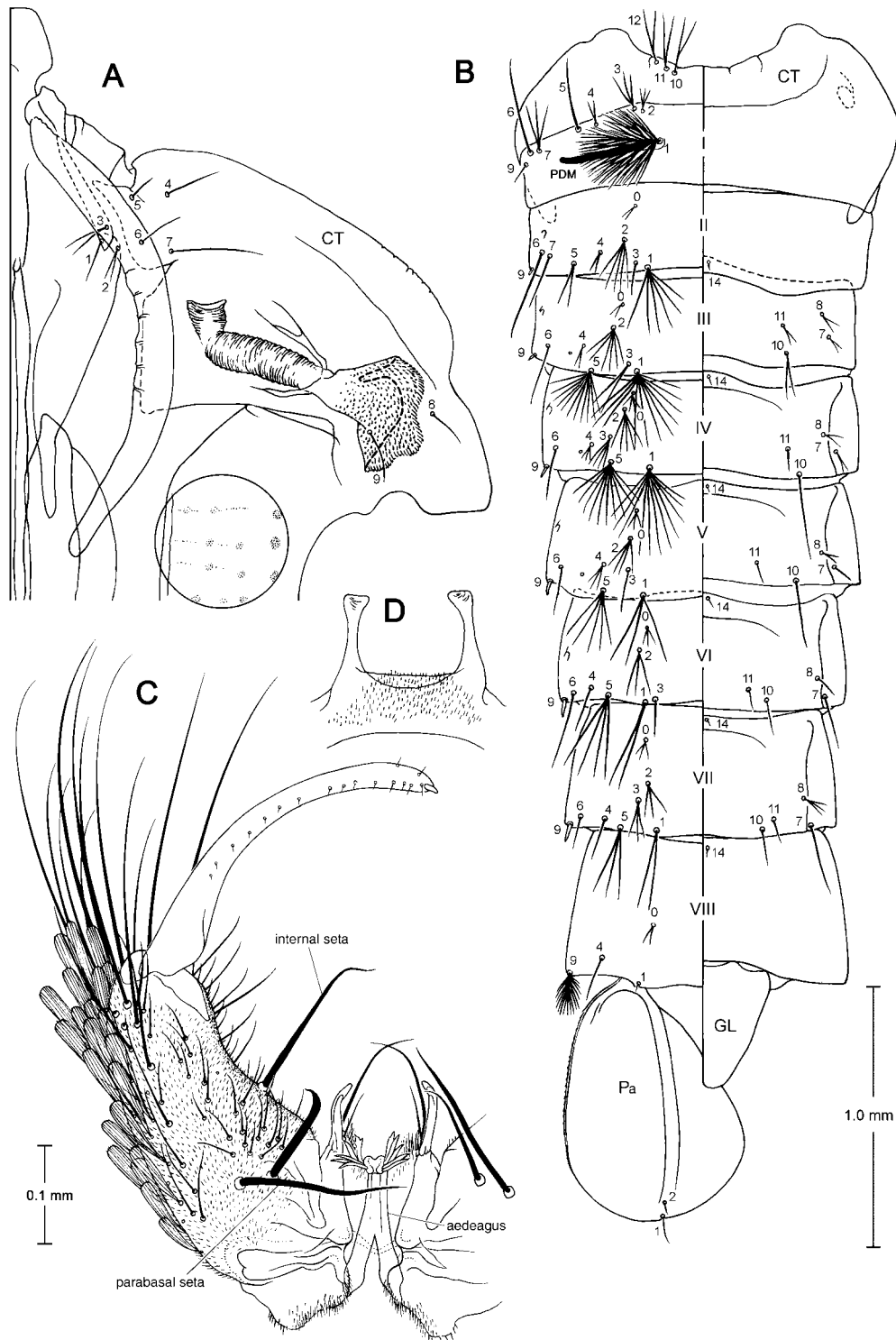
base of  $R_{4+5}$  dark-scaled; tips of  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_{4+5}$  usually with pale fringe. Media (M) mostly pale-scaled; bifurcation of  $M_{1+2}$  and  $M_{3+4}$  dark-scaled; tips of  $M_{1+2}$  and  $M_{3+4}$  with dark fringe. Cubitus (Cu) with basal dark spot, length 0.25–0.45 (mean = 0.34);  $Cu_1$  with 4 dark spots, length of first basal spot 0.20–0.30 mm (mean = 0.26 mm), second spot 0.30–0.40 mm (mean = 0.34 mm), third spot 0.45–1.00 mm (mean = 0.64 mm), distal fourth spot 0.20–0.30 mm (mean = 0.26 mm);  $Cu_2$  with distal dark spot only, length 0.20–0.30 mm (mean = 0.25 mm); tip of  $Cu_1$  with dark fringe; tip of  $Cu_2$  with pale fringe. Anal vein (1A) with 2 dark spots, basal spot length 0.20–0.35 mm (mean = 0.30 mm), distal spot length 0.45–0.60 mm (mean = 0.49 mm), tip of 1A with dark fringe. *Halter*. Scabellum and pedicel light brown to dark with grayish pollinosity; capitellum dark brown to black. Descriptive statistics for ratios of costal and  $R-R_1$  wing spot lengths/length of wing measured from humeral crossvein are shown in Table 4, with holotype male wing measurements in brackets. *Abdomen*. Terga and sterna dark brown to black with grayish pollinosity, covered with pale brown to golden brown setae.

**TABLE 4.** *An.kleini*: descriptive statistics for ratios of veins C and  $R-R_1$  wing spot lengths/length of wing measured from humeral crossvein\*.

Wing spot	Range	Mean $\pm$ SD
<b>Costa (C)</b>		
Basal dark to sector dark (BD+PHD+HD+PD+SD)	0.49 - 0.60	0.56 $\pm$ 0.03 [0.56]
Subcostal pale (SCP)	0.07 - 0.16	0.11 $\pm$ 0.03 [0.07]
Preapical dark (PD)	0.11 - 0.27	0.21 $\pm$ 0.05 [0.27]
Preapical pale (PP)	0.04 - 0.12	0.06 $\pm$ 0.02 [0.06]
Apical dark (AD)	0.03 - 0.04	0.04 $\pm$ 0.00 [0.04]
<b>Vein <math>R-R_1</math></b>		
Basal dark to presector dark (BD+PHD+HD+PSD)	0.24 - 0.27	0.25 $\pm$ 0.01 [0.26]
Sector pale (SP)	0.07 - 0.19	0.10 $\pm$ 0.03 [0.09]
Sector dark (SD)	0.21 - 0.26	0.23 $\pm$ 0.01 [0.26]
Subcostal pale (SCP)	0.06 - 0.11	0.09 $\pm$ 0.01 [0.06]
Preapical dark (PD)	0.07 - 0.24	0.20 $\pm$ 0.05 [0.22]
Preapical pale (PP)	0.06 - 0.16	0.08 $\pm$ 0.03 [0.07]
Apical dark (AD)	0.03 - 0.05	0.04 $\pm$ 0.01 [0.04]

\*Nine wings from the holotype and paratypes; [ ], holotype male.

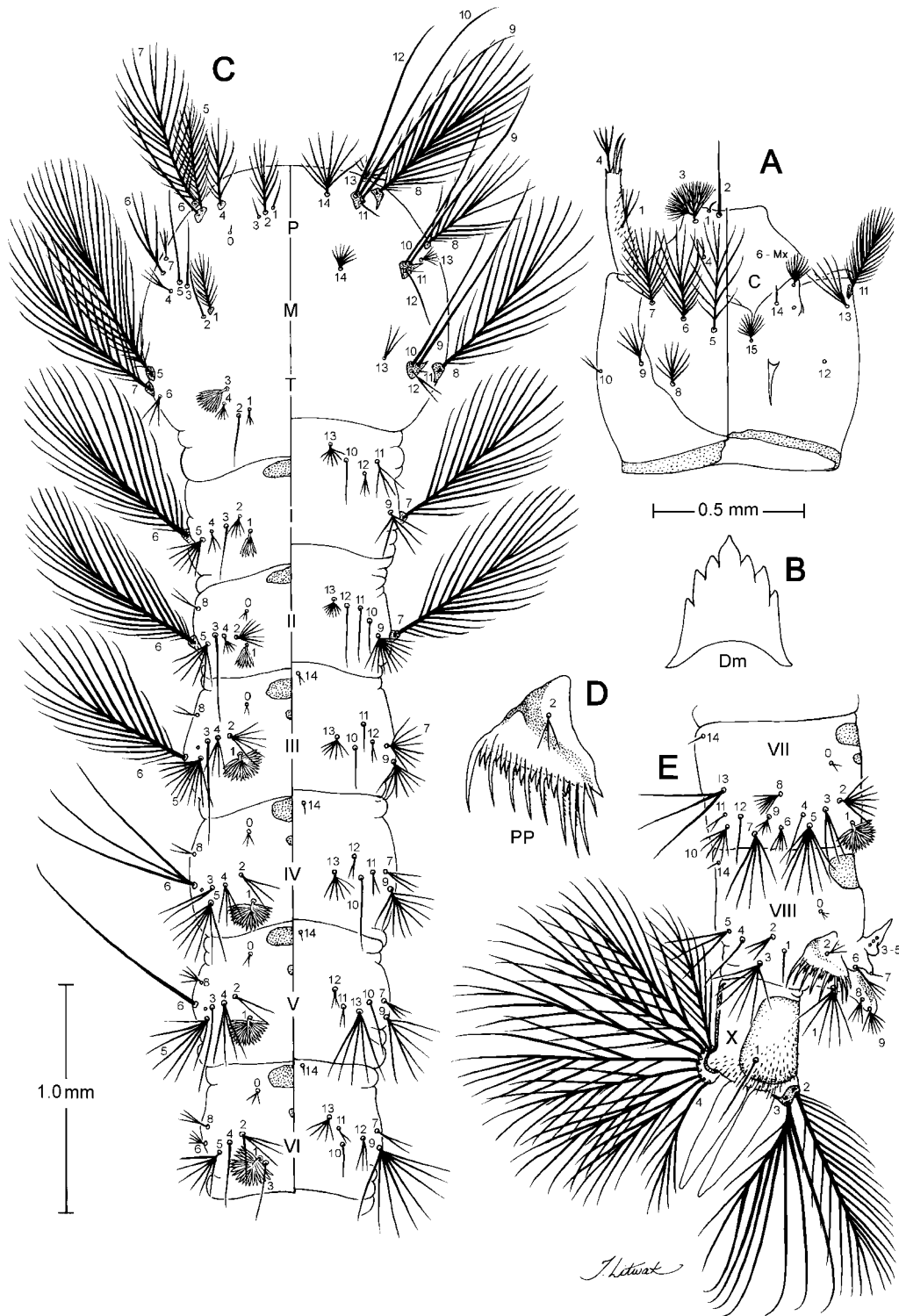




**FIGURE 5.** *Anopheles kleini*. (A) Pupa, cephalothorax. (B) Pupa, metathorax and abdomen, left side dorsal, right side ventral. (C) Male genitalia. (D) Tergum IX. Abbreviations used include CT = cephalothorax, GL = genital lobe, Pa = paddle, PDM = posterior dark mark.

**Male** (Figs. 5 C, D). As in female except for following sexual differences. Maxillary palpus 0.97–1.04 length of proboscis (mean = 0.99;  $n = 9$  for this and  $n = 6$  for 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 pale scales from middle to apex; palpomere 3 dark-scaled with long yellowish 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 2.40–3.00 mm (mean = 2.69 mm,  $n = 9$ ), dark brown-scaled. Anal vein with single dark spot. Tergum IX bare, with pair of elongate caudally directed capitate tergal lobes; length of lobe from base to tip 0.93 distance between 2 lobes; middle of lobe narrower, 0.57 width of lobe tip and 0.08 width of lobe base. Gonocoxite 2.09–2.12x as long as wide at widest point; 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 from base of gonocoxite; parabasal 2 base 0.05 from base of gonocoxite; internal seta slender, base 0.18–0.28 distance from base of gonocoxite. Gonostylus widened at base and narrowed toward middle and tip; gonostylus 1.03 length of gonocoxite; gonostylus 23.33x 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 5 per side; 3 mesal leaflets broadest, with broad, thin, nearly transparent inner margins; other leaflets with narrow, thin, nearly transparent inner margins; most mesal leaflet with 0–3 aciculae.

**Pupa** (Figs. 5 A–B). Position and development of setae as figured; range and modal number of branches, and number of branches of holotype male as in Table 5. Integument with light to moderate dark pigmentation. Exuviae colorless to dark brown. *Cephalothorax*. Mesothoracic wing with distinct rows of round dark spots. Trumpet with thin, lightly serrated pale brown rim; meatus with simple cleft, and its subbasal part with numerous spinules; trumpet length 0.46–0.80 mm (mean = 0.53 mm,  $n = 11$  for this and  $n = 10$  for following measurements and counts except where indicated), width 0.35–0.50 mm (mean = 0.43 mm, measured at base of pinna), index 1.02–1.72 (mean = 1.23); tracheoid area 0.91 length of trumpet. *Abdomen*. Abdominal tergum I with 2 elongate posterior dark marks (PDM); each PDM narrows mesally at base, with maximum width (0.02–0.05 mm, mean = 0.04 mm) towards distal tip, length 0.34–0.74 mm (mean = 0.40 mm), about 0.02–0.04 (mean = 0.03 mm,  $n=17$ ) width of abdominal segment I, and longer than the longest branch



**FIGURE 6.** *Anopheles kleini*, larva. (A) Head, left side dorsal, right side ventral. (B) Dorsomen-tum (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.

**TABLE 5.** Pupal setal branching for *An. kleini*: range mode ( ) based on counts made on 5-20 setae of the holotype and paratypes; [ ], male holotype

Seta no.	Cephalothorax	Abdominal segment				
	CT	I	II	III	IV	V
0	-	-	2-3(2) [-]	2-4(2) [2]	2-4(4) [-]	2-4(3) [3]
1	1-2 (1) [1]	12-29(19) [13]	3-12(6) [7, 8]	8-18(11) [12]	7-16(10) [12]	2-8(3) [4]
2	1-3(2) [2]	2-3(3) [3]	3-8(5) [6]	3-7(6) [5]	2-5(4) [4]	3-5(4) [4]
3	1-3(2) [3]	1-5(3) [3]	1-2(1) [1]	2-5(2) [2]	1-5(4) [4]	1-2(1) [2]
4	1-2(1) [2]	2-4(2) [4]	1-4(2) [1]	1-3(2) [3]	1-4(3) [4]	1-3(3) [3]
5	1-4(1) [4]	1-5(2) [2]	1-3(2) [2]	5-14(12) [10]	6-14(11) [11]	5-13(5) [8]
6	1-2(1) [1]	1-2(1) [1]	1-3(2) [2]	1-2(1) [2]	1 [1]	1 [1]
7	1 [1]	1-6(3) [3]	1-3(1) [1]	1-4(2) [-]	1-4(2) [4]	1-2(1) [1]
8	1 [1]	-	-	1-2(2) [2]	1-2(2) [2]	1-2(2) [2]
9	1 [1]	1-2(1) [2]	1 [1]	1 [1]	1 [1]	1 [1]
10	1-4 [2]	-	-	1-2(2) [2]	1-3(1) [1]	1-2(1) [1]
11	2-3(2) [2]	-	-	1-3(2) [2]	1-2(1) [2]	1-2(1) [2]
12	1-3(2) [2]	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	1 [1]	1 [1]	1 [1]

Seta no.	Abdominal segments				Paddle
	VI	VII	VIII	IX	Pa
0	3 [3]	2-5(3) [5]	1-4(2) [2]	-	-
1	1-3(2) [1]	1-2(2) [1]	-	1 [1]	1 [1]
2	3-5(3) [3]	2-4(3) [3]	-	-	1 [1]
3	1-2(1) [1]	1-5(3) [2]	-	-	-
4	1-2(1) [2]	1-2(1) [2]	1-3(1) [1]	-	-
5	3-10(4) [9]	3-5(3) [5]	-	-	-
6	1 [1]	1-2(1) [2]	-	-	-
7	1 [1]	1-3(1) [1]	-	-	-
8	1 [1]	1-5(3) [4]	-	-	-
9	1 [1]	1 [1]	1 [1]	-	-
10	1-2(1) [2]	1-2(1) [2]	-	-	-
11	1-3(1) [1]	1-3(1) [1]	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	1 [1]	1 [1]	-	-

of seta 1-I. Seta 1-I fan-like with 12–29 aciculate dendritic branches; 6-I with 1 or 2 branches; 7-I with 1–6 branches; 9-I with 1 or 2 branches. Setae 1, 5-III–VII well developed; 1-V 0.80–1.17 (mean = 1.04) length of 5-V; 1-VI 0.71–1.29 (mean = 0.96,  $n = 4$ ); 1-VII 0.79–1.17 (mean = 0.99,  $n = 9$ ); 3-VI aligned with and mesal of 1-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, 2.25–4.67 (mean = 2.95) length of 9-II; 9-IV strong, lightly pigmented and slightly pointed, 1.44–1.86 (mean = 1.56) length of 9-III; 9-V–VIII long, lightly pigmented and slightly pointed; 9-V 1.00–2.00 (mean = 1.29) length of 9-IV; 9-VI 1.00–1.19 (mean = 1.08) length of 9-V; 9-VII 3.89–5.00 (mean = 4.41) length of 9-VI; 9-VIII 0.16–0.27 (mean = 0.22) length of 9-VII; 9-VI 0.15–0.16 (mean = 0.15,  $n = 3$ ) length of segment VI; 9-VII 0.06–0.08 (mean = 0.07,  $n = 9$ ) length of segment VII; 9-VIII 0.05–0.54 (mean = 0.12,  $n = 8$ ) length of segment VIII. Seta 9-I positioned near anterolateral edge of tergum; 9-II on posterolateral edge of tergum; 9-III–VIII near posterolateral edge of tergum. Segment VII 1.00–1.85 (mean = 1.15) length of segment VI; segment VIII 1.15–1.27 (mean = 1.20,  $n = 6$ ) length of segment VI; segment VIII 0.11–1.22 (mean = 1.02,  $n = 8$ ) length of segment VII. Segment VII 0.008–0.009 (mean = 0.009) width of segment VI (width at posterior margins); segment VIII 0.10–0.96 (mean = 0.77,  $n = 6$ ) width of segment VI; segment VIII 1.06–1.03 (mean = 0.90,  $n = 8$ ) width of segment VII. Width/length of segment VI 2.15–2.76 (mean = 2.44), segment VII 1.15–2.16 (mean = 1.99), segment VIII 0.001–0.007 (mean = 0.006). Paddle length 0.88–1.06 mm (mean = 0.96 mm), width 0.49–0.71 mm (mean = 0.63 mm), length/width ratio 1.34–1.97 (mean = 1.53); refractile index 0.76–0.85 (mean = 0.80,  $n = 8$ ); paddle seta 1-Pa simple or forked; 2-Pa simple; 1-Pa thicker and 1.74x longer than 2-Pa. Width/length of genital lobe of female 1.78–1.86 (mean = 1.82), male 0.73–1.00 (mean = 0.88,  $n = 8$ ); numerous spicules present on subapical and apical margins of genital lobe of female, absent in male.

**Larva** (Fig. 6). Position and development of setae as figured; range and modal number of branches and number of branches of holotype male as shown in Table 6. *Head*. Length 0.78–1.02 mm (mean = 0.90 mm,  $n = 9$  for this and following measurements and counts except where indicated), width 0.74–0.90 mm (mean = 0.81 mm). Antennal length 0.32–0.36 mm (mean = 0.33 mm), slightly tapered toward apex, 4.44–6.15 (mean = 4.97) longer than wide; with spicules longer and more numerous ventrally and in vicinity of seta 1A; spicule length 0.004–0.02 mm (mean = 0.02 mm,  $n = 20$ ). Seta 1-A with 2–7 (mode = 5,  $n = 11$ ) branches, length 0.08–0.24 mm (mean = 0.14 mm), inserted 0.11–0.17 mm (mean = 0.15 mm) from base of antenna; 2-A single, pointed, length 0.08–0.12 mm (mean = 0.10 mm,  $n = 8$ ); 3-A single, pointed, length 0.12–0.14 mm (mean = 0.13 mm,  $n = 7$ ); 4-A with 3–5 branches (mode = 5,  $n = 6$ ); 5-A short, spine-like, 0.12–0.23 (mean = 0.14) length of seta 1-A; 6-A spine-like 0.91x as long as seta 5-A. Seta 2-C single 1.04–1.72 (mean = 1.53,  $n = 12$ ) length of 3-C; seta 2-C close to mate of opposite side 0.002–0.008 mm (mean = 0.003 mm,  $n = 15$ ); 3-C densely dendritic with 11–23 main branches (mode = 11), longest branch 0.16–0.24 mm (mean = 0.18 mm,  $n = 13$ ), clypeal index (distance between

**TABLE 6.** Larval setal branching for *An. kleini*: range mode ( ) based on counts made on 5-20 setae of the holotype and paratypes; [ ], male holotype.

Seta no.	Head	Thorax			Abdominal segments	
	C <sup>a</sup>	P	M	T	I	II
0	-	-	-	-	-	1-3(3) [3]
1	1 [1]	1-5(1)[1, 2]	13-22(15) [17]	3-4(3) [3]	8-15(8) [8]	7-12(12) [7]
2	1 [1]	5-10(8) [6, 8]	1-4(1) [1]	1 [1]	5-7(5) [6]	5-9(7) [5]
3	11-23(11) [14]	1-2 [2, 1]	1 [1]	9-15(12) [9]	1 [1]	1 [1]
4	2-7(2) [2, 4]	8-16(10) [8, 11]	3-8(3) [5]	1-5(5) [4]	3-8(3) [3]	3-6(5) [4]
5	11-15(11) [-]	15-20(18) [20]	1 [1]	25-29(28) [-]	4-11(6) [4]	6-11(6) [6]
6	11-18(15) [17]	1 [1]	3-6(4) [6]	2-4(3) [-]	13-23(21) [13]	16-26(17) [16]
7	12-21(15) [14]	21-22(21) [-]	3-3(2) [2]	17-25(21) [-]	19-23(19) [23]	19-27(22) [19]
8	6-10(8) [9, 7]	19-25(23) [23]	12-15(13) [15]	18-27(27) [-]	-	2 [2]
9	5-8(5) [6]	1 [1]	1 [1]	1 [1]	5-9(6) [6]	4-12(11) [8]
10	1-3(2) [2, 1]	1 [1]	1 [1]	1 [1]	1 [1]	1-5(1) [1]
11	24-41(35) [39]	1-5(1) [5]	1 [1]	1 [1]	2-5(4) [4]	1-2(1) [1]
12	3-5(4) [3, 4]	1 [1]	1 [1]	2 [2]	2-3(3) [3]	1-3(1) [1]
13	4-11(5) [8]	6-12(8) [12]	4-7(5) [7]	3 [3]	5-10(7) [10]	6-10(8) [9]
14	1-4(1) [3]	5-11(9) [-]	5-9(8) [9]	-	-	1 [1]
15	4-11(8) [9]	-	-	-	-	-

Seta no.	Abdominal segments						
	III	IV	V	VI	VII	VIII	X
0	2-3(2) [3]	1-3 (3) [3]	2-3(2) [2]	1-3(3) [3]	1-3(2) [3]	2-3(3) [3]	-
1	14-20(15) [14]	14-20(17) [15]	15-22(17) [17]	14-18(15) [15]	13-17(15) [15]	1 [1]	1 [1]
2	2-6(6) [6]	2-3(3) [3]	2-6(2) [4]	4-6(5) [4]	5-7(7) [6]	3-11(3) [11]	16-51(18) [19]
3	1 [1]	2-4(2) [2]	1-3(1) [2]	1 [1]	1-3(3) [3]	5-10(7) [10]	6-22(6) [12]
4	3-4(4) [3]	4-7(4) [4]	4-7(4) [4]	1-2(1) [2]	1 [1]	1 [1]	9 [9]
5	9-13(11) [11]	5-7(7) [7]	5-8(7) [8]	4-10(6) [8]	7-12(8) [9]	2-4(3) [4]	-
6	16-32(16) [16]	3-5(3) [3]	1-2(1) [1]	3-6(4) [6]	2-6(5) [5]	1-S	3-7(6) [6]
7	3-9(8) [9]	2-3(3) [3]	3-5(3) [3]	2-4(2) [2]	4-9(5) [5]	2-S	3-6(3) [3]
8	2-3(2) [3]	2-4(2) [-]	1-5(4) [4]	2-5(3) [4]	3-8(8) [8]	6-S	2-6(3) [-]
9	5-11(7) [5]	6-11(9) [9]	6-9(6) [9]	5-11(8) [10]	2-8(5) [8]	7-S	1-2(1) [1]
10	1-2(1) [1]	1 [1]	1 [1]	1-3(1) [2]	3-7(6) [3]	8-S	4-7(4) [5]
11	2-4(2) [2]	2-4(2) [3]	2-3(2) [2]	1-3(1) [2]	2-5(2) [2]	9-S	3-7(7) [3]
12	1-2(1) [2]	2-4(2) [4]	1-3(3) [2]	1-2(1) [1]	1 [1]	-	-
13	6-9(7) [8]	4-9(6) [9]	5-10(5) [5]	5-10(6) [7]	2-4(3) [4]	-	-
14	1 [1]	1-2(1) [2]	1-2(1) [2]	1-2(1) [2]	1-2(1) [2]	1-2(1) [2]	-
15	-	-	-	-	-	-	-

<sup>a</sup>C, head; P, prothorax; M, mesothorax; T, metathorax.

bases 2-C and 3-C on 1 side/distance between bases of 2-C) 9.00–32.00 (mean = 21.33,  $n = 12$ ). *Thorax*. Seta 1-P with 1–5 branches (mode = 1,  $n = 13$ ); 9, 10, 12-P single; 9-12-P setal support plate spine length 0.03–0.06 mm (mean = 0.04 mm,  $n = 8$ ). Setae 9-12-M single; 9-M 1.25–1.42 (mean = 1.30,  $n = 5$ ) length 10-M; 9-12-M setal support plate spine length 0.02–0.04 mm (mean = 0.03 mm,  $n = 5$ ). Setae 9-11-T single; 9-T 1.38–1.50 (mean = 1.45,  $n = 6$ ) length of 10-T; seta 12-T with 2 branches; 9-12-T setal support plate spine length 0.03–0.04 mm (mean = 0.03 mm,  $n = 14$ ); 13-T with 3 branches. *Abdomen*. Seta 1-I with 8–15 branches (mode = 8,  $n = 8$ ); 1-II 7–12 branches (mode = 12). 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; 3-II, V, VI single; 3-I, III, IV single. Seta 1-X single, 0.56–0.95 (mean = 0.71,  $n = 6$ ) 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 19–25 spines (mode = 19,  $n = 13$ ); arrangement of spines alternating long and short, with 8–9 (mode = 8,  $n = 13$ ) long spines and 10–17 (mode = 13,  $n = 12$ ) short spines; long spines 2.50–5.50 (mean = 3.59,  $n = 20$ ) length of short spines. Two posterolateral spiracular lobe plates present, each plate with elongate, slender, sclerotized projection from inner caudal margin.

**Type material.** Holotype male with associated slide-mounted larval and pupal exuviae, and male genitalia; H. C. Kim coll., reared from a female collected from CDC light trap at a cow shed, 2 August 2002, Cheongpyeong (36° 40'N 128° 22'E), Gyeongsangbuk-do, South Korea, WRBU ACC No. 1684, KS 02-6(2)-6. Deposited in the NMNH, Smithsonian Institution, Washington, D.C. Paratypes. SOUTH KOREA: GYEONGSANG BUK-DO, Cheongpyeong, same data as holotype male, 8 F PeLe, KS 02-6(2)-11,12,14, 5,18-21; 2 M PeLe, KS 02-6(2)-9,10; 1 F, KS 02-6(2); GYEONGGI-DO, Majeong-ri, Munsan-up, Pajusi (37° 50'N 126° 49'E), W. J. Lee coll., 5 June 2004, reared from a female, 5 F, KS 04-2(1)-7, 8, 13-15; 3 F, KS 04-2(17)-3-5. DNA vouchers are deposited in the NMNH, Smithsonian Institution, Washington, D.C.

**Diagnosis.** *Anopheles kleini* 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 (like *An. belenrae*); vein Cu<sub>2</sub> with apical pale fringe spot (like *An. sinensis*, *An. sineroides*, *An. pullus*, but unlike *An. belenrae* and *An. lesteri*); subcostal pale (SCP) spot narrow; humeral crossveins without scales (unlike *An. pseudosinensis*); mid-coxa with upper patch of pale scales (like *An. sinensis* and *An. belenrae*, but unlike *An. lesteri*); hindtarsomeres 1–4 with narrow apical pale bands, hindtarsomere 4 without basal pale band (unlike *An. peditaeniatus*). *Adult male*. Male genitalia with dorsal lobe of claspette having 3 closely appressed setae of about equal length. Aedeagus with 5 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 thin, lightly serrated pale brown rim (like *An. sinensis* and *An. belenrae*, and unlike

*An. lesteri* having thick serrated rim). Wing sheath with distinct rows of round dark spots (like *An. sinensis* and *An. belenrae*, and unlike *An. lesteri* having checkered dark stripes). Setae 9-III-VII single, with narrowly rounded apex (like *An. lesteri* and *An. belenrae*); seta 5-V with 5–13 branches. *Larva*. Setae 2-C, 3, 5-M single; 3-P single or forked; 3-C with 11–23 branches; 4-M with 3–8 erect branches; 9-M about 1.5x length of 10-M, T; 5-III with 9–13 branches; 9-III with 5–11 branches; 13-IV with 4–9 branches; 1-X strong, single less than length of saddle (unlike *An. lesteri* about 2x or more longer than the saddle); pecten with 8–9 long spines and 10–17 short spines.

**Etymology.** The species is named after Colonel Terry A. Klein, medical entomologist, for his numerous contributions to mosquito research in Asia.

**Remarks.** The specimens used in this study were progeny reared from adults that were collected in cow sheds at villages with rice paddies. Nothing is known about the natural habitats of the immature stages. The ribosomal DNA ITS2 sequence of *An. kleini* is provided by Li *et al.* (2005, Figure 1, *Anopheles* “unknown 2”).

## Discussion

With the recent increase of malaria cases in South Korea, major collection efforts for the *Anopheles* Hyrcanus Group were conducted, and specimens from that country were accumulated for both morphological and molecular studies. However, type specimens of many species of the Hyrcanus Group are non-existent, and collections of this group from type localities located in other countries also are not available. Presently, it is not possible to provide a meaningful morphological comparison of all species of the *Anopheles* Hyrcanus Group because other members of the group have not been studied in detail in all life stages. Except for the work of Reid (1968), Harrison and Scanlon (1975) and Tanaka *et al.* (1979), no modern comprehensive morphological study persists for the members of the group, and most of the previous literature is either limited in scope, or pertains to species of questionable identification (Rueda *et al.* 2005). Adults of *An. belenrae* can be separated morphologically from those of *An. lesteri*, *An. sinensis* and other species (see section on adult diagnosis). However, *Anopheles kleini* is very difficult to distinguish morphologically from *An. sinensis*. Although both new species share some similar morphological features with *An. sinensis* and related species of the Hyrcanus Group, they are genetically distinct from each other. Based on ribosomal DNA ITS2 sequence, *An. belenrae* and *An. kleini* have genetic distances of 10.6% from each other, and 9.1% and 10.7%, respectively, with *An. sinensis*. Wilkerson *et al.* (2003) also noted ITS2 sequence to separate *An. belenrae* (as “unknown sp. Korea”) from *An. sinensis*, *An. lesteri*, and *An. pullus* specimens collected from South Korea and other countries. PCR assay based on the above sequence, 7 multiplexed primers (as discussed in Li *et al.* 2005) worked unambiguously to separate these 2 new species (*An. belenrae* as “unknown 1” and *An. kleini* as “unknown 2”) from each other and related species (i.e., *An. sinensis*, *An. lesteri*, and *An. pullus*).



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