# Anopheles prachongae, a new species of the Gigas Complex of subgenus Anopheles (Diptera: Culicidae) in Thailand, contrasted with known forms of the complex 

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#### Abstract

Anopheles (Anopheles) prachongae, a new species of the Gigas Complex from northern Thailand, is described and illustrated in the adult, pupal and larval stages, and bionomics and chaetotaxy tables are provided for the immature stages. The species is distinguished from Anopheles baileyi, the only other species of the complex known to occur in Thailand, and contrasted with other taxa of the complex that occur in the Oriental Region. Available morphological data indicate that An. gigas sumatrana is unique and is therefore formally afforded species status. The three other Sumatran subspecies may be conspecific. The taxonomic status of the non-Sumatran subspecies, i.e. crockeri (Borneo), formosus (Philippines), refutans (Sri Lanka) and simlensis (south-central Asia), is questioned but their status is unchanged pending further study.


Key words: Anopheles, baileyi, mosquito, prachongae n. sp., taxonomy, systematics

## Introduction

Giles (1901) described Anopheles gigas from adult males and females collected at an elevation of 6,000 feet ( 1828.8 m ) in the Nilgiri Hills of southwestern India. Between 1909 and 1954, eight "varieties" of An. gigas were described from mountainous localities in the Oriental Region, including formosus Ludlow, 1909 (Philippines), simlensis James, 1911, in James \& Liston (1911) as a species of genus Patagiamyia (India), refutans Alcock, 1913 (Sri Lanka), sumatrana Swellengrebel \& Rodenwaldt, 1932 (Sumatra), danaubento Mochtar \& Walandouw, 1934 (Sumatra), oedjalikalah Nainggolan, 1939 (Sumatra), pantjarbatu Waktoedi Koesoemawinangoen, 1954 (Sumatra) and baileyi Edwards, 1929 (Tibet). Colless (1955) described a subspecies, An. gigas crockeri, from northern Borneo (Sabah, Malaysia). Although the eight nominal varietal forms were treated as subspecies at one time or another in various publications, Stone et al. (1959) and Knight \& Stone (1977) treated them as varieties as originally proposed. Thus, Knight \& Stone (1977) listed 10 forms of An. gigas, including the nominotypical form, eight varieties and one subspecies, in their catalog of the Culicidae. Harrison et al. (1991) subsequently elevated var. baileyi to species status, thus leaving seven formally designated varieties. Applying provisions contained in Article 45 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999) that regulate the rank of species-group names, Harbach \& Howard (2007) showed that the seven varieties must be afforded subspecific status. Hence, prior to this report, the Gigas Complex included two species, An. baileyi and An. gigas; the latter comprising eight geographic subspecies, i.e. danaubento, formosus, gigas, oedjalikalah, pantjarbatu, refutans, simlensis and sumatrana.

Anopheles gigas was recorded in Thailand by Barnes (1923) with uncertainty as variety formosus, by Thurman (1959) as variety sumatrana based on specimens collected between 1950 and 1956, and by Stojanovich \& Scott (1966) as subspecies baileyi, which they included in a list and illustrated key to the Anopheles of Thailand. The first two records were considered doubtful by Peyton \& Scanlon (1966), Scanlon et al. (1968) and Rattanarithikul
\& Harrison (1973). In the absence of specimens for study, the restriction of formosus to the Philippines (Reid, 1968) and the restriction of sumatrana to Sumatra, Indonesia (Bonne-Wepster \& Swellengrebel, 1953; Reid, 1968), Harrison \& Scanlon (1975) excluded gigas from their revision of the Anopheles (Anopheles) of Thailand. Anopheles gigas was listed as absent in Thailand by Apiwathnasorn (1986), but the following year Tsukamoto et al. (1987) recorded the collection of gigas sensu lato on Doi Inthanon, Thailand's highest mountain located in Chiang Mai Province. More detailed study of specimens collected on the same mountain in 1978 and 1981 led Harrison et al. (1991) to identify the taxon as gigas baileyi, which, as noted above, they elevated to species status.

Based on the examination of type specimens of An. gigas s.s., An. baileyi and An. gigas spp. simlensis, ssp. refutans and spp. crockeri in the Natural History Museum, London, review and comparison of published descriptions and the distributions of all forms of the Gigas Complex (references listed above), we recognize and herein formally name and describe a new species of the complex from Thailand. The new species is distinguished, in particular, from An. baileyi, the only other member of the complex known to occur in Thailand.

## Material and methods

Specimens of the new species described below were reared individually from larvae to provide adults with associated larval and pupal exuviae. Adults were studied using stereomicroscopy and emulated natural light (daylight). Larval and pupal chaetotaxy and dissected male genitalia were studied using differential interference contrast optics. Images of wings were taken with a Canon EOS 550D digital camera mounted on a Leica M125 Stereo Microscope and images of the male genitalia and larval and pupal structures were taken with the same type of camera mounted on a Zeiss Axioskop 20 compound microscope; Helicon Focus version 3.03 software (Helicon Soft Ltd, Kharkov, Ukraine) was used to obtain extended-focus images. The morphological terminology used herein is defined in the Anatomical Glossary of the Mosquito Taxonomic Inventory (http://mosquito-taxonomicinventory.info/). The symbols $q, O^{\lambda}, \mathrm{L}$, Le and Pe used in the Specimens examined section represent female(s), male(s), fourth-instar larvae, larval exuviae and pupal exuviae, respectively. Measurements (e.g. length of pupal paddle) and counts (e.g. number of setal branches) are given as a range followed by the mean or mode, respectively, in parentheses.

## Anopheles (Anopheles) prachongae Rattanarithikul \& Harrison, n. sp.

Anopheles n. sp. near An. gigas of Rattanarithikul et al., 2006 (Thailand, $\ell^{*} L^{*}$ keys, larval habitats).
Diagnosis. Anopheles prachongae is very similar to other members of the Gigas Complex. Females resemble the other members in having basal, humeral, presector, subcostal and apical pale spots on the costal vein of the wings (a preapical pale spot is only present on the costa of An. gigas simlensis). Combinations of characters that distinguish females of An. prachongae and the other members of the Gigas Complex are contrasted in Table 1. Morphological differences of the larval and pupal stages are contrasted in Table 2. Features that distinguish An. prachongae and An. baileyi, the only members of the Gigas Complex known to occur in Thailand, are contrasted in Table 3.

Female. A fairly large brown mosquito with conspicuously spotted wings and narrowly banded tarsi. Head: Vertex largely with erect brown scales, with well-marked patch of erect pale scales before interocular space; interocular space with pale setae and narrow pale scales. Antenna about 0.75 length of proboscis; pedicel with mixture of brown and paler scales on mesal, dorsal and lateral surfaces; flagellomere 1 with mesal patch of brown and paler scales, other flagellomeres without scales. Proboscis length about 2.7 mm , about 1.1 length of forefemur, entirely dark-scaled, slightly shaggy in proximal 0.5 , labella also dark. Maxillary palpus slightly shorter than proboscis, dark-scaled with hint of pale scales dorsally at apices of palpomeres 3 and 4 , palpomere 5 entirely darkscaled, palpomeres 1 and 2 particularly shaggy. Thorax: Scutum brown laterally, with broad central pale longitudinal stripe consisting of silvery white tomentum and fine golden setae; anterior promontory with erect brownish pale scales medially; scutellum with golden piliform scales along bases of large golden setae in a complete transverse posterior row. Paratergite, mesopostnotum and postpronotum bare. Antepronotum with golden
TABLE. 1. Comparison of morphological characteristics of adult females of the Gigas Complex. Sumatran forms are highlighted in pale gray. Apparent diagnostic features are in larger boldface type.

| Taxon | Type locality | Maxillary palpus, apex | Wing, costa, basal dark spot | Wing, preapical pale spot | Wing, $\mathrm{R}_{4+5}$ $\mathrm{M}_{3+4}$ pale fringe spots | Wing, pale fringe spot between tips of 1 A and CuA | Wing, pale fringe spot between tips of CuA and $\mathrm{M}_{3+4}$ | Wing, anal vein, distal pale spot | Midfemur, dorsal preapical pale spot |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| An. baileyi | Tibet | Dark | Short | Present on $\mathrm{R}_{1}$ | Absent | Absent | Absent | Absent | Present |
| An. prachongae | Thailand | Dark | Long | Present on $\mathrm{R}_{2}$ | Present | Absent | Absent | Present | Present |
| An. gigas |  |  |  |  |  |  |  |  |  |
| ssp. crockeri | Borneo (Sabah) | Dark | Long | Absent | Present | Present | Absent | Present | Absent |
| ssp. danaubento | Sumatra | Pale | Long | Present on $\mathrm{R}_{1}-\mathrm{R}_{2}$ | Absent | Present | Absent | Present | Absent |
| ssp. formosus | Philippines | Pale | Short | Present on $\mathrm{R}_{2}$ | Absent | Present | Absent | Present | Absent |
| ssp. gigas | India | Dark | Long | Absent | Present | Present | Absent | Present | Absent |
| ssp. oedjalikalah | Sumatra | Pale | Long | Present/absent on $\mathrm{R}_{2}$ | Absent | Present | Absent | Present | Absent |
| ssp. pantjarbatu | Sumatra | ? | ? | ? | ? | ? | ? | ? | ? |
| ssp. refutans | Sri Lanka | Sometimes pale | Long? | Absent? | Absent | Present | Absent | Present? | Absent |
| ssp. simlensis | India | Dark | Short | Present on costa and $\mathrm{R}_{1}$ | Present* | Present | Absent | Absent | Present |
| ssp. sumatrana | Sumatra | Dark | Short | Absent | Absent | Absent | Present | Present | Absent |
| Celebes form | Sulawesi | Pale | Long | Present on $\mathrm{R}_{1}$ | Present | Present | Absent | Present | Absent |

*Christophers (1931) described two forms ( a and b ) of ssp. simlensis (as var. simlensis) in the northern Kashmir region of India. Pale fringe spots $\mathrm{M}_{2}$ and $\mathrm{M}_{3+4}$ are absent in form a, which, as Christophers noted, "...somewhat closely approximates to var. baileyi and many males show a condition of the fringe which is indistinguishable from that in var. baileyi."
TABLE 2. Comparison of distributions and characteristics of pupae and larvae of recognized forms of the Gigas Complex. Sumatran forms are highlighted in pale gray. Apparent diagnostic features are in larger boldface type.

| Taxon | Distribution* | Pupa, seta 5IV.VII | Pupa, paddle, apex | Larva, seta 2-C | Larva, seta 3-C | Larva, seta 4-C | Larva, seta 1-P | Larva, pecten, large spines |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| An. baileyi | Bangladesh, Cambodia?, China, India, Laos, Myanmar, Nepal, Taiwan, Tibet, Thailand, Vietnam | Branched, about as long as seta 1 | Truncate | Single, $\approx 0.3$ length of head | Long, single | Usually single (1,2) | $4-10$ <br> branches | 8-11(9) |
| An. prachongae | Thailand | Branched, about as long as seta 1 | Emarginate | Single, $\approx 0.3$ length of head | Long, branched | Usually double (1-3) | $3-8$ <br> branches | 5-7(6) |
| An. gigas |  |  |  |  |  |  |  |  |
| ssp. crockeri | Sabah, Malaysia | Branched, markedly longer than seta 1 | Emarginate | Single, long, > 0.5 length of head | Long, 1-3 branches, usually at tip | Stout, usually single or bifid at tip | $4-6$ <br> branches | ? |
| ssp. danaubento | Sumatra | ? | ? | ? | Long, branched | 0.5-0.7 length of 3-C, single or few branches | $7-9$ <br> branches | ? |
| ssp. formosus | Philippines | Branched, distinctly shorter than seta 1 | Emarginate | Single, occasionally split into two | Long, single or 2-6 branches | $\begin{aligned} & \approx 0.5 \text { length of } 3-C, \\ & 2-8 \text { branches } \end{aligned}$ | $5-10$ <br> branches |  |
| ssp. gigas | India | ? | ? | Single | Long, 2-6 branches | $\approx$ length of 3-C, single, sometimes branched | $3-8$ <br> branches | 6-8 |
| ssp. oedjalikalah | Sumatra | ? | ? | ? | Long, branched | $\approx 0.5$ length of $3-C$, single or weakly branched | $7-9$ <br> branches | ? |
| ssp. pantjarbatu | Sumatra | ? | ? | ? | $?$ | $\approx 0.5$ length of 3-C, 3 or 4 branches) | $8-10$ <br> branches | ? |
| ssp. refutans | Sri Lanka | Single, almost as long as seta 1 | ? | ? | Long, usually 2 or 3 branches, sometimes single | ? | ? | ? |
| ssp. simlensis | Bangladesh, China, India, Nepal, Pakistan | Branched, about as long as seta 1 | Slightly convex? | Single | Long, 2-5 apical branches, may be single | $\begin{aligned} & \approx 0.5 \text { length of } 3-C, \\ & 2-5 \text { branches } \end{aligned}$ | ? | ? |
| ssp. sumatrana | Sumatra | ? | ? | Single | Short, stout, forked | 3-branched | 8 branches | ? |
| Celebes form | Sulawesi | ? | ? | Single, sometimes forked or 3branched at tip | Long, $\approx 0.8$ <br> length of 2-C, <br> with 3-5 distal <br> branches | $\approx 0.5$ length of $3-\mathrm{C}, 3$ or 4 branches | $4-6$ <br> branches | 5 or 6 |

[^0]setae and cluster of brown scales among setae on dorsoanterior surface. Pleura with brown and pale horizontal bands, upper brown band on postspiracular area, upper mesokatepisternum and upper mesepimeron, median brown band on subspiracular area, area between upper and lower mesokatepisternal setae and mid to lower area of mesepimeron, lower brown band at level above coxae extending across mesokatepisternum, mesotrochantin, mesomeron and metameron; pleura without scales, with golden setae as follows: 9 upper proepisternal, 9 prespiracular, 11 or 12 prealar, 7 upper and 6 lower mesokatepisternal and 13-20 upper mesepimeral. Wing (Fig. 1A): Length $4.8-5.1 \mathrm{~mm}(\bar{X}=4.9 \mathrm{~mm})$, width $1.1-1.2 \mathrm{~mm}(\bar{X}=1.2 \mathrm{~mm})$; humeral crossvein without scales; pale scaling creamy white to yellow, dark scaling dark brown to nearly black, especially on costa, subcosta and vein $\mathrm{R}_{1}$. Pattern of dark and pale spots as shown in Fig. 1A (cf. wing of An. baileyi in Fig. 1B); costa with long basal dark spot (about 0.3 mm ), short humeral pale spot, long presector pale spot and distinct subcostal and apical pale spots; preapical pale spot absent on costa and vein $R_{1}$, weakly to distinctly developed on vein $R_{2}$; veins $R_{1}$ and $R_{2}$ with apical pale spots adjoining apical pale spot on costa; apices of veins $R_{4+5}, M_{1}$ and $M_{2}$ with pale scales adjoining pale fringe spots, vein $M_{3+4}$ with few inconspicuous apical pale scales and sometimes an adjoining faint pale fringe spot; pale fringe spot absent between apices of veins 1 A and CuA and apices of veins CuA and $\mathrm{M}_{3+4}$; vein 1 A with long pale spot on distal 0.5 . Halter: Integument of scabellum pale; pedicel and capitellum dark-scaled. Legs: Mainly dark-scaled; coxae pale with pale setae; femora narrowly pale at base and less so at apex, midfemur with small dorsal preapical pale spot; tibio-tarsal and tarsal joints (i.e. apices and bases of tarsomeres) with narrow pale bands, pale scales less distinct or absent at bases of tarsomeres 4 and 5, tarsomere 5 pale at tip. Abdomen: Terga brown, sterna paler except basomedially; scales absent, setae golden.


FIGURE 1. (A) Right wing of a female of Anopheles prachongae; (B) right wing of a female of An. baileyi. 1A, anal vein; BD, basal dark spot; CuA, anterior cubitus; $M_{1}, M_{2}, M_{3+4}$, medial veins; $R_{1}, R_{2}, R_{3}, R_{4+5}$, radial veins.

Male. Similar to female except as follows. Head: Proboscis longer, $2.8-3.3 \mathrm{~mm}(\bar{X}=3.1 \mathrm{~mm}), 1.4-1.6$ length of forefemur. Maxillary palpus with dark brown and light yellow scales; with pale band at junction of palpomeres 2 and 3, apex of palpomere 3 with long dark scales and tuft of long golden setae on either side of mesal surface; club constricted between palpomeres 4 and 5 ; lateral surface of palpomere 4 largely pale-scaled, mesal surface with dense covering of long golden setae; proximal 0.25 and distal 0.5 of palpomere 5 pale-scaled, setae rather inconspicuous. Wing: Scaling of veins posterior to radius reduced; preapical pale spot fully developed on costa and veins $\mathrm{R}_{1}$ and $\mathrm{R}_{2}$, increasingly longer from costa to vein $\mathrm{R}_{2}$. Genitalia (Fig. 2C): Tergum IX not strongly sclerotized,
relatively indistinct, lateral lobes widely separated by very narrow interlobar bridge, bridge nearly obsolete in middle. Gonocoxite with 2 parabasal setae at base of dorsomesal surface (pre-rotation sense), lateral parabasal long, relatively slender and acutely tapered, mesal parabasal 0.67 length of lateral parabasal, stout, distally flattened and apically hooked; lateral and ventral surfaces of gonocoxite with numerous very long setae (about length of gonocoxite), much shorter setae on dorsomesal and ventromesal surfaces (shorter than width of gonocoxite), dorsolateral surface with long slender scales among long setae, scales longer toward apex of gonocoxite, apices of some scales distinctly truncate; internal seta on middle of ventromesal surface similar but slightly thinner and distinctly longer than lateral parabasal seta, occasionally 2 internal setae present; gonostylus long, about 1.3 length of gonocoxite, slender, curved, slightly broader at base, ventromesal margin with complete line of minute setae, proximal 0.3 of ventrolateral surface with patch of minute spicules; gonostylar claw short, pigmented, inserted on dorsal side of apex, gonostylus with a short seta inserted proximal to base of claw. Ventral lobe of claspette with 3-6 relatively long simple setae; dorsal lobe of claspette with 4 flattened mesally curved setae. Aedeagus distinct, strongly sclerotized, length about 0.11 mm , bearing 5 or 6 pairs of apical leaflets, longest leaflet 0.025 mm , edge of leaflets without serration or serration unapparent; proctiger membranous, highly aculeate; ventrolateral paraprocts faintly sclerotized but distinctly demarcated.

TABLE 3. Salient anatomical differences that distinguish the adults, pupae and fourth-instar larvae of Anopheles prachongae and An. baileyi.

| Character | An. prachongae | An. baileyi |
| :--- | :--- | :--- |
| Adults |  |  |
| Wing, costa, basal dark spot | Long | Short |
| Wing, vein $R_{4+5}$, apex | Pale | Dark |
| Wing, $\mathrm{R}_{4+5}$ pale fringe spot | Present | Absent |
| Wing, vein $\mathrm{M}_{1}$, apex | Pale | Dark |
| Wing, $\mathrm{M}_{1}$ pale fringe spot | Present | Absent |
| Wing, vein $\mathrm{M}_{2}$, apex | Pale | Dark |
| Wing, Vein 1 A (anal vein) | Long pale spot on distal 0.5 | Entirely dark-scaled |

## Male genitalia

| Tergum IX | Indistinct, weakly sclerotized | Distinct, strongly sclerotized |
| :--- | :--- | :--- |
| Tergum IX, interlobar bridge | Very narrow | Broad |
| Aedeagus, length | $\sim 0.10 \mathrm{~mm}$ | $\sim 0.15 \mathrm{~mm}$ |
| Aedeagus, leaflets, length | $25 \mu \mathrm{~m}(0.025 \mathrm{~mm})$ | $45 \mu \mathrm{~m}(0.045 \mathrm{~mm})$ |

## Pupae

Setae 10,11-II
Seta 9-VI
Paddle, apex

| Absent | Usually present |
| :--- | :--- |
| Normally straight | Normally bent |
| Emarginate | Truncate |

## Fourth-instar larvae

Seta 3-C
Seta 12-T
Seta 1-II
Seta 1-III-VII, sum of branches on one side
Pecten, large spines
Seta 1-X, insertion
Anal papillae, length

3-6(3) branches
Single
1-5(4) branches
83-119 ( $\bar{X}=110)$
5-7(6)
In notch at edge of saddle
$0.80-1.41 \mathrm{~mm}$ ( $\bar{X}=1.04 \mathrm{~mm}$ )

Single
2,3(2) branches
6-11(6) branches
$122-148(\bar{X}=131)$
8-11(9)
On margin of saddle
$0.55-0.65 \mathrm{~mm}(\bar{X}=0.60 \mathrm{~mm})$

Pupa (Fig. 2A,B). Character and positions of setae as figured; numbers of branches in Table 4 (cf. chaetotaxy of An. baileyi in Table 5). Cephalothorax: Lightly to moderately pigmented, with patches of darker pigmentation especially around bases of maxillary palpi, lateral areas of scutum on either side of median keel and metathoracic wings. Maxillary palpus of female (length $\sim 0.85 \mathrm{~mm}$ ) shorter than palpus of male (length about 1.0 mm ). Setae normally all single, setae $1,5,7,8-\mathrm{CT}$ occasionally split at apex; seta 7-CT short, about same length as seta 6-CT. Trumpet: Laticorn, moderately pigmented, large, transverse length from meatal cleft to apex of pinna $0.46-0.55 \mathrm{~mm}$ ( $\bar{X}=0.53 \mathrm{~mm}$ ), without secondary cleft and tragus, rim thin and uniform, without tracheoid area at base. Abdomen: Length $3.5-4.2 \mathrm{~mm}(\bar{X}=4.0 \mathrm{~mm})$; lightly to moderately pigmented with variable patches of darker pigmentation especially on anterolateral areas of terga. Seta 0-II-VIII single, inserted anterior and slightly mesad of seta 2; seta 1-III-VII shorter than following tergum, 1-II,IV usually multi-branched, 1-V (usually) and 1-VI,VII single, 1-IX minute, usually double ( $1-3$ branches); seta 5-IV-VII branched, nearly as long as seta 1 ; seta 7-VI distinctly longer than 7-VII (also in An. baileyi); seta 8-III-VII inserted on mesal side of fold line; seta 9-II-VII progressively longer and transforming from peg-like and straight to acutely spine-like and inwardly bent on succeeding posterior terga, generally shorter than corresponding seta in An. baileyi (Table 5), 9-VIII with strong central stem and 15-17(15) lateral branches. Genital lobe: Moderately tanned; male - length about 0.5 mm ; female - length about 0.3 mm , distal and lateral margins of ventral surface distinctly spiculate. Paddle: Lightly pigmented, buttress and base of midrib slightly darker; asymmetrical, outer part larger than inner part, apex emarginate; length $0.93-1.21 \mathrm{~mm}(\bar{X}=1.10 \mathrm{~mm})$, width $0.66-0.82 \mathrm{~mm}(\bar{x}=0.75 \mathrm{~mm})$, index $1.39-1.54(\bar{X}=1.46)$, outer margin with minute serration extending approximately $0.4-0.7$ from base; refractile border about 0.7 length of paddle, proximal part without serration. Seta 1Pa inserted at base of apical emargination, single, generally stiff and straight, length $<0.1$ length of paddle length; seta 2-Pa minute, inconspicuous.

Larva, fourth-instar (Fig. 3). Character and positions of setae as figured; numbers of branches in Table 6 (cf. chaetotaxy of An. baileyi in Table 7). Head: Length $\sim 0.8 \mathrm{~mm}$, very slightly wider than long; lightly pigmented with variable darker patches around bases of setae 5,6-C and posteriorly on dorsal apotome and lateralia; collar and dorsomentum darkly pigmented. Seta 2-C close-set, separated at base by less than width of alveolus, long, single; seta 3-C shorter than 2-C, with 3-6(3) short branches on distal 0.5 ; seta 4-C small, usually 2-branched at mid-length (1-3); setae 5-8,11,13-C plumose; seta 9-C not plumose, with 3-6(6) branches (usually 5 or 6). Antenna: Lightly pigmented; entirely spiculate; length $0.32-0.36 \mathrm{~mm}(\bar{x}=0.34 \mathrm{~mm})$. Seta 1-A inserted about 0.3 from base of antenna, relatively long, about 0.35 length of antenna, with $5-10(8)$ branches; seta $4-A$ longer than seta $1-\mathrm{A}$, about 0.45 length of antenna, with 5-12(6) branches. Thorax: Integument hyaline, smooth. Seta 1-P branched distally, with 3-8(5) branches; seta 2-P somewhat plumose, with 8-12(10) branches, borne in sclerotized tubercle; setae 1,3-P not borne on tubercles; setae 4,5,7,8,14-P, 1,8,14-M and 5,7,8-T plumose; seta 6-M large, usually 2- or 3-branched (1-5), branches arise well beyond base; seta 7-M often 2-branched (1-3) near mid-length; seta 3-T with 5-9(7) slender branches; setae $9,10,12-\mathrm{T}$ single; seta13-T usually double or triple, sometimes single. Abdomen: Integument hyaline; ventral surface of all segments with minute spicules. Anterior tergal plates on segments I-VIII, about 0.2 width of segment, distinctly smaller on segment II and notably larger on segment VIII; median accessory tergal plates present on segments III-VII, present or absent on segment II; submedian accessory tergal plates absent. Seta 1-I,II not palmate, similar to seta 3-T but 1-II with longer main stem and $1-5(4)$ branches, $1-\mathrm{III}-\mathrm{VII}$ fully palmate (Fig. 3), with 33-54 leaflets, leaflets entirely dark, acuminate, with smooth or weakly notched edges, without shoulders and filament; setae 6,7-I,II and 6III large and plumose, 6-IV,V long, 6-IV usually 2-branched (1-3), 6-V normally single, occasionally double, 6-VI usually single ( $1-4$ branches), about 0.5 length of 6 -IV,V. Pecten plate moderately pigmented, with $5-7(6)$ long spines and $12-15(14)$ short spines (in pattern of variable numbers of alternating large and small spines), total number of spines 18-22(19). Saddle lightly pigmented with darker borders and relatively strong spicules on posterolateral margins, length $0.39-0.43 \mathrm{~mm}(\bar{X}=0.40 \mathrm{~mm})$. Seta 1-X longer than saddle, inserted in notch at edge of saddle; seta 3-X with apically hooked branches; seta $4-X$ (ventral brush) with 9 offset pairs of setae with branches arising noticeable distance from grid, most anterior of $4 \mathrm{a}-\mathrm{X}$ about 0.6 length of longest setae ( $4 \mathrm{c}-\mathrm{g}-\mathrm{X}$ ), with 11-13 branches, most posterior of 4iX shorter, $0.4-0.5$ length of longest setae, with $5-8$ branches, setae $4 \mathrm{~b}-\mathrm{h}-\mathrm{X}$ with $11-15$ branches, longest branches $0.6-0.7$ length of seta. Anal papillae very long, about 3 times length of saddle, $0.80-1.41 \mathrm{~mm}(\bar{X}=10.4 \mathrm{~mm})$.

Etymology. We are very pleased to name this species in honor of our good friend and colleague Mrs. Prachong Panthusiri, who produced an extraordinary number of beautiful mosquito illustrations for numerous publications while working for the Southeast Asian Treaty Organization (SEATO) and the successional Armed Forces Research Institute of Medical Sciences (AFRIMS) laboratories in Bangkok.
TABLE 4. Numbers of branches for setae of pupae of Anopheles prachongae ( 5 specimens). Modes in parentheses.

| $\begin{aligned} & \hline \text { Seta } \\ & \text { No. } \end{aligned}$ | Cephalothorax <br> CT | Abdominal segments |  |  |  |  |  |  |  |  | Paddle <br> Pa |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | I | II | III | IV | V | VI | VII | VIII | IX |  |
| 0 | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - | - |
| 1 | 1 | $\sim 80$ | 1-3(2) | 1-5(4) | 2-5(3) | 1,2(1) | 1 | 1 | - | 1-3(2) | 1 |
| 2 | 1,2(1) | 2,3(3) | 4,5(4) | 3,4(3) | 2 | 2 | 2 | 2 | - | - | 1 |
| 3 | 1 | 1-4(2) | 1,2(1) | 1 | 1-3(2) | 1 | 1 | 1 | - | - | - |
| 4 | 1 | 2,3(3) | 1-3(3) | 1-4(2) | 1-3(2) | 1,2(2) | 1 | 1 | 1 | - | - |
| 5 | 1,2(1) | 2,3(2) | 2,3(3) | 4-6(5) | 4-9(5) | 4-6(5) | 3-7 | 3-5(3) | - | - | - |
| 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2(1) | - | - | - |
| 7 | 1,2(1) | 1-3(2) | 1,2(1) | 1-3(2) | 1-3(2) | 1,2(2) | 1 | 1 | - | - | - |
| 8 | 1,2(1) | - | - | 1,2(2) | 1,2(1) | 1,2(1) | 1,2(1) | 1-3(1,2) | - | - | - |
| 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 15-17(15) | - | - |
| 10 | 1,2(1) | - | - | 1 | 1 | 1 | 1,2(1) | 1 | - | - | - |
| 11 | 2-4(2) | - | - | 1,2 | 1 | 1 | 1 | 1 | - | - | - |
| 12 | 2-4(3) | - | - | - | - | - | - | - | - | - | - |
| 13 | - | - | - | - | - | - | - | - | - | - | - |
| 14 | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | - | - |

TABLE 5. Numbers of branches for setae of pupae of Anopheles baileyi (4 specimens). Modes in parentheses.

| $\begin{aligned} & \hline \text { Seta } \\ & \text { No. } \end{aligned}$ | Cephalothorax CT | Abdominal segments |  |  |  |  |  |  |  |  | Paddle <br> Pa |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | I | II | III | IV | V | VI | VII | VIII | IX |  |
| 0 | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - | - |
| 1 | 1,2(2) | $\sim 70$ | 3-5(3) | 2-6(3) | 2,3 | 1 | 1 | 1 | - | 2,3(3) | 1 |
| 2 | 1,2(1) | 3-5(3) | 3,4(4) | 3-5(3) | 1-3(2) | 2 | 2 | 1,2(2) | - | - | 1 |
| 3 | 1,2(2) | 1-3(1) | 1,2(1) | 1,2 | 3,4 | 1,2(1) | 1 | 1,2(1) | - | - | - |
| 4 | 1,2(2) | 3,4(3) | 2,3(2) | 2,3(3) | 1-4(1,3) | 1,2(2) | 1 | 1 | 1 | - | - |
| 5 | 1-3(2) | 1,2(2) | 2-4(3) | 4-7(6) | 4,5(5) | 3-6(6) | 4-7(6) | 4-7(5) | - | - | - |
| 6 | 1 | 1,2(1) | 1,2 | 1-3(2) | 1 | 1 | 1 | 1,2(2) | - | - | - |
| 7 | 1,2(1) | 1,2(2) | 1,2(2) | 2-5(2) | 2-4(2,3) | 1,2 | 1 | 1,2)1) | - | - | - |
| 8 | 1,2(1) | - | a-2* | 1-3(2) | 1,2 | 1 | 1,2(1) | 1-3(2) | - | - | - |
| 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 11-15 | - | - |
| 10 | 1-4(1) | - | $0,2(2) \dagger$ | 2 | 1 | 1 | 1 | 1,2(1) | - | - | - |
| 11 | 2,3(3) | - | 0-2(1) $\ddagger$ | 1,2(1) | 1 | 1 | 1 | 1 | - | - | - |
| 12 | 2,2(2) | - | - | - | - | - | - | - | - | - | - |
| 13 | - | - | - | - | - | - | - | - | - | - | - |
| 14 | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | - | - |

[^1]

FIGURE 2. (A-C, E) Anopheles prachongae: A, pupa, left side of cephalothorax, dorsal to right; B, pupa, dorsal (left) and ventral (right) aspects of metathorax and abdomen; C, male genitalia, dorsal (tergal) aspect (note presence of two internal seta on left gonocoxite); E, apex of paddle. (D) Apex of paddle of An. baileyi. Ae, aedeagus; CL, claspette; CT, cephalothorax; Gc, gonocoxite; Gs, gonostylus; InS, internal seta; LAe, leaflets of aedeagus; Pa, paddle; PBS, parabasal setae; Ppr, paraproct; Pr, proctiger; RB, refractile border; I-IX = abdominal segments I-IX; 1-14 = setal numbers for specified areas, e.g. seta 3-I.


FIGURE 3. Fourth-instar larva of Anopheles prachongae. 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. A, antenna; APP, anal papilla; C, cranium; P, prothorax; PP, pecten plate; Pt, pecten; M, mesothorax; MAPT, median accessory tergal plate; S, spiracular lobe; Sa, saddle; T, metathorax; TP, tergal plate; $\mathrm{I}-\mathrm{VIII}, \mathrm{X}=$ abdominal segments I-VIII and $\mathrm{X} ; 1-15=$ setal numbers for specified areas, e.g. seta $5-\mathrm{C}$.

Bionomics. Like the other forms of the Gigas Complex, An. prachongae is a montane species. The immature stages which gave rise to the type series (see below) were collected at an elevation $1,420 \mathrm{~m}$. Immature stages have been collected from stream-pool, stream-margin, seep or seepage-spring and rock-hole habitats (Rattanarithikul et al., 2006).

Distribution. Anopheles prachongae is only known from its type locality in the Phetchabun Mountain Range in Loei Province of northern Thailand, bordering Sainybuli and Vientiane Provinces of Laos. The Phetchabun Range consists of two parallel mountain chains in Chaiyaphum, Loei, Phetchabun and Phitsanulok Provinces of Thailand. The chains are a southern extension of the Luang Prabang Range that mainly encompasses Sainyabuli Province of northwestern Laos and Nan and Uttaradit Provinces of northern Thailand. Elevations above 1,000 m are covered by evergreen forest, to the height of the highest mountain in the range, Phu Soi Dao, with an altitude of $2,120 \mathrm{~m}$. Since the type specimens of An. prachongae were collected at an altitude of $1,420 \mathrm{~m}$, the species is likely to be found in ecologically similar areas within the Luang Prabang Range.

Specimens examined. Anopheles prachongae-Holotype, of (TH 976-27), with LePe on microscope slide: THAILAND, Loei Province, Phu Luang District, Huai Pong ( $17^{\circ} 7^{\prime} \mathrm{N} 101^{\circ} 32^{\prime}$ E), elevation 1,420 m, 11 Jan 1989, stream pool, coll. Rampa et al. Paratypes, same data as holotype: $1 q$ (TH 968-12) with LePe on microscope slide; $2 \sigma^{\AA}$ (TH 970-11; TH 976-36) both with LePe and dissected genitalia on separate microscope slides; 1才 (TH 97652) with LePe on microscope slide; 2L (TH 970-C; TH 976-D) on individual microscope slides. The type series is deposited in the National Museum of Natural History (USNM), Smithsonian Institution, Washington, DC. Anopheles baileyi-2 2 (TH 161-31; TH 161-63) with LePe on microscope slides, $1 \delta^{\wedge}$ (TH 161-46) with LePe and dissected genitalia on separate microscope slides, $1 \delta$ (TH 161-111) with Pe and dissected genitalia on separate microscope slides; 1L (TH 161-D) on microscope slide: THAILAND: Chiang Mai Province, Amphoe Chom Tong, Doi Inthanon, sphagnum bog ( $18^{\circ} 35^{\prime} \mathrm{N} 98^{\circ} 29^{\prime} \mathrm{E}$ ), elevation 2,540 m, 23 Apr 1981, coll. Harrison; 1L (TH 214-I) on microscope slide, same locality as TH 161, 30 Mar 86, coll. Rampa \& team. These specimens are also deposited in the USNM.

## Discussion

Members of the Gigas Complex occur in mountainous areas in the Oriental Region, the Manchurian Subregion of the Palaearctic Region and the Austro-Malayan Subregion of the Australasian Region. All forms of the complex are recorded from altitudes at or usually above 1000 m (Christophers, 1933; Kundig, 1934; Bonne-Wepster \& Swellengrebel, 1953; Colless, 1955). Most of the subspecies of An. gigas are known only from specific or limited geographic areas, and whether all are truly allopatric and morphologically distinct is unknown. Their true status cannot be determined until significantly more material is available from many localities for comparative study, especially the larval and pupal stages. At present, the currently recognized forms are distinguished principally on the presence and absence of pale markings on the wings of adult females (Table 1), and to a lesser extent on a few select setal characters of the known larval and pupal stages (Table 2). Based on the data shown in Tables 1, 2 and 3, we observe that An. baileyi, An. gigas and An. prachongae are distinct species; however, it is unlikely that all of the Sumatran forms of An. gigas (highlighted in pale gray in Tables 1 and 2), known only from their type localities, represent separate subspecies. There seems little doubt that An. gigas sumatrana is a distinct entity as it is the only form of the complex in which larval seta 3-C is short, stout and forked and the wings of the adults have a pale fringe spot between the tips of veins CuA and $\mathrm{M}_{3+4}$ (characters highlighted in grey in Tables 1 and 2). In fact, based on these two unique (diagnostic) characteristics, as well as the combination of three other characters of females, i.e. apex of maxillary palpus dark, pale fringe spot present between the apices of veins CuA and $\mathrm{M}_{3+4}$ and absent between the apices of veins 1 A and CuA , we are compelled to hereby formally recognize sumatrana as a distinct species of the complex. On the other hand, the available data do not support the separate subspecific status of danaubento, oedjalikalah and pantjarbatu. Further collection and study of Sumatran specimens may reveal that these three nominal forms represent a single entity. If this proves to be the case, then danaubento has priority over the other two names.

We also question the taxonomic status of the other subspecies of An. gigas, which exhibit distinct combinations of morphological characters (Table 1, except simlensis which is diagnosed by the presence of a preapical pale spot on the costa) and have, as far as known, allopatric distributions (Table 2): crockeri occurs in Borneo, formosus in
the Philippines, refutans in Sri Lanka and simlensis in northern areas of south-central Asia. It is noteworthy that Christophers (1931) encountered two forms of simlensis in the northern Kashmir region of India based on the presence/absence of fringe spots at the apices of veins $M_{2}$ and $M_{3+4}$ (see Table 1 and Christophers, 1933). The nominotypical member of the Gigas Complex, currently denoted as subspecies An. gigas gigas, occurs in southwestern India. The combination of morphological data and allopatric isolation would seem to support the recognition of these nominal taxa as separate species, and the unnamed form in Sulawesi, the Celebes form of Kundig (1934), could also be a distinct species, but it seems prudent to continue to rank them as subspecies pending further study, with emphasis on the immature stages. It must be emphasized that An. gigas, i.e. the nominotypical member of the complex, will remain a valid species regardless of what discoveries are made concerning the status of the geographical forms that are currently recognized as subordinate taxa of this species.

Speciation of montane fauna can take place across elevation gradients and within or between mountain ranges (e.g. Willmott et al., 2001; Hall, 2005). Species that have evolved to utilize habitats at cooler higher elevations generally have fragmented distributions; thus, many montane species are endemic to certain mountain ranges. This suggests that the evolutionary history of the Gigas Complex has been shaped primarily by upward adaptation to higher elevations. If so, it seems plausible that the ancestor of the Gigas Complex adapted to the cooler temperate conditions of mountainous terrain prior to the repeated formation and disappearance of land bridges between the mainland and islands of Southeast Asia that occurred during the Pleistocene, from 0.01-2.6 million years ago (mya). The increasing isolation and adaptation of populations to climatic conditions of higher altitudes resulted in restricted gene flow, or the cessation of gene flow between forms confined to islands. The problem with this scenario is that there are no species obviously related to the Gigas Complex at elevations below 1000 m , but perhaps members of the complex have undergone little morphological differentiation while ancestral forms diversified at lower elevations, which is connoted by pre-Pleistocene speciation events within subgenus Cellia during the early and mid-Pliocene from 3.2-4.5 mya (Morgan et al., 2009). It is interesting to note that Reid (1968) suggested subgenus Cellia may have arisen from an ancestral form that resembled a species like Anopheles gigas. Similarities in the ornamentation of the wings and legs suggest that members of the Gigas Complex may be nearer to the ancestral form that gave rise to subgenus Cellia.

As noted in the description, males of An. prachongae occasionally have two internal setae on the ventromesal margin of the gonocoxite (see left gonocoxite in Fig. 2C). This unusual condition is noteworthy because as far as known only a single internal setae is present in other species of subgenus Anopheles.

This is the first time that the larval and pupal stages of members of the Gigas Complex have been studied in detail, and fully illustrated in the case of the new species described above. The information presented herein will hopefully set the stage for further study and changes in the taxonomy of the Gigas Complex. Many potentially useful larval and pupal characters, as well as finer details of the male genitalia, remain to be investigated in most members of the complex, but it seems likely that such characters, especially if coupled with DNA sequence data, are needed to elucidate and delimit independent lineages within the complex.

Finally, we take this opportunity to point out that the mosquito fauna of Thailand is more completely known than for any other tropical country. In 2010, 459 species were known to occur in Thailand (Rattanarithikul et al., 2010). Since then a total of seven new species, including An. prachongae, have been described from Thailand. The six other species include An. (Cellia) rampae, a species in the Maculatus Group (Somboon et al. 2011); Nyctomyia pholeocola, a cavernicolous species of tribe Aedini (Harbach et al. 2013), originally placed in genus Nyx, preoccupied (Harbach, 2013); Nc. biunguiculata (Harbach \& Taai (2014); An. dissidens, An. saeungae and An. wejchoochotei, three species in the Barbirostris Complex of subgenus Anopheles (Taai \& Harbach (2015). This brings the total number of species known to occur in Thailand to 466 -remarkably representing $13 \%$ of the currently recognized species of Culicidae.

## Acknowledgements

We gratefully acknowledge the following individuals who participated in the field trip that resulted in the collection of specimens examined during this study: Kol Mongkolpanya (deceased), Chamnong Noigamol, Somporn Chanaimongkol, Pradith Mahapibul and Dr. Ronald Rosenberg, formerly of AFRIMS.

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[^0]:    *Based on assessment of available taxonomic literature, with careful consideration of information contained in composite descriptions of An. gigas s.l.

[^1]:    *Alveolus only or single or 2-branched seta.
    $\ddagger$ Usually present; usually single when present.

