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A NEW SPECIES OF THE HYRCANUS GROUP OF *ANOPHELES*, SUBGENUS *ANOPHELES*, A SECONDARY VECTOR OF MALARIA IN COASTAL AREAS OF SOUTHERN VIETNAM

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ABSTRACT. *Anopheles (Anopheles) nimpe,* a new species of the Hyrcanus Group from the coastal areas of southern Vietnam, is described and illustrated in the adult, pupal, and larval stages. Partial DNA sequence data from paratypes are included for the mitochondrial cytochrome c oxidase I and 16S rRNA genes. The species is distinguished from other members of the group occurring in Southeast Asia.

KEY WORDS *Anopheles nimpe,* Culicidae, mosquitoes, new species, Vietnam

INTRODUCTION

As currently defined, the Hyrcanus Group includes nearly three quarters of the species that comprise the Myzorhynchus Series of *Anopheles* subgenus *Anopheles.* With the addition of the new species described in this paper, the group includes 28 formally recognized species in the Oriental and southern Palaearctic regions. Ten of these species, 8 in China, 1 in Japan, and 1 in Vietnam, were recognized after the Southeast Asian members of the group were described in detail by Reid (1968) and Harrison and Scanlon (1975). Whether these species occur outside of the countries where they were discovered is unknown, and their affinities with other members of the group are likewise unknown. In fact, relationships, based on morphologic similarity, have been hypothesized for only 9 species of the group, those that comprise the Lesteri (5 species) and Nigerrimus (4 species) Subgroups (Harrison 1972). The other species of the group, including the new one described here, are unplaced within the Hyrcanus Group because they cannot be segregated into supraspecific taxa on the basis of available information. As pointed out by Reid (1968) and Harrison (1972), much additional taxonomic work needs to be done on the group to differentiate the species, determine their distributions, and establish their relationships to one another. The complexity of the taxonomic problem lends itself to an integrated approach, using morphologic, molecular, and bionomic data. The description of the new species below is a contribution toward this goal.

MATERIALS AND METHODS

This study is based on specimens collected in coastal areas of southern Vietnam. Wild-caught larvae and the progeny of wild-caught adults were individually reared to provide adults with associated larval and pupal exuviae. Observations of the adults were made under simulated natural light. Larval and pupal chaetotaxy were studied using a combination of bright-field and differential interference contrast microscopy. Measurements and counts were made from available specimens. Numbers in parentheses represent modes of the reported ranges. Except for wing spot nomenclature (Wilkerson and Peyton 1990), the morphologic terminology used in the species description follows Harbach and Knight (1980, 1982). The new species is recognized on the basis of correlated anatomical features in associated life stages. Diagnostic and differential characters were confirmed in all available specimens. Type specimens are deposited in The Natural History Museum (BMNH), London, and the National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi.

DNA was extracted from pinned male paratypes (EP2-1, E3) following a phenol–chloroform protocol (adapted from W. Tabachnick, personal communication) and resuspended in 100 µl of 10 mM Tris-HCl, pH 8.5. Partial DNA sequence data for the mitochondrial genes cytochrome c oxidase I (COI) (472 bp) and 16S rRNA (504 bp) were obtained from the paratype EP2-1, and can be obtained from GenBank under the accession numbers AF216285 and AF216284, respectively. The COI gene fragment was amplified using the universal insect primers C1-J-1718 and C1-N-2191, and the primers LR-N-13398 and LR-J-12888 were used to amplify the 16S rRNA amplicon (Simon et al. 1994). In both cases, the last five digits indicate the position of the 3′ end of the primers with respect to the *Drosophila yakuba* (X03240) mtDNA genome (Clary and Wolstenholme 1985). Purified DNA was amplified by polymerase chain reaction (PCR) using the following reaction mix (50 µl): 2 µl DNA, 25.5 µl double-distilled H_2O, 2.5 µl 2.5 mM $MgCl_2$, 0.1 µl Taq polymerase (BioLine, London, England), and 5 µl each of primers at 5 µM, deoxynucleotide phosphates (dNTPs), and NH_4

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buffer (BioLine). The PCR thermocycler program consisted of a 2-min denaturation at 94°C; 34 cycles of 94°C, 53°C, and 72°C for 30 sec each; followed by a 5-min extension at 72°C. Sequence data were obtained after PCR purification using a commercially available PCR purification kit (QIAgen Ltd, Sussex, England) and cycle sequencing reactions were read by an ABI 377 automated sequencer (Applied Biosystems, Perkin-Elmer Corporation, Wellesley, MA). Sequence data were edited and aligned on Macintosh® computers using Sequencher™ 3.0 (Genes Codes Corporation, Ann Arbor, MI).

TAXONOMIC TREATMENT

Anopheles (*Anopheles*) *nimpe* Nguyen, Tran, and Harbach

Anopheles (*Anopheles*) sp 1 of IMPE 1987:13 (adult key); Nguyen Duc Manh et al. 1991:54, 55 (taxonomy); Nguyen Tang Am 1993:495, 496 (bionomics, control); Nguyen Tang Am et al. 1993:465–468, 470, 472 (bionomics); Đoàn Hanh Nhân 1993:38 (vector incrimination); Vu Thi Phan 1998:49 (medical importance).

Anopheles (*Anopheles*) sp. 1 of Nguyen Duc Manh et al. 1993:215 (mention).

Diagnosis. Adults of *An. nimpe* are distinguished from other members of the Hyrcanus Group in having narrow apical tarsal bands, apex of wing with long pale fringe spot, preapical pale spot of costa and vein R_1 absent or weakly developed, humeral crossvein with dense patch of dark scales, preapical dark spot of vein R_1 without pale scales, and veins CuA and 1A largely dark-scaled with long basal dark spot on CuA (usually not well defined) ending at nearly same level as base of upper dark spot on 1A. Pupae have the trumpet rim thin and uniform, seta 5-III–VII inserted on intersegmental membrane, seta 8-VII inserted lateral to fold line, seta 9-VIII with well-developed branches, paddle with marginal serrations rarely reaching 0.75 its length, and seta 1-Pa with 2–7 branches arising at base. Larvae are not so clearly distinguished: seta 1-A usually with fewer than 6 branches, seta 8-C has fewer than 12 branches (3–8, mode 6), seta 9-C with 3–6(4) branches, and seta 14-P relatively short and with 5 or fewer branches.

Female. *Head:* Vertex with pale erect scales behind frontal tuft, dark erect scales posterolaterally; frontal tuft with long pale setae. Clypeus with patch of dark scales on either side. Antenna length about 1.5 mm; pedicel with small pale scales on lateral surface; basal 2 or 3 flagellomeres with pale scales on mesal surface. Proboscis entirely dark-scaled, scales semierect proximally and appressed distally; labella paler than prementum; length about 2.0 mm, 1.25× length of forefemur, slightly longer than maxillary palpus (about 1.1×). Maxillary palpus (in dorsal view) with pale scales on apex of palpomere 5, narrow pale bands (incomplete ventrally) across joints between palpomeres 4–5, 3–4, and 2–3; palpomere 2 usually without pale scaling on mesal surface, occasionally with few indistinct pale scales in this location. *Thorax:* Integument brown; scutum usually with darker median line extending back to prescutellar area, often with darker lateral line on posterior dorsocentral areas, and much darker spot just behind scutal fossa of either side; scutum sparsely covered with fine golden piliform scales; anterior promontory with long pale setae and piliform scales mesally, dark setae and scales laterally; dark setae on acrostichal, dorsocentral, lateral prescutal, scutal fossal, antealar, and supraalar areas. Scutellum with long dark setae and golden piliform scales; integument dark medially, pale laterally. Mesopostnotum and postpronotum bare. Antepronotum with patch of dark erect scales on dorsoanterior margin, dark setae laterally. Pleura with dark setae on upper proepisternum, prespiracular area, prealar knob, upper and lower mesokatepisternum, and upper mesepimeron. *Wing* (Fig. 1): Length about 3.4 mm; mainly dark-scaled; small subcostal pale spot on costa, apex of subcosta, and R_1; preapical pale spot on costa and R_1 weakly developed or absent, especially on costa; remigium dark-scaled, sometimes with few indistinct pale scales; humeral crossvein with dark scales; vein R with scattered pale scales between base and small sector pale spot; R_1 usually with few scattered pale scales between sector and subcostal pale spots, always entirely dark-scaled between subcostal and preapical pale spots, apex with dark scales; veins R_2, R_{4+5}, M_1, M_2, and M_{3+4} usually with some pale scaling, as proximal spots on R_2, M_1, and M_2, and speckling on R_{4+5} and M_{3+4}; CuA predominantly dark-scaled with dark scales more concentrated in "basal" and apical spots, basal dark spot (generally not sharply delimited) long, terminating at or just before level of base of upper dark spot on vein 1A; 1A also usually predominantly dark-scaled, dark scales concentrated in "upper" and apical spots; apical pale fringe spot long, extending from R_1 to R_{4+5} or slightly beyond (Figs. 1A, 1C), sometimes indefinite and seemingly ending before R_{4+5} (Fig. 1D); posterior margin of wing without pale fringe spot at apex of CuA. *Halter:* Pedicel mainly pale, scabellum and capitellum dark, capitellum dark-scaled. *Legs:* Coxae without pale scales, forecoxa with few dark scales at base; femora, tibiae, and tarsi mainly dark-scaled, posterior or ventroposterior surfaces of femora, tibiae, and 1st tarsomeres with dirty yellowish scaling, mid- and hindtibiae with few pale scales dorsally at apex; foretarsomeres 1–3 with apical pale bands approximately equal to width of tarsomeres; midtarsomeres 1–3 and hindtarsomeres 1–4 with narrow dorsoapical pale spots. *Abdomen:* Integument dark

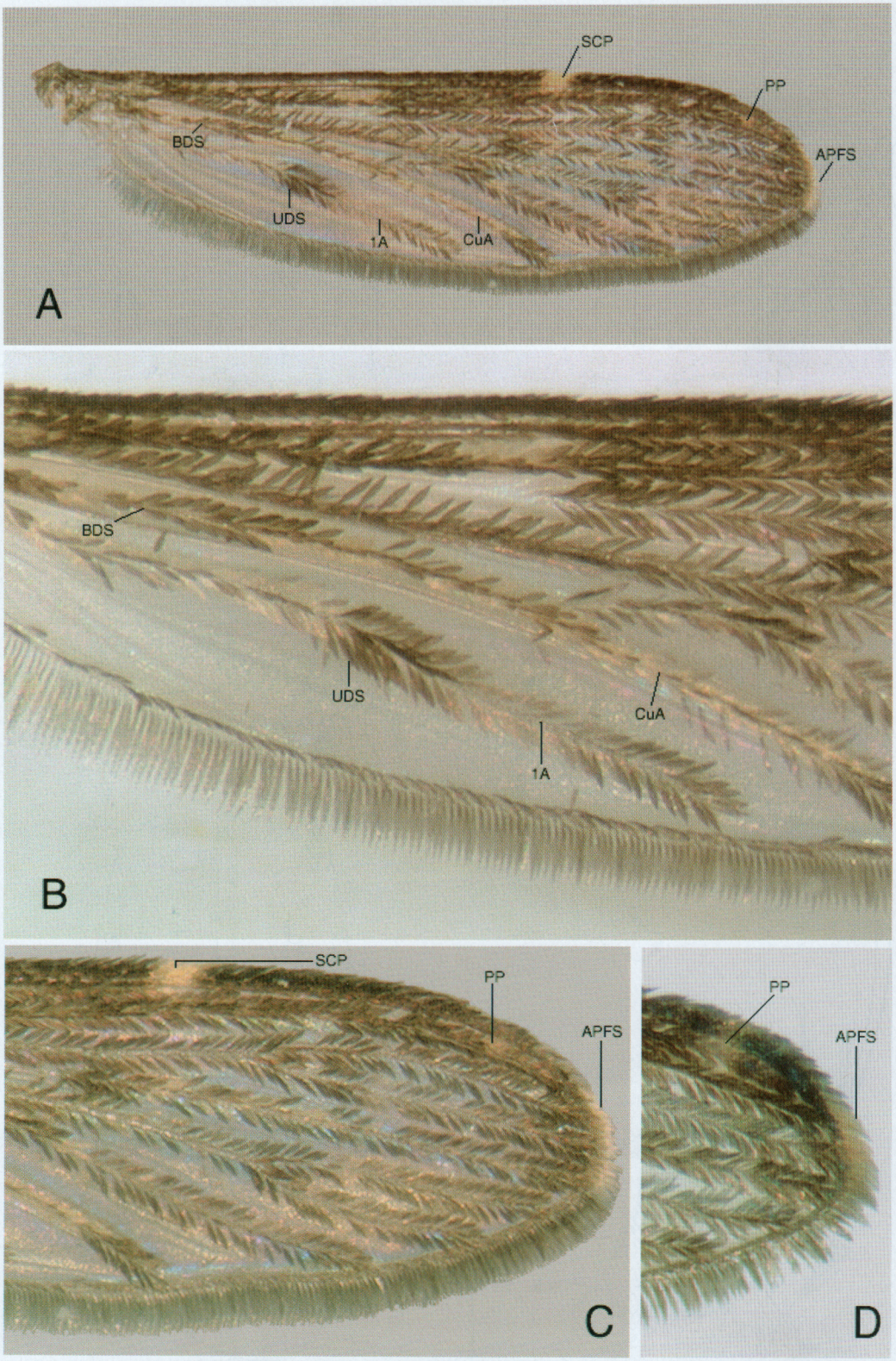

Fig. 1. Wing of *Anopheles* (*Anopheles*) *nimpe*. A–C, Paratype female (EP2-8); D, holotype female (EP1-1), apex of left wing flipped 180°. APFS, apical pale fringe spot; BDS, basal dark spot; CuA, vein CuA; PP, preapical pale spot; SCP, subcostal pale spot; UDS, upper dark spot; 1A, vein 1A (anal vein).

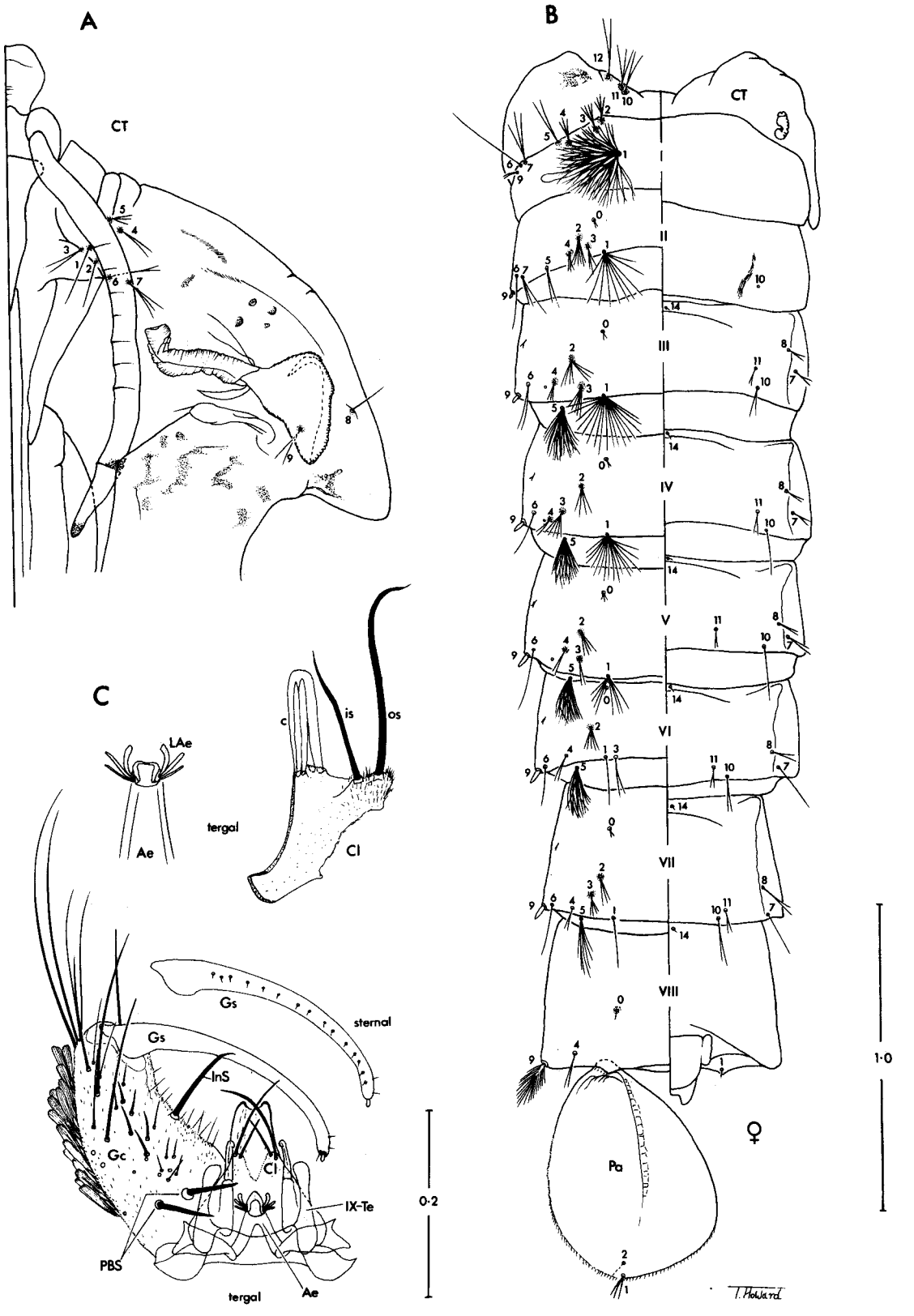
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Table 1. Range of numbers of branches for pupal setae of *Anopheles (Anopheles) nimpe*. Mode in parentheses.

Seta no.	Cephalothorax CT	Abdominal segments I	II	III	IV	V	VI	VII	VIII	Paddle Pa
0	—	—	1–3(2)	1–3(2)	1–3(2)	1–3(2)	1–3(2)	1–3(2)	1–3(1)	—
1	1–3(1)	70–115	4–16(8)	10–33(18)	11–30(17)	2–17(6)	1–3(1)	1,2(1)	—	2–7(4)
2	1,2(2)	2–6(3)	3–7	3–8(6)	2–5(4)	3–6(4)	2–7(4)	2–5(4)	—	1–3(1)
3	1–3(2)	2–6(3)	1–3(2)	2–5(3)	3–7(5)	1–3(2)	1–3(2)	2–4(3)	—	—
4	1–3(2)	2–5(3)	1–3(3)	1–3(2)	1–3(2)	1–4(2)	1–3(2)	1,2(2)	1-3(2)	—
5	1–4(2)	1–4(2)	1–4(2)	9–38(14)	10–39	11–40(17)	4–31(13)	3–15(5)	—	—
6	1–3(2)	1–3(1)	1,2(1)	1–4(2)	1	1,2(1)	1–3(1)	1–3(2)	—	—
7	1–4(2)	1–6(1)	2–4(3)	1–3(2)	1–4(2)	1–3(2)	1,2(1)	1–3(1)	—	—
8	1,2(1)	—	—	1–3(2)	1–3(2)	1–3(2)	1–3(2)	1–4(3)	—	—
9	1–3(2)	1–4(2)	1	1	1	1	1	1	12–23	—
10	1–4(2)	—	0,1(0)	1,2(2)	1,2(1)	1	1–3(1)	2,3(2)	—	—
11	2–4(3)	—	—	1–4(2)	1–3(2)	1–3(2)	1–5(2)	1–3(2)	—	—
12	2,3(2)	—	—	—	—	—	—	—	—	—
14	—	—	—	1,2(1)	1,2(1)	1	1	1,2(1)	1,2(1)	—

with long golden-brown setae; without scales except sternum VII sometimes with few narrow dark erect scales near posterior margin (without conspicuous tuft of dark scales that characterizes other members of the Hyrcanus Group).

Male. Resembles the female except as follows. *Head:* Maxillary palpus with indistinct line of pale scales on mesal surface of palpomere 2 and lateral patches of pale scales at base of palpomere 4, across joint between palpomeres 4 and 5, and on distal portion of palpomere 5. *Genitalia* (Fig. 2): Gonocoxite with pale scales on lateral and dorsal surfaces; inner and outer parabasal setae straight or nearly so, outer seta slightly longer than inner seta; internal seta rather long, relatively stout, and slightly curved or bent apically; tergum IX with pair of long slender caudally directed lobes with slightly expanded tips; aedeagus with 3 pairs of leaflets, leaflets without teeth or serrations; ventral lobe of claspette with 2 long setae, outer seta nearly twice as long as inner seta and noticeably curved or bent; dorsal lobe of claspette with club formed of at least 3 setae on inner-sternal side and 2 flat appressed setae on outer-tergal side.

Pupa. (Fig. 2). Character and positions of setae as figured; numbers of branches in Table 1. *Cephalothorax:* Lightly to moderately pigmented, all setae usually with ring of darker cuticle around alveoli; mesothoracic wing with blurred lattice pattern of darker spots; antenna usually with apex and most distal joint darkly pigmented. *Trumpet:* Without secondary cleft or tragus; rim thin and uniform, without thickened saw-toothed areas. *Abdomen:* Lightly to moderately pigmented, terga III–VII without central dark spot, sterna II–IV darker anteriorly, integument at bases of setae 2–4-II–V, 2-VI, 2,3-VII, and 0-VIII darkly pigmented; length about 3.2 mm. Seta 10-II, or more often its alveolus, usually present; 5-III–VII inserted on intersegmental membrane posterior to tergum; 8-VII inserted on lateral side of fold line; 9-VIII with well-developed branches (12–23). *Paddle:* Lightly pigmented; asymmetrical, outer part larger than inner part; refractile border about 0.6 paddle length; length about 0.70 mm, width about 0.53 mm, index about 1.3. Seta 1-Pa short, with 2–7(4) branches arising at base.

Larva, 4th-stage. (Fig. 3). Character and positions of setae as figured; numbers of branches in Table 2. *Head:* Slightly longer than wide, length about 0.70 mm, width about 0.65 mm; unevenly pigmented, with mottled pattern of moderately dark tanning, collar and dorsomentum darkly pigmented. Seta 2-C single, simple; 3-C dendritic, with 21–39(24) branches arising from distinct basal stem; 4-C small, double or triple; 5,6,8,9-C with relatively few branches, 5-C with 11–16(12), 6-C with 9–16(14), 8-C with 3–8(6), and 9-C with 3–6(4) branches. *Antenna:* Lightly pigmented; mesal and ventral surfaces strongly spiculate; length about 0.27 mm. Seta 1-A moderately long, usually with fewer than 6 branches (1–7, mode 3). *Thorax:* Integument hyaline, smooth. Seta 1-P without setal support plate, single or branched apically; 2-P on

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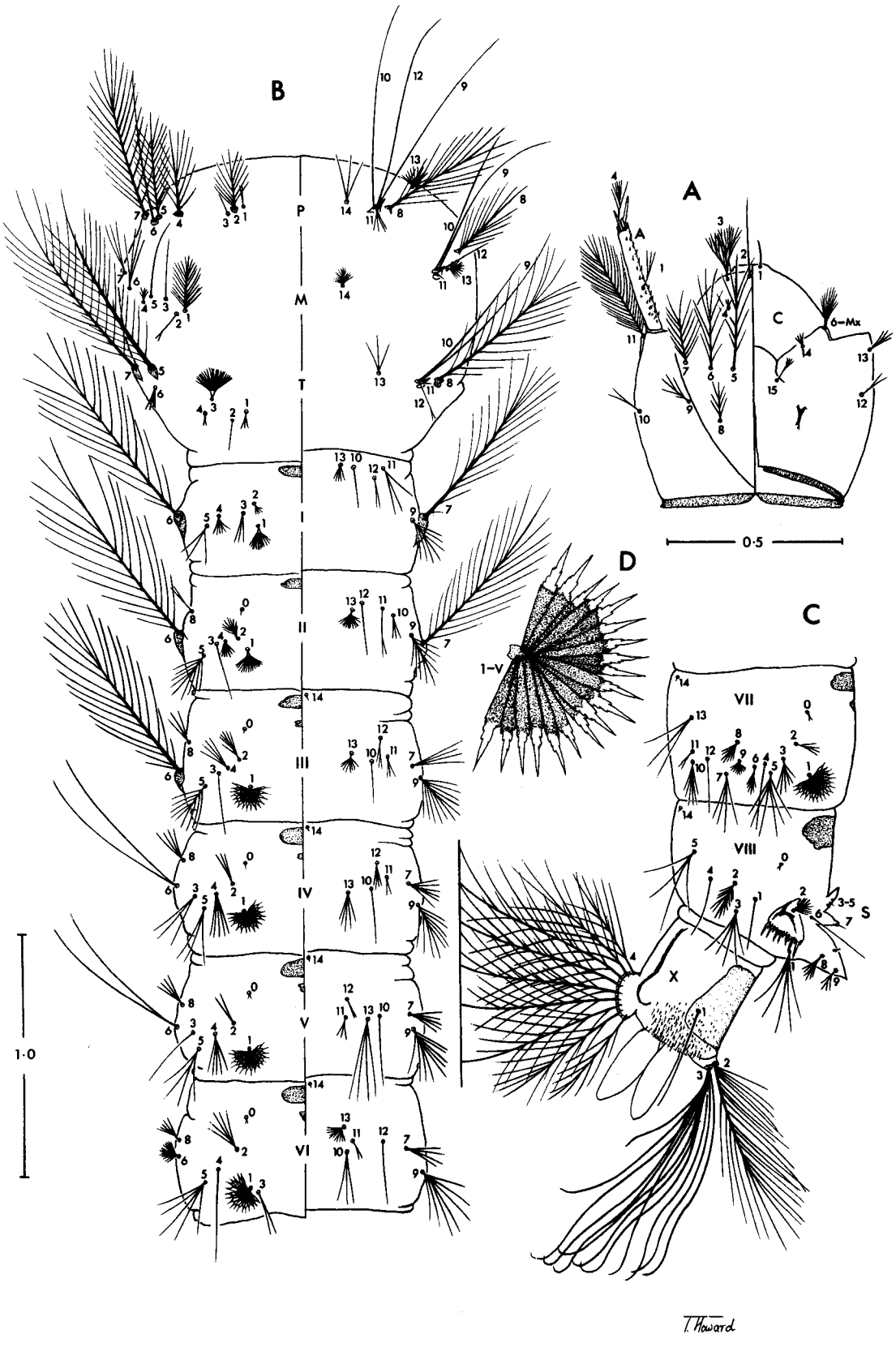
Fig. 2. Pupa and male genitalia of *Anopheles (Anopheles) nimpe*. Pupa: A, left side of cephalothorax, dorsal to right; B, dorsal (left) and ventral (right) aspects of metathorax and abdomen. C, Male genitalia, aspects as indicated. Ae, aedeagus; c, club on dorsal lobe of claspette; Cl, claspette; CT, cephalothorax; Gc, gonocoxite; Gs, gonostylus; InS, internal seta; is, inner seta on ventral lobe of claspette; LAe, leaflets of aedeagus; os, outer seta on ventral lobe of claspette; Pa, paddle; PBS, parabasal setae; I–VIII, abdominal segments I–VIII; IX-Te, tergum IX; 1–14, setal numbers for specified areas, e.g., seta 3-I. Scales in mm.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setal support plate, with 7–17 branches; 11-P significantly larger than 11-M,T, with 3–5(4) branches; support plate of pleural setal group 9–12-P with strong spine; 14-P with relatively few branches (2–5, usually 3); 4-M with 2–5(4) branches arising from central stem, not sinuous; 3-T palmate with simple lanceolate leaflets (not differentiated into a blade with apical filament), with 8–23(15) unpigmented branches. *Abdomen:* Integument hyaline, smooth except for fine spicules ventrally on segments II–VIII; tergal plate of segment VIII roughly hexagonal, with anterior margin wider than posterior margin and greatest width about 1.7 length. Seta 1-I,II similar to seta 3-T, palmate with simple lanceolate leaflets; 1-III–VII fully palmate, leaflets with blades darkly pigmented proximal to shoulders, shoulders not well developed, filaments not pigmented; 6-I most often with fewer than 21 branches (14–28, mode 19), 6-II most often with more than 21 (18–27, mode 23); 5-II usually with fewer than 12 branches (6–12, mode 9); 9-III most often with more than 10 branches (6–13, mode 11); 13-IV intermediate in length between 13-III and 13-V, with 2–11(5) branches and approximately 0.5 length of seta 10-IV. Pecten plate lightly to moderately pigmented, with 7 or 8 long and 8–12(9) small spines. Saddle moderately pigmented, length about 0.65 mm. Seta 1-X slightly longer than saddle.

Molecular characterization. The 472 bp partial sequence for the mtDNA COI fragment sequence comprised the following nucleotide bases: 38.8% T, 29.5% A, 16.5% C, and 15.2% G (see GenBank accession AF216285). Higher insect mitochondrial DNA sequences are unique in their high AT content (Jermiin and Crozier 1994), and the COI fragment for *An. nimpe* (68.2% AT) falls within the range of previously sequenced COI genes of Culicidae, for example, *Anopheles quadrimaculatus* Say L04272 (65.4%) and *An. gambiae* Giles L20934 (68.4%). A FASTA search of available sequences (Pearson and Lipman 1988) revealed closest homology at 90.4% with *An. gambiae* (Beard et al. 1993).

The 504 bp mtDNA 16s rRNA amplicon comprised 40.7% T, 33.7% A, 16.9% G, and 8.7% C nucleotides, with an AT bias of 74.4% (see GenBank accession AF216284). A FASTA search revealed closest homology with *An. quadrimaculatus* (L04272) and *An. gambiae* (L20934), with 97.5% and 96.0% shared bases, respectively.

Etymology. The species is named in recognition of the National Institute of Malariology, Parasitology and Entomology (NIMPE-Hanoi) and its support of mosquito taxonomy in relation to malaria control. For purposes of nomenclature, the specific name *nimpe* is to be regarded as an arbitrary combination of letters without gender.

Systematics. The Hyrcanus Group is an extremely complex assemblage of species that occurs in the southern Palaearctic and Oriental regions, with the majority of species known from China and Southeast Asia. Despite recent studies of the group in Thailand (Harrison and Scanlon 1975), China (Xu Jin-jiang and Feng Lan-chou 1975, Lu Baolin et al. 1997), and Vietnam (Nguyen Duc Manh et al. 1993), present knowledge of the species in eastern Asia is surprisingly inadequate and fragmentary. The group as a whole is distinct in the adult and immature stages, but individual species are often difficult or impossible to distinguish in 1 or more life stages. As currently interpreted, the group includes 28 species: 5 placed in the Lesteri Subgroup, 4 in the Nigerrimus Subgroup, and the other 19, including *An. nimpe,* are unplaced within the group (Harbach 1994). Most species of the last group were described in recent years by Chinese workers without careful study of species that occur outside of China. Needless to say, the taxonomic status and affinities of these nominal species are unclear and the entire group is in need of comprehensive revision. To further complicate the situation, the group undoubtedly includes a number of unrecognized species; for example, we are aware of at least 2 additional new species in Vietnam that require formal description. In order to aid identification, we intend to include keys to the species of the Hyrcanus Group in Vietnam in a later paper that will describe the other new species.

Anopheles nimpe seems to show closer affinities with *An. crawfordi* Reid, *An. lesteri* Baisas and Hu, *An. paraliae* Sandosham, and *An. sinensis* Wiedemann than with other members of the Hyrcanus Group, but it cannot be confused with any of these species in the adult, larval, or pupal stages. Three of these species, *An. crawfordi, An. paraliae,* and *An. sinensis,* are known from southern Vietnam and occur in various degrees of sympatry with *An. nimpe. Anopheles lesteri,* in addition to its presence in China, the Philippines, Okinawa, and Japan (Harrison and Scanlon 1975), is recognized in northern Vietnam (IMPE 1987, unpublished records).

Adults of *An. nimpe* key to *An. crawfordi* in Nguyen Thuong Hien (1968) and to *An. sinensis* in Stojanovich and Scott (1965, 1966); larvae key to *An. sinensis* in the former and to the combination of *Anopheles nigerrimus* Giles and *An. sinensis* in

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Fig. 3. Fourth-stage larva of *Anopheles* (*Anopheles*) *nimpe,* reconstructed from exuviae. A, Head, dorsal (left) and ventral (right) aspects of left side; B, thorax and abdominal segments I–VI, dorsal (left) and ventral (right) aspects of left side; C, abdominal segments VII–X, left side; D, palmate seta 1-V showing pigmentation of leaflets; E, abdominal segments VII–X, left side. A, antenna; C, cranium; P, prothorax; M, mesothorax; S, spiracular lobe; T, metathorax; I–X, abdominal segments I–X; 1–15, setal numbers for specified areas, e.g., seta 5-C. Scales in mm.

Table 2. Range of numbers of branches for 4th-stage larval setae of *Anopheles (Anopheles) nimpe*. Mode in parentheses.

Seta no.	Head	Thorax			Abdominal segments									
	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	S	X
0	—	—	—	—	—	1,2(2)	1,2(2)	1,2(2)	1–3(2)	1–3(2)	1–3(2)	1,2(2)	—	—
1	1	1–4(1)	19–28(24)	1–4(3)	8–24	10–24(19)	13–22(18)	15–22(18)	16–23(18)	14–26(17)	14–26	1,2(1)	3–6(3)	1
2	1	7–17	1,2(2)	1,2(1)	1–8(5)	4–9	3–7(5)	1–3(2)	1,2(2)	1–9(4)	2–8(5)	6–14(12)	4–9(6)	16–26(20)
3	21–39(24)	1	1	8–23(15)	2,3(2)	1,2(1)	1,2(1)	2–4(3)	1	1,2(1)	1–6(4)	5–9	0[1]	9–13
4	2,3(3)	11–19(13)	2–5(4)	1–4(2)	4–8(5)	3–7(5)	2–6(4)	3–5(4)	3–6(5)	1,2(1)	1,2(1)	1	1	9–15
5	11–16(12)	11–29	1	19–31(28)	2–4(3)	6–12(9)	6–10(8)	3,4(3)	3–5(4)	4–7(5)	4–8(6)	3–5(4)	1	—
6	9–16(14)	1	2–5(3)	2–5(3)	14–28(19)	18–27(23)	16–29(23)	2,3(2)	2,3(2)	4–13(6)	5–12(6)	—	1–3(2)	—
7	10–20(18)	16–28(24)	2–5(3)	16–29(23)	14–23(19)	16–26(23)	3–6(3)	3–6(4)	3–5(4)	2–5(3)	2–5(3)	—	1	—
8	3–8(6)	10–29(19)	10–24(12)	18–29	—	2,3(2)	2–4(3)	3–5(4)	3–5(4)	3–7(4)	4–7(6)	—	3–6(4)	—
9	3–6(4)	1	1	1	3–8(6)	5–15	6–13(11)	8–15(11)	5–13(8)	5–8(7)	3–10(4)	—	2–5(4)	—
10	1–3(2)	1	1	1	1	1–4(3)	1–4(1)	1	1–3(1)	2–4(3)	2–6	—	—	—
11	19–40(32)	3–5(4)	1,2(1)	1	2–4(2)	1,2(1)	2–4(3)	2,3(2)	1–4(2)	1–3(2)	1–3(2)	—	—	—
12	1,2(2)	1	1	1–3(2)	2,3(2)	1,2(1)	2–4(3)	2–4	1–3(2)	1	1	—	—	—
13	1–5(1)	6–12(9)	4–13(8)	2,3(3)	3–6(5)	5–12(6)	5–10(7)	2–11(5)	3–5(4)	6–14	2–4(3)	—	—	—
14	2–5(2)	2–5(3)	9–18(11)	—	—	—	1	1	1,2(1)	1–3(2)	1–3(1)	1	—	—
15	1–4(3)	—	—	—	—	—	—	—	—	—	—	—	—	—

[1] Alveolus only.

the latter. No life stage (females, males, larvae, or pupae) key to a terminal species in Harrison and Scanlon (1975). Adults of *An. nimpe* differ from these 3 species, as well as *An. lesteri* and *An. paraliae,* in having the preapical pale spot of the costa and vein R_1 absent or weakly developed. Pupae are easily distinguished from these species by the unique insertion of seta 5-III–VII on the intersegmental membrane, the insertion of seta 8-VII on the lateral side of the fold line, and the distinct development of seta 1-Pa. The larval stage of *An. nimpe* is not as strongly differentiated as the adult and pupal stages, but larvae can be distinguished from those of the other species in having setae 1-A, 8-C, 9-C, and 14-P with fewer branches.

Sequence data for the 16S rRNA gene of *An. nimpe* revealed relatively high homology with distantly related taxa within *Anopheles,* thus suggesting that this gene region has high levels of conservation due to functional or structural constraints. Results of recent studies on *Culicoides* (Linton et al., unpublished) indicated that the COI gene is useful in defining both intra- and interspecific relationships. The COI sequences are widely used and have proven to be informative across a broad range of divergences in insects, for example, tetranychid mites (Navajas et al. 1996) and Coleoptera (Howland and Hewitt 1992; for review see Caterino et al. 2000). Brown et al. (1994) reported high levels of congruence between COI sequence data and morphologic characters in *Greya* (Lepidoptera). In addition, the role of COI as a functional protein coding region, adhering to structural (Saraste 1990) and amino acid constraints (Lunt et al. 1996), provides a solid framework around which to investigate relationships between both closely related and distant taxa. We advocate use of this gene region in any future studies of the molecular systematics of the Hyrcanus Group.

Bionomics. Larvae of *An. nimpe* are found in bodies of brackish water where *Anopheles* (*Cellia*) *sundaicus* (Rodenwaldt) and *An.* (*Cel.*) *subpictus* Grassi also occur. However, larvae of the last 2 species inhabit masses of floating plants (*Ceratophyllum* and *Najas* spp.) exposed to full or partial sunlight, whereas larvae of *An. nimpe* are found among emergent grasses and plants shaded from the sun. Females of *An. nimpe* have been collected biting humans both indoors and outdoors, but they have never been found resting indoors (Nguyen Duc Manh and colleagues, unpublished observations). The species has been incriminated as a secondary vector of malaria by enzyme-linked immunosorbent assay detection of *Plasmodium falciparum* and *P. vivax* in head–thoracic sections of specimens (Đoàn Hanh Nhân 1993, Nguyen Tang Am 1993).

Distribution. *Anopheles nimpe* is only known from coastal areas of southern Vietnam, from the most southerly province of Cà Mau northward to

the Cân Giò, Binh Chánh, and Duyên Hai districts of Ho Chi Minh City.

Material examined. One hundred one specimens (17♀, 15♂, 6♂ genitalia, 32 larval exuviae [Le], 31 pupal exuviae [Pe]) from 32 larval rearings. *Holotype,* ♀ (EP1-1), with LePe on separate microscope slides, VIETNAM: Cà Mau Province, Đâm Dói District, Tan Tiên Commune, Village TK97, 22.x.98 (*Lê Xuân Hói*) (BMNH). *Paratypes,* 6♂ (EP1-2; EP2-1 [used for DNA extraction], -2, -5, -6, -11), 5♀ (EP1-3, -4; EP2-8, -9, -10), 6♂ genitalia, 11Le, 10Pe (EP-11 without Pe), same data as holotype (BMNH); Ho Chi Minh City, Binh Chánh District, Phong Phu Commune, ii.1988 (*Pham Xuân Đinh et al.*), 4♂ (D12, E1, E3 [used for DNA extraction], E6), 3♀ (C12, C27, E2), 7LePe on separate microscope slides (BMNH); 5♂ (B8, C2, C6, D6, D7), 8♀ (B2, B11, C4, C5, C7, C29, D16, E10), 14LePe on separate microscope slides (NIMPE-Hanoi).

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