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Keys to the Anopheline Mosquitoes (Diptera: Culicidae) of Pakistan

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J. Med. Entomol. 39(1): 28–35 (2002)

ABSTRACT This article provides taxonomic keys for the identification of the fourth-instar larvae and females of 24 species of anopheline mosquitoes (seven species in subgenus *Anopheles* and 17 species in subgenus *Cellia*) recorded from Pakistan. The keys are based on literature sources as well as the examination of field and museum collections.

KEY WORDS *Anopheles*, females, larvae, keys, Pakistan

THE MOSQUITO FAUNA of Pakistan is poorly studied. Modern taxonomic descriptions and keys to species of the major genera found in that country are lacking, with the exception of regional keys to female *Anopheles* (Glick 1992) that include 20 species, and keys and descriptions to adult and immature life stages of subgenus *Culex* (Harbach 1980) that include 10 species from Pakistan. Despite malaria being an important public health problem, there are no recently published keys to all the known anopheline mosquitoes of that country. Ansari and Shah (1950) described species in the Punjab, and Talibi and Qureshi (1956) described those in the area that includes present-day Pakistan and Bangladesh. Aziz et al. (1988) published keys to 15 species of female *Anopheles* in the Islamabad-Rawalpindi area. Copies of an unpublished pictorial key to female anophelines of Pakistan developed by Bashir Ahmad (a senior technician at the malaria entomology unit in Lahore) are still available. To the authors' knowledge, keys to the larval stages have never been developed for the Pakistani anopheline fauna.

A checklist of mosquitoes published by Aslamkhan (1971) includes 22 species, two subspecies and one variety within the land area presently known as Pakistan. Several nomenclatural changes have occurred since the publication of that checklist. *Anopheles habibi* Mulligan & Puri is now regarded as a junior synonym of *An. claviger* (Meigen) (Glick 1992). *Anopheles maculatus* ssp. *willmori* (James) is regarded as a separate species (Rattanarithikul and Green 1987). Also, Aslamkhan (1971) listed *An. gigas* Giles, *An. gigas simlensis* (James), *An. stephensi*, and *An. stephensi mysorensis* Sweet & Rao in Pakistan. However, Glick (1992), following Christophers (1933) and Reid (1968), recognized only *An. gigas simlensis*, and did not recognize subspecies within *An. stephensi*, which occurs in several morphological "forms" (Sweet and Rao 1937, Subbarao et al. 1987) whose taxonomic status is unclear.

The need for up-to-date taxonomic keys hardly needs to be emphasized, with the resurgence of malaria worldwide. Although *An. culicifacies* s.l. Giles is acknowledged as the major vector in Pakistan, the status of other anophelines in relation to malaria transmission is uncertain. Hussain (1951) lists *An. culicifacies* s.l. as the main vector in the Punjab, North West Frontier Province (NWFP), Sind, Baluchistan and the Karachi area, with *An. stephensi* Liston as a suspected vector in these areas and *An. superpictus* Grassi as a suspected vector in Baluchistan and parts of the NWFP. Hussain and Talibi (1956) and Rahman and Muttalib (1968) incriminated *An. stephensi* as a malaria vector in periurban areas of Karachi, but Mahmood et al. (1984) and Mahmood and MacDonald (1985) concluded that *An. stephensi* and *An. subpictus* s.l. Grassi would be unlikely vectors in rural Pubjab owing to low survivorship. Aziz et al. (1988) state that no proper studies to incriminate vectors have been done in Pakistan, and thus species such as *An. stephensi*, *An. fluviatilis* s.l. James, *An. annularis* s.l. van der Wulp, *An. subpictus* s.l., *An. superpictus*, and *An. pulcherrimus* Theobald are still suspected to be vectors in different parts of the country. Recent studies by Rowland et al. (1997, 2000) reinforce a concern raised earlier by Pervez and Shah (1988) that *An. stephensi* plays a more important role in transmission than previously suspected in Pakistan.

Because morphotaxonomy is the basis on which routine malaria vector surveillance and control operations rest, we thought it timely to produce keys to the females and fourth-instar larvae of anopheline mosquitoes in Pakistan to assist malaria control personnel and others interested in research on local mosquitoes.

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Materials and Methods

Based on the existing literature, the following 24 species are recognized from Pakistan.

Subgenus *Anopheles: barbirostris* s.l. van der Wulp, *barianensis* James, *claviger* (Meigen), *gigas simlensis* (James), *lindesayi* Giles, *nigerrimus* Giles, and *peditaeniatus* Leicester.

Subgenus *Cellia: annularis* s.l. van der Wulp, *culicifacies* s.l. Giles, *dthali* Patton, *fluviatilis* s.l. James, *maculatus* s.l. Theobald, *moghulensis* Christophers, *multicolor* Cambouliou, *pallidus* Theobald, *pulcherrimus* Theobald, *sergenti* (Theobald), *splendidus* Koizumi, *stephensi* Liston, *subpictus* s.l. Grassi, *superpictus* Grassi, *theobaldi* Giles, *turkhudi* Liston, and *willmori* (James).

Morphological characters used in the keys are based on observation of specimens and previous usage in published literature. Specimens (adults, larval exuviae with associated adults, and preserved larvae) in Pakistan were examined from the south Punjab and Northwest Frontier Province areas in 1999–2000. Material from western Asia and the Oriental Region deposited in the British Museum of Natural History was examined in August 2000. Immature stages of all except *An. willmori,* and adults of all anopheline species recorded in Pakistan were examined during the study. Reference sources consulted during the preparation of the keys were Christophers (1933), Mattingly and Knight (1956), Du Bose and Curtin (1965), Reid (1968), Harrison and Scanlon (1975), Ramachandra Rao (1984), Glick (1992), and Amerasinghe (1990, 1992).

The terminology used in the keys follows Harbach and Knight (1980). Figs. 1 and 2 illustrate morphological characters of larval and adult anopheline mosquitoes as an aid to using the keys.

I. Key to Fourth-Instar Larvae

Abbreviations include the following: A, antenna; C, cranium; P, prothorax; M, mesothorax; T, metathorax; I–X, abdominal segments.

1. Seta 2-C inserted at least as far apart as distance between 2-C and 3-C on one side; seta 1-A always single, setae 5–7-C branched (subgenus *Cellia*) 2
   Seta 2-C inserted close together, closer than distance between 2-C and 3-C on one side; setae 1-A and 5–7-C branched; if seta 1-A single, then setae 5–7-C reduced, single (subgenus *Anopheles*) 16

2(1). Tergal plates on abdominal segments III-VII very large, ≥0.5 width of segment, and enclosing median accessory tergal plate; thorax with long pleural setae 9-P,T branched, all other long pleural setae single. . . . . *fluviatilis* s.l.
   Tergal plates on abdominal segments III-VII smaller, < 0.5 width of segment, and not enclosing median accessory tergal plate; thoracic pleural setae 9-P,T and other long pleural setae various 3

3(2). Thorax with long pleural setae 9,10,12-P and 9,10-M single or occasionally bifid; 9,10-T branched; seta 3-C ≥0.5 length of 2-C; seta 4-C nearly as long as 3-C, placed posteriorly and wide apart . . *subpictus* s.l.
   Thorax with at least pleural seta 9-P,T branched; 9,10-M and 10-T single or branched 4

4(3). Thorax with long pleural setae 9-P,T branched; 10-T single 5
   Thorax with long pleural setae 9-P,T and 10-T branched 7

5(4). Seta 4-C much shorter than 2-C; setae 1,2-P with equally prominent basal tubercles; seta 9-M simple; tips of setae 2,3-X branches various 6
   Seta 4-C as long as 2-C; seta 1-P with smaller basal tubercle than 2-P; seta 9-M distinctly branched; tips of setae 2,3-X branches hooked . . . . . *sergentii*

6(5). Seta 4-C distinctly shorter than 3-C; basal tubercles of 1,2-P close together; tips of seta 2-X branches not hooked, those of seta 3-X hooked (Fig. 1c) . *culicifacies* s.l.
   Seta 4-C as long as 3-C; basal tubercles of seta 1,2-P well separated; branches of setae 2,3-X with distinctly hooked tips . *dthali*

7(4). Setae 9,10-M branched; abdominal palmate seta 1-III weak, 1-IV–VI strong (Fig. 1b, inset), filaments of palmate leaflets very short and blunt; seta 1-VII not palmate . . . . . *turkhudi*
   Seta 9-M branched, 10-M simple; seta 1-III-VII palmate, with leaflets and filaments variably developed 8

8(7). Seta 3-C with long lateral branches, brush-like; abdominal seta 1-I palmate, well developed, with obvious leaflets 9
   Seta 3-C single or with a few short lateral branches, not brush-like; abdominal seta 1-I not-palmate or very weakly palmate 10

9(8). Seta 8-C single, sometimes bifid distally . . . . . *annularis* s.l.
   Seta 8-C split near base into 2–10 branches. . . . . *pallidus*

10(8). Basal tubercles of setae 1,2-P joined; seta 3-T distinctly palmate . . . . . *moghulensis*
   Basal tubercles of setae 1,2-P separate; seta 3-T not-palmate or very weakly palmate. 11

11(10). Setae 2,3-C single, smooth (Fig. 1a, inset); anal papillae reduced to stumps; filaments of abdominal palmate setae ≥0.67 length of blade . . . . . *multicolor*
   Setae 2,3-C single, finely or distinctly frayed; anal papillae normal; filaments of abdominal palmate setae ≤0.5 length of blade 12

12(11). Setae 3,4-C each with 3–6 branches . . . . . *pulcherrimus*

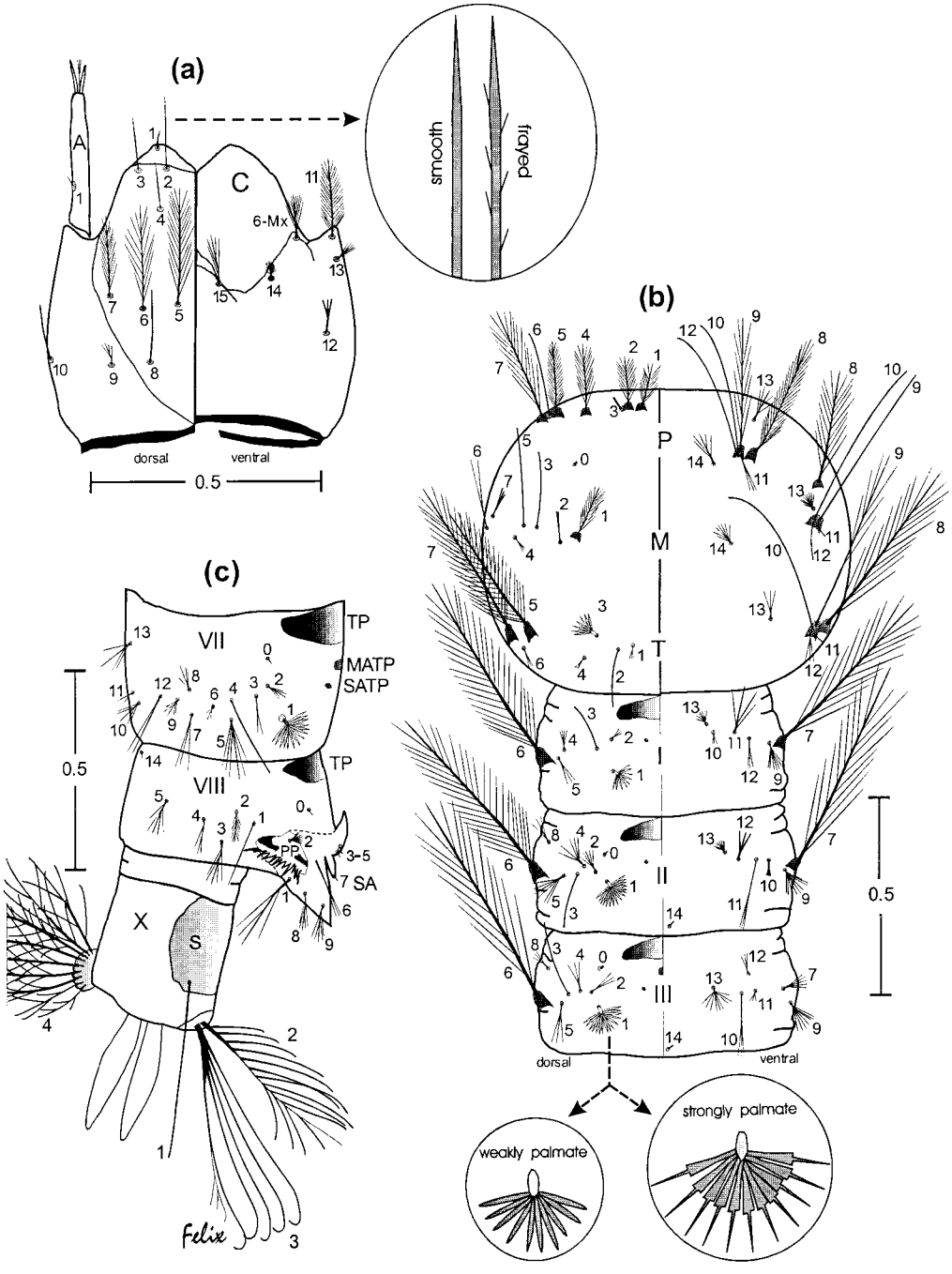

Fig. 1. Anopheline larval chaetotaxy (based on the fourth-instar larva of *An. culicifacies*). (a) Dorsal (left) and ventral (right) aspects of head. (b) Dorsal (left) and ventral (right) aspects of thorax and abdominal segments I-III. (c) Lateral aspect of segments VII-X. A, antenna; C, cranium; M, mesothorax; MATP, median accessory tergal plate; P, prothorax; PP, pecten plate; S, saddle; SA, spiracular apparatus; SATP, submedian accessory tergal plate; T, metathorax; TP, tergal plate; I-III, VII, VIII, X, abdominal segments.

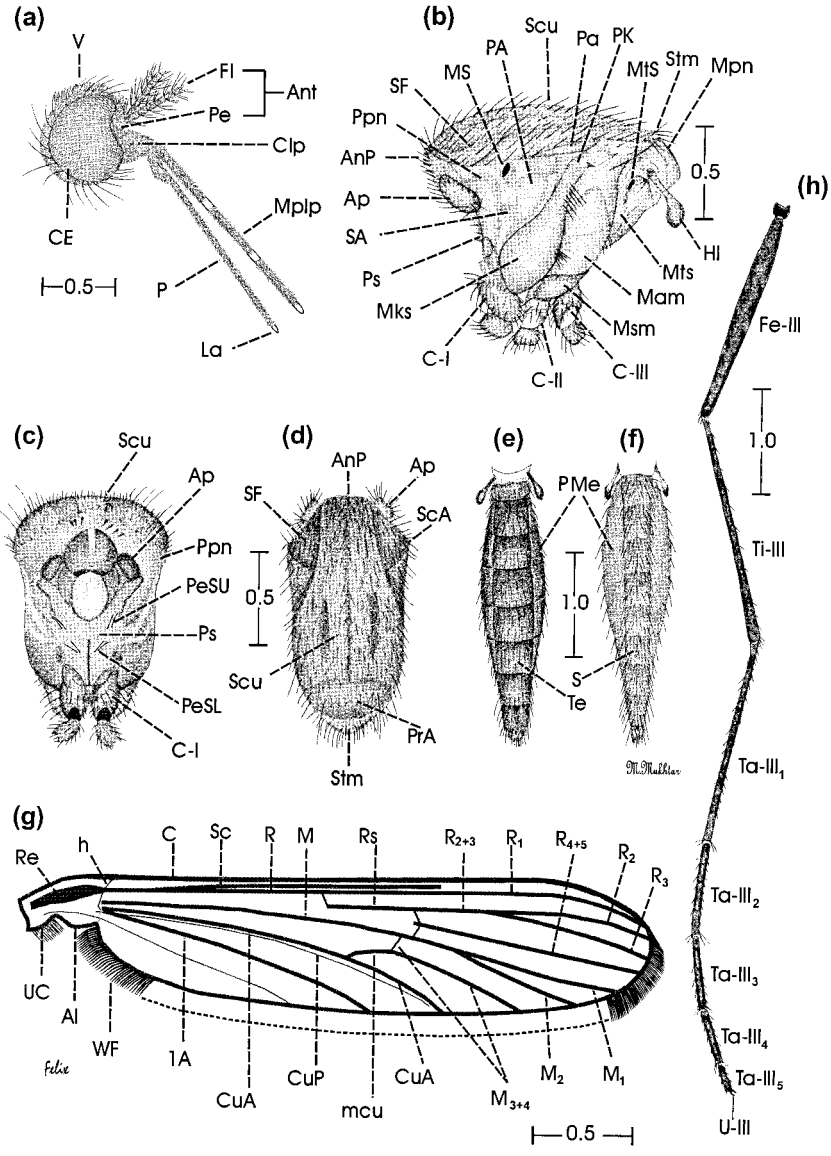

Fig. 2. Anopheline adult morphology. (a) Lateral view of head. (b) Lateral view of thorax. (c) Front view of thorax with head removed. (d) Dorsal view of thorax. (e) Dorsal view of abdomen. (f) Ventral view of abdomen. (g) Outline of wing with venation. (h) Lateral view of hindleg. 1A, anal vein; Al, alula; AnP, anterior promontory; Ant, antenna; Ap, antepronotum; C, costal vein; CE, compound eye; Clp, clypeus; CuA, anterior cubitus; CuP, posterior cubitus; C-I, coxa of foreleg; C-II, coxa of midleg; C-III, coxa of hindleg; Fe-III, hindfemur; Fl, flagellum; h, humeral crossvein; Hl, halter; La, labelum M, media; Mam, mesanepimeron; mcu, mediocubital crossvein; Mks, meskatepisternum; Mplp, maxillary palpus; Mpn, mesopostnotum; MS, mesothoracic spiracle; Msm, mesomeron; MtS, metathoracic spiracle; Mts, metepisternum; M1, media-one; M2, media-two; M3+4, media-three-plus-four; P, proboscis; PA, postspiracular area; Pa, paratergite; Pe, pedicel; PeSL, lower proepisternal seta; PeSU, upper proepisternal seta; PK, prealar knob; PMe, pleural membrane; Ppn, postpronotum; PrA, prescutellar area; Ps, proepisternum; S, sternum; SA, subspiracular area; Sc, subcosta; ScA, scutal angle; Scu, scutum; SF, scutal fossa; Stm, scutellum; Re, remigium; R, radius; Rs, radial sector; R1, radius-one; R2, radius-two; R3, radius-three; R2+3, radius-two-plus-three; R4+5, radius-four-plus-five; Ta-III$_1$ - Ta-III$_5$, hindtarsomeres 1–5; Te, tergum; Ti-III, hindtibia; U-III, hindunguis; UC, upper calypter; V, vertex; WF, wing fringe.

Setae 3,4-C single or occasionally bifid, never multiple branched 13
13(12). Setae 2,3-C smooth or finely frayed (Fig. 1a, inset); abdominal seta 1-II strongly palmate (Fig. 1b inset) 14
Setae 2,3-C distinctly frayed; abdominal seta 1-II weakly palmate 15
14(13). Seta 3-C smooth; seta 4-C length ≤ 3-C; basal tubercle of seta 1-P well developed. . . . . *stephensi*
Seta 3-C finely frayed; seta 4-C length >3-C; basal tubercle of seta 1-P weak . . . . . *superpictus*
15(13). Setae 2,3-C slender . . . . . *splendidus*
Setae 2,3-C stout . . . . . *maculatus* s.l. . . . . *theobaldi* . . . . . *willmori*
16(1). Branches of seta 1-A not extending much beyond middle of antennal shaft, whole seta usually <0.5 length of shaft (Angusticorn section) 17
Branches of seta 1-A extend close to or beyond the end of antennal shaft, whole seta usually >0.5 length of shaft (Laticorn section) 20
17(16). Seta 1-A single; setae 2–4-C single; setae 5–7-C minute, single; abdominal setae 5 and 9 branched, spinous . . . *barianensis*
Seta 1-A branched, setae 2–4-C variably developed; setae 5–7-C large, branched; abdominal setae 5 and 9 not spinous 18
18(17). Extremely large larva (7–10 mm); seta 8-C multi-branched; seta 1-II not-palmate. . . . . *gigas*
Smaller larva (<7 mm); seta 8-C single or bifid; seta 1-II strongly palmate 19
19(18). Seta 4-C branched; seta 3-T not-palmate . . . . . *claviger*
Seta 4-C single; seta 3-T distinctly palmate. . . . . *lindesayi*
20(16). Seta 1-P with long branches from near base (Barbirostris Group); seta 3-C with 20–95 thick branches, broom-like; seta 1-II palmate, darkly pigmented. . . . . *barbirostris* s.l.
Seta 1-P single or with 2–5 short branches near tip; seta 1-II palmate, unpigmented (Hyrcanus Group) 21
21(20). Seta 4-m on both sides of larva with sinuate, spreading branches arising close together at base . . *peditaeniatus* (Note 1)
Seta 4-m on both sides of larva with stiff, erect branches arising along stem . . . . . *nigerrimus* (Note 1)

II. Key to Females

Abbreviations include the following: 1A, anal vein; C, costa; CuA, anterior cubitus; mcu, mediocubital crossvein; R, radius.

1. Wing veins entirely dark-scaled 2
Wing veins with contrasting pale and dark areas at least on veins C and R-R_1 . . . 3
2(1). Lower proepisternal seta present (Fig. 2c); scutum with very narrow, piliform, pale scales on median area; pale knee spots absent or indistinct on femora and tibiae. . . . . *claviger*
Lower proepisternal seta absent; scutum with narrow to moderately broad pale scales on median area; distinct pale knee spots present on femora and tibia, especially on hindleg . . . . . *barianensis*
3(1). Leading margin of wing with at least four separate dark areas involving either or both veins C and R-R_1 (subgenus *Cellia*) . 4
Leading margin of wing with <4 separate dark areas involving either or both veins C and R-R_1 (subgenus *Anopheles*) . . . 21
4(3). Femora and tibiae with pale-scaled spots 5
Femora and tibiae without pale-scaled spots 10
5(4). Hindtarsomeres 3–5 entirely white-scaled. 6
Hindtarsomeres 3–5 not entirely white-scaled 7
6(5). Maxillary palpus with four pale bands; abdominal terga densely covered with broad pale scales and prominent posterolateral dark scale-tufts on all segments . . . . . *pulcherrimus* (in part) (Note 2)
Maxillary palpus with three pale bands, the two apical bands broad and basal band more narrow; abdominal terga with narrow scales . . . . . *splendidus*
7(5). Hindtarsomere 5 entirely dark. . . . . *stephensi* (Note 3)
Hindtarsomere 5 entirely pale 8
8(7). Hindtarsomere 4 entirely pale . *theobaldi*
Hindtarsomere 4 pale at base and apex, dark at center 9
9(8). Abdominal terga II-VIII largely covered by pale scales, with small dark scale-tufts on posterolateral corners of terga VII and VIII, occasionally on IV and V . *willmori*
Abdominal terga with pale scales at most on V-VIII, occasionally on IV, dark scales on posterolateral corners of VII or VIII or both, rarely on VI, but no tufts . . . . . *maculatus* s.l.
10(4). Hindtarsomeres 3–5 entirely white-scaled. 11
Hindtarsomeres 3–5 not entirely white-scaled 13
11(10). Maxillary palpus with four pale bands; abdominal terga densely covered with broad pale scales and prominent posterolateral dark scale-tufts on all segments . . *pulcherrimus* (in part) (Note 2)

Maxillary palpus with three pale bands; abdominal terga with narrow pale scales and dark posterolateral or apical scales only on distal segments 12

12(11). Wing extensively dark-scaled; vein CuA mostly dark, with dark scales at origin of mcu; pale band on apical 0.25 or less of hindtarsomere 2 . . . . . *annularis* s.l.

Wing paler-scaled; vein CuA mostly pale-scaled, without dark scales at origin of mcu; pale band on apical 0.33–0.50 of hindtarsomere 2 . . . . . *pallidus*

13(10). Wing with pale spots confined to veins C and R-R_1; erect head scales narrow, straw-colored; scutum with seta only, no scales . . . . . *dthali*

Wing with pale spots present on most veins; erect head scales broad, pale on vertex and dark brown laterally and posteriorly; scutum with obvious pale scales in addition to seta 14

14(13). Palpomere 5 dark at apex 15

Palpomere 5 pale at apex, sometimes entirely pale 16

15(14). Scutal fossa with scattered pale scales; base of vein C pale-scaled; vein 1A with three small dark spots or with the outer 2 spots joining to form long distal dark spot . . . . . *multicolor*

Scutal fossa without scales, or few scales at extreme upper margin; base of vein C dark; vein 1A with two dark spots, distal spot long, entire vein often appearing mostly dark-scaled; wing spots sometimes indistinct on veins posterior to R-R_1 . . . . . *turkhudi*

16(14). Scutum with narrow pale scales on median area; upper proepisternal seta present (Fig. 2c) 17

Scutum with broad pale scales on median area; upper proepisternal seta absent . 20

17(16). Scutal fossa with scattered pale scales; foretarsomeres with pale bands crossing the joints; hindtarsomeres 3 and 4 pale at apex; vein 1A with two small dark spots. . . . . *subpictus* s.l. (Note 4)

Scutal fossa without scales; foretarsomeres dark or with only narrow apical bands not crossing joints; hindtarsomeres 3 and 4 dark; vein 1A with proximal small and distal long dark spots 18

18(17). Vein R_{4+5} with distinct large median pale area; wing fringe with at least four pale spots on posterior margin . . . . . *fluviatilis* s.l.

Vein R_{4+5} dark-scaled except at base and apex; wing fringe various 19

19(18). Vein R with dark spot just distal to humeral crossvein; wing fringe usually with one or two inconspicuous pale spots on posterior margin, rarely more . . . . . *culicifacies* s.l. (Note 5)

Vein R without basal dark spot just distal to humeral crossvein; wing fringe usually with at least four pale spots on posterior margin . . . . . *sergentii*

20(16) Vein 1A with three dark spots . . . . . *moghulensis*

Vein 1A with two dark spots, distal spot long . . . . . *superpictus*

21(3). Basolateral area of clypeus without scales. 22

Basolateral area of clypeus with patch of dark, laterally projecting scales 24

22(21). Veins C and R-R_1 entirely dark except at apex; maxillary palpus entirely dark; hindfemur with broad subapical pale band; hintarsomeres 4 and 5 entirely dark . . . . . *lindesayi*

Veins C and R-R_1 with at least one pale spot or scattered pale scales before apex; maxillary palpus with pale bands; hindfemur without broad subapical pale band; hindtarsomeres 4 and 5 with narrow pale bands 23

23(22). Veins C and R-R_1 with three distinct pale areas before apex; at least three pale spots on wing fringe, the last between veins CuA and 1A broad; abdomen without scales . . . . . *gigas*

Veins C and R-R_1 with scattered pale scales on small spots on one or the other vein, but not both; only two distinct fringe spots; abdominal sterna with median pale-scale patches and scattered lateral pale scales . . . . . *barbirostris* s.l.

24(21). Humeral crossvein with patch of dark scales; remigium mostly dark-scaled; pale bands on hindtarsomeres 4 and 5 variable, often absent . . . . . *nigerrimus* (Note 6)

Humeral crossvein bare; remigium mostly pale-scaled; pale bands on hindtarsomeres 4 and 5 present, often crossing joints . . . . . *peditaeniatus* (Note 6)

Notes

1. The major character for separating larvae of *An. peditaeniatus* from *An. nigerrimus* is the condition of seta 4-M. Other characters, such as the branching of seta 8-C (4–9 *in An. peditaeniatus* versus 12–24 in *An. nigerrimus*) and seta 9-C (3–7 in *An. peditaeniatus* versus 8–14 in *An. nigerrimus*) given in keys to Sri Lankan anophelines (Amerasinghe 1992) are unreliable, there being considerable overlap in branching ranges in the Pakistani material examined. Some preserved larval material from the Punjab has seta 4-M in an intermediate condition (i.e., stiff branches originating from the base, or different branching characteristics on the two sides of the same larva) to that seen in typical *An. peditaeniatus* or *An. nigerrimus.* The status of this material is

presently unclear and needs further investigation (see Note 6).

2. The majority of *An. pulcherrimus* females have unspotted femora and tibiae, but occasional specimens with spotted legs are encountered.
3. Based on egg dimensions and the number of ridges on the egg float, three forms of *An. stephensi* have been recognized. They are the "type" form, the form "*mysorensis*," and an "intermediate" form (Sweet and Rao 1937, Subbarao et al. 1987). In India, all three forms occur in periurban areas, whereas only the intermediate and *mysorensis* forms occur in rural areas (Subbarao et al. 1988). Within Pakistan, the *mysorensis* form dominates in Karachi and surrounding villages (Afridi et al. 1958), but the varietal status of *An. stephensi* elsewhere in the country is currently unknown. Nalin et al. (1985) cite evidence showing that in areas such as Karachi (Pakistan) and Salem, Tamil Nadu (India), where *An. stephensi* is a malaria vector, its populations possess chromosomal inversions, whereas nonvector populations from Lahore (Pakistan) and Pondicherry (India) do not possess these inversions. It is not known how the chromosomal inversion patterns relate to varietal status.
4. *Anopheles subpictus* consists of at least four sibling species (A, B, C, and D) (Suguna et al. 1994) that cannot be reliably separated on morphological characters. It is still unknown which species of this complex occur in Pakistan.
5. *Anopheles culicifacies* consists of at least five morphologically indistinguishable sibling species (A, B, C, D, and E) (Kar et al. 1999). Sibling species A and B occur in rural Punjab, with sibling A (the likely major malaria vector) predominating (Mahmood et al. 1984, Mahmood and MacDonald 1985). The identity of populations in other areas of Pakistan is unknown.
6. One of the characters used in other keys (e.g., Amerasinghe 1990, Harrison and Scanlon 1975) to differentiate populations of Southeast Asian *An. peditaeniatus* and *An. nigerrimus* is that in *An. peditaeniatus* the basal 0.33 and preapical area on vein R-R_1 is mainly pale-scaled, whereas it is mainly dark-scaled in *An. nigerrimus.* In specimens from Pakistan examined by us, however, these areas were mainly dark-scaled in *An. peditaeniatus* as well. Glick (1992) and Harrison and Scanlon (1975) also point out that the extent of pale banding on the hindtarsomeres is variable in *An. peditaeniatus,* and Glick (1992) suggests that early records of *An. nigerrimus* from Pakistan may in fact refer to *An. peditaeniatus.* These two species belong to the Southeast Asian segment of the *hyrcanus* species group and are closely related to the nominate species, *An. hyrcanus* (Pallas) and other described Palaearctic forms. The geographic separation of these two segments of the *hyrcanus* group in the west apparently runs north from the Indian Ocean up through Baluchistan to the Afghanistan mountains and east to the Himalaya mountains (Harrison and Scanlon 1975). Thus, it is possible that members of the Palaearctic segment of this group also may occur in Pakistan.

Acknowledgments

We thank Mohammad Abdur Rab (Healthnet International, Peshawar, Pakistan) for providing specimens of adult anophelines for examination; Kapila Fonseka, Sarath Lionelrathne, Tipu Naveed, Tariq Mehmood, Tariq Nazir, and Asim Munawar for field collections in the Punjab Province, Pakistan; and Ralph E. Harbach (Department of Entomology, The Natural History Museum, London, UK) for making available the museum collections for examination. We also thank two anonymous reviewers for suggestions that helped to improve the manuscript.

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Received for publication 22 January 2001; accepted 26 April 2001.