## Anopheles (Cellia) nemophilous, a New Species of the Leucosphyrus Group from Peninsular Malaysia and Thailand (Diptera: Culicidae)

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**ABSTRACT**. Anopheles (Cellia) nemophilous, a new species of the Leucosphyrus Group from Peninsular Malaysia and Thailand, is described and illustrated in the adult, pupal and larval stages. The species is contrasted with Anopheles dirus and introlatus, two morphologically similar species. The zoogeography and biology of the species is discussed. The Dirus Complex is characterized in the adult stage.

**INTRODUCTION.** Although the species Anopheles (Cellia) nemophilous is formally described and named here, it is by no means a newly discovered form for the Leucosphyrus Group. Colless (1956), in his classic review of the group, briefly described what he believed to be a distinct geographic variant of *leucosphyrus* Doenitz from a small series of specimens (7 females, 10 larval and pupal skins from Central Malaysia and 2 females from "other areas") and assigned to it the vernacular name "Fraser's Hill form". At the same time he described the "Kepong form" from the same general region, suggesting that it was "quite distinct" from the "Fraser's Hill form", yet quite similar in the larval stage and that pending further investigation both were treated as "presumed hybrids" (as a possible result of zones of hybridization between leucosphyrus leucosphyrus and leucosphyrus balabacensis Baisas). Colless (1957) reporting on considerable new study material, elevated leucosphyrus balabacensis to "specific rank" and redescribed the "Kepong form" as balabacensis introlatus Colless. However, since no new material of the "Fraser's Hill form" was available to him, he continued to call it a form, but this time he was less certain of its affinities with leucosphyrus because of his obvious strong feelings about its close affinity with balabacensis and the new subspecies introlatus. Nevertheless, he suggested that the "Fraser's Hill form" might be a distinct altitudinal subspecies or species, or a phenotypic variant produced by growth at high altitude and that further material would be required before any formal recognition could be granted. Reid (1968) also recognized the "Fraser's Hill form" and said that it appeared to be a mountain variant of balabacensis introlatus, but noted some significant morphological differences in both the adult and pupal stages and that additional material was needed to determine "whether the two were distinct".

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The theory of a high altitude variant was based on the small number of collections then available to Colless and Reid. According to Reid (1968), the common Central Malaysian form (*introlatus*) occurred up to about 1,000 ft. and the "Fraser's Hill form" had been encountered only at 3,000 and 4,000 ft. Based on our study of the many collections and specimens reported here, there is no basis for this theory except for a reduction in the pale basal band on hindtarsomere 4 which is sometimes absent on specimens from Fraser's Hill. However, too few specimens are available from this area to evaluate this character. Specimens from all other localities including high elevations in Thailand always show a clear pale band. While it is clear from the available evidence that *nemophilous* has been found at considerably higher elevations in Malaysia and Thailand (1,220 and 1,500 m) than that recorded for any other member of the Leucosphyrus Group, it has also been collected commonly in peninsular Thailand at much lower levels between 100 and 550 m without showing signs of variation any greater than the others.

For several years we have been aware that the "Fraser's Hill form" of Colless (1956) represented a distinct species on purely morphological grounds. This was based on a long term critical study of the material reported here at the Walter Reed Biosystematics Unit (WRBU). Both of us have personally collected or directed the collecting by others of considerable study material of this species including reared adults with associated immature skins from Fraser's Hill in central Peninsular Malaysia and many new sites in Thailand dating from 1964 to 1969. Additional collections from Thailand were obtained through the special efforts of Bruce A. Harrison and Ralph E. Harbach formerly of the U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, who led teams on special trips in 1979, 1980 (BAH) and 1987 (REH) to the southern peninsular province of Phangnga (Thailand) for collecting the "Fraser's Hill form" for this study and to obtain material for colonization and other studies at the AFRIMS and Mahidol University, Bangkok. More recently, Visut Baimai, Mahidol University, a long term collaborator with the WRBU and AFRIMS on the systematics of the Leucosphyrus Group, sent cytotype vouchers of his dirus, species F (=FHF) from Padang Besa, southern Thailand (see distribution). Throughout this period we have referred to this species as the Fraser's Hill Form (FHF) and many collection records and specimens in the WRBU collections bear the designation "FHF". However, for the past two years or more we have also referred to the species as dirus, species F since we now consider the species to be a member of the currently recognized Dirus Complex. The use of a letter designation was more compatible with letter designations used by collaborators at the AFRIMS and Mahidol University (Baimai et al. 1984, 1988a, 1988b). We emphasize this point for historical value, since many references to these species are made in correspondence, reports, manuscripts and on specimen labels which are permanent records.

We agreed to collaborate on describing this species as early as 1980, but in view of the considerable apparent controversy and confusion created by the earlier description of *dirus* Peyton and Harrison (1979), and the subsequent description and elevation of *takasagoensis* Morishita to species status by Peyton and Harrison (1980), we agreed to postpone the description of this and other known but undescribed species of the Dirus Complex until cytogenetic and hybridization studies on each were completed at the AFRIMS and Mahidol University. These studies have now been accomplished (Baimai et al. 1988a). This is the first of several papers which will describe morphologically, the species of the Dirus Complex referred to in Baimai et al. (1984, 1988a, 1988b).

In a preliminary reference to this complex, Peyton and Harrison (1980) called it the Balabacensis Complex, partly because of convention and partly because of an incomplete knowledge of several other species of the Leucosphyrus Group and an incomplete understanding of the complexities of the group that were yet to be discovered in a more comprehensive study of the Thailand fauna by one of us (ELP). The vernacular name "Dirus Complex" is now becoming widely used (op. cit.) without definition other than an implied similarity-resemblence to dirus Peyton and Harrison. Since this is of considerable significance to a better understanding of the systematics of the complex as well as the entire Leucosphyrus Group, and since it is desirable to seek stability in the present inconsistent use of terms and names for informal taxonomic categories, we provide here for the first time a brief morphological and geographic description of the Dirus Complex. The previously included species, balabacensis, introlatus (Hii et al. 1988, to species status) and leucosphyrus are excluded from the Dirus Complex by present definition and will be treated separately at a later date.

Species currently recognized as members of the Dirus Complex include the following: dirus Peyton and Harrison (species A), dirus, species B to E, nemophilous (species F) and takasagoensis. Geographically, all but takasagoensis and dirus (species A) are known to occur exclusively on the mainland of the Oriental region. In addition to a wide distribution on the mainland, *dirus* (species A) is known to also occur on the offshore islands of Con Son, Vietnam and Hainan, China and takasagoensis is known only on the island of Republic of China. None are currently known to occur south of  $3^{\circ}$  30' N and six occur north of  $14^{\circ}$  N. Morphologically, the most significant adult character is the absence of an accessory sector pale (ASP) spot on the costa and usually also the subcosta (an occasional male in some species will exhibit an ASP spot on the costa, but always less than 6% of specimens). Additional adult characters that serve to characterize the complex are as follows: presector dark (PSD) spot on wing vein R with one or more pale spots on at least one wing (except occasional specimens of nemophilous), hindtarsomere 4 with a distinct basal pale band or dorsal patch (except rarely absent on *nemophilous*), proboscis approximately equal to, or shorter than the forefemur (ratio 0.85-1.15 and at most, only slightly longer than the palps).

METHODS. The terminology used follows Harbach and Knight (1980) with minor modifications as noted. A description of methods for defining and interpreting pale colors used in adult descriptions is treated separately under "discussion". The middle dark (MD) spot on the costa of the wing is changed to the subcostal dark (SD) spot. Measurement of the palpus was made from the point between the palpifer and flagellomere 1 to the apex of palpomere 5. The proboscis was measured from the same basal point as the palpus but the point on the proboscis is also visible as a shallow transverse indentation (groove) basoventrally and just slightly basad of the labial basal setae which were used by Colless (1956) and others as the basal point from which both measurements were made. We believe that measuring from the basal setae is too imprecise since the setae are often scattered, in a patch or, not too infrequently broken or completely missing, leading to an arbitrary point of reference. Consequently, our measurements will generally differ slightly from those presented by Colless. The pupal toothed margin (TM) index follows that of Colless (1956) as it provides for a use-

ful character which can be easily compared to the measurements presented in that publication. The toothed-margin index "expresses the ratio (a/b) of two lengths, (a) from the tip of the inner basal tubercle to the base of the most distant marginal tooth, and (b) from the same point to the base of the paddle hair" [1-P] (see Fig. 4). We have also adopted several very useful means of expressing character states and frequencies from Colless (1956) which were used most effectively by him in presenting variations. This also provides for direct comparisons with the data presented in that publication. In our publication we propose a standardized method for expressing and quantifying the degree of basal extention of the presector dark (PSD) spot on vein R in relation to the spots on the costa, by establishing numerical levels (see Fig. 2) and presenting the data as an expression of the condition as it is encountered on both wings of an individual and the frequency this condition was encountered to the total individuals examined, e.g., level 1:1 (96/132). This method has been used extensively throughout the descriptions of all stages for expressing other character states. Format and abbreviations follow that of earlier publications on the Leucosphyrus Group by Peyton and Harrison (1979, 1980) and Hii et al. (1988).

We have made a minor modification in the illustrative presentation of larval seta 14-III-VII in order to change what we feel is a misrepresentation. We follow the definition (not necessarily the illustrations) in Harbach and Knight (1980) which is derived in part from Belkin (1962) in which seta 14 is "a minute ventral seta near the anterior margin at the longitudinal midline of abdominal segments III-VII" (pupa) or I-VII (typically absent from I and II) "close to the longitudinal midline far forward on the intersegmental membrane" (larva). Setae 14 of the larva and pupa are homologous according to Belkin (1962) and are therefore of the same segments and relative position (anterior). Most larval illustrations of recent years show no association of seta 14 with segment VII and all others from II to VI are graphically portrayed as located at varying distances cephalad of the posterior margin of each segment (except Belkin 1968 and Berlin 1969, in part), but 14-VIII is always portrayed as anterior. In the illustrations of Harbach and Knight (1980) larval seta 14-II-VI is portrayed as anterior (Figs. 60, 67 and 72), posterior (Figs. 61, 66 and 68) and intersegmental (anterior) on others (Figs. 69-71). This is not in error; it is a matter of how the division of each segment and intersegmental membrane is drawn. In the first paper on larval chaetotaxy by Belkin (1950), seta 14-III-VIII is portrayed as it is in this paper (anterior). We do not differ with the definition or morphological position of seta 14-III-VIII (op. cit.), but we alter the illustrative portrayal of the division of segments to more accurately show the true morphological association of seta 14 to each segment and to its homolog on the pupa.

We have selected two collections from Ban Bang Ra Ko in the southern peninsular province of Phangnga, Thailand to represent the type series and to establish this area as the type locality for the species, in preference to using the small but excellent series of specimens from the higher elevation of Fraser's Hill, Malaysia. We believe the species is more firmly and widely established at the lower elevations in peninsular Thailand and in particular the Phangnga area (see distribution). The specimens from peninsular Thailand more closely represent the species from all areas, north or south and show a high degree of homogeneity.

ETYMOLOGY. The name used for this species is an English word of Greek origin, indicative of the obvious preference of the species for wooded-forested areas. It is

defined in Webster's Twentieth Century Dictionary, Unabridged as: "a. [Gr. nemos, a wooded pasture and *philos*, loving] having a love for or living in the woods." The selection of an English word conforms to article 11(b),(ii) of the ICZN and in this form it is indeclinable and the original spelling is to be retained, with termination unchanged, article 31(b) ICZN.

Anopheles (Cellia) nemophilous n. sp.

## (Figs. 3-5)

Anopheles leucosphyrus, Fraser's Hill form of Colless 1956: 62 (9<sup>\*</sup>, P<sup>\*</sup>, L<sup>\*</sup>, taxonomy).

Anopheles balabacensis balabacensis, Fraser's Hill form of Colless 1957: 131 (A, P, L, taxonomy); Reid 1968: 298 (Q<sup>\*</sup>, P<sup>\*</sup>, L<sup>\*</sup>, taxonomy); Baimai et al. 1984: 53 (listed).

Anopheles (Cellia) balabacensis introlatus of Scanlon et al. 1967: 82 (in part, misidentification of specimen reported from Ranong province, Thailand, 10° N).

Anopheles (Cellia) dirus of Baimai et al. 1988a (in press). (cytogenetics, species F).

Most adult characters exhibit considerable variation and none are singularly diagnostic. There is at least some degree of overlap of character states with those of closely related species of the complex. This is particularly evident between populations from the northern and southern regions where there is a small but noticable difference in the number of pale spots on the radial vein of the wing and the pale band on hindtarsomere 4. Consequently, some specimens may prove to be more difficult or impossible to distinguish from those of other closely related species (see details under discussion). A combination of several differential (discontinuity of variation pattern not complete, 5% or more of overlap) characters should allow for the recognition of a good portion of specimens from most areas. The immature stages possess useful characters, and the larval stage is reliably diagnostic for the species. Therefore, reared adults with associated pupal and larval skins can always be correctly identified to this species. Generally, the most significant differential characters of the adult are the following: the level to which the presector dark (PSD) spot on vein R extends basally (see Fig. 2); number of pale spots on the presector dark, sector dark (SD) and preapical dark (PD) spots on radial vein; color of pale scales on wing, particularly those on anterior veins but also others and the pale spots and bands on fore- and hindtarsomeres, (see discussion).

**FEMALE** (Fig. 3). Head. Proboscis brown scaled, often with a few inconspicuous pale scales ventroapically basal to labella, length 1.85-2.36 ( $\bar{x}$ =2.17) mm, ratio of length to forefemur 1.00-1.15 ( $\bar{x}$ =1.09); palpus 1.55-2.29 ( $\bar{x}$ =1.96) mm, ratio of length to proboscis 0.84-0.99 ( $\bar{x}$ =0.90), ratio to forefemur 0.93-1.01 ( $\bar{x}$ =0.98), ratios of lengths of palpomeres: 3/4 1.50-1.85 ( $\bar{x}$ =1.62), 3/5 2.08-2.88 ( $\bar{x}$ =2.40), palpomeres 2-4 with narrow pure-white apical bands, 5 with a broader apical white band varying from 0.86 to 2.00 ( $\bar{x}$ =1.46) the length of the basal dark band (range and means based

on 34 specimens); cibarial teeth (Fig. 3) long, thin, numerous, 16-20 ( $\bar{x}$ =16.5); maxillary teeth 16-20 ( $\bar{x}$ =17.6) (10 specimens). Thorax. Pleural setae as follows: upper proepisternal (PeSU) 2:2-3:4 (x=2.3) most commonly 2:2 (35/60), prespiracular (PsS) 0-4 ( $\bar{x}$ =2.1), prealar (PaS) 3-6 ( $\bar{x}$ =4.4), upper mesokatepisternal (MkSU) 3-7 ( $\bar{x}$ =4.4), lower mesokatepisternal (MkSL) 2-6 ( $\bar{x}$ =2.6), upper mesepimeral (MeSU) 3-8 ( $\bar{x}$ =5.3). Wing. (Figs. 2,3). Length 3.11-4.05 ( $\bar{x}$ =3.63) mm; prehumeral pale (PHP), humeral pale (HP) and presector pale (PSP) spots of costa usually prominent, occasionally reduced to very small spot or rarely absent, subcostal pale (SCP) spot usually not longer than length of preapical pale (PP) spot (250/309) range 0.41-2.20 ( $\bar{x}$ =0.91), color of costal pale spots usually pale yellowish or creamy white, but PHP, HP and PSP spots sometimes darker yellowish or golden, SP spot always the brightest spot on costa when compared to the others, though usually dull-dingy white or light creamy-white, not pure-white, SCP, PP, AP spots and apical pale fringe scales each usually progressivly darker cream or yellowish to apex of wing, (white scales on the halter are used here as the standard for pure-white scales for comparison against other shades of pale scales in this and related species); pale spots on vein R, R<sub>1</sub> usually correspond in color to those on costa; pale spots on posterior veins dull-white; PSD spot on vein R usually not extending basally beyond level 1 (see Fig. 2 for standardized levels) on both wings (96/132) or extending to the following levels: 2:1 (13/132), 2:2 (6/132), 3:1 (3/132, 3:2 (8/132), 3:3 (6/132); PSD spot of R usually with 1-3 pale spots ( $\bar{x}$ =0.93) but occasionally without a pale spot on both wings (15/132) or without on at least one wing (50/132), commonly 1:1-1:2 on both wings (75/132) and less commonly 2:2 (6/132), the maximum number observed, 2:3 (1/132), these spots sometimes consisting of 1-3 scales: SD spot of R with 1-3 pale spots ( $\bar{x}$ =1.46), one of which (the most basal) is the accessory sector pale (ASP) spot which is always present though sometimes reduced and most often the only spot present on at least one wing (94/132), the more distal pale spots when present, are varied, but the more median spot is most often absent and when present often consisting of only a few (2-4) pale scales, spots distributed as follows: ASP spot only 1:1 (57/132), 1:2 (35/132), 1:3 (2/132), 2:2 (31/132), 2:3 (7/132); basal dark spot of SD spot of R between SP and ASP spot infrequently with a few pale scales or spot at middle, (these are not treated here as part of the commonly occurring pale spots on the SD spot of R as they are infrequently found in all species), the basal dark spot of SD spot of R is usually longer than SP and ASP spots of R, ratio of basal dark/SP 0.25-3.00 ( $\bar{x}$ =1.29) and basal dark/ASP 0.22-6.00 ( $\bar{x}$ =1.70); PD spot of R with 0-4 ( $\bar{x}$ =1.84) pale spots, usually with 2 or more on at least one wing (110/132) with most (96/132) between 1:2-2:3; ASP spot never present on costa, and only occasionally present on subcosta (29/144), always developed on vein R, sometimes greatly reduced, usually SP spot of R longer, ratio of SP/ASP-R 0.45-6.00 ( $\bar{x}$ =1.33); yein R<sub>2</sub> with 2-6 ( $\bar{x}$ =4.0) pale spots excluding fork which is also pale; vein R<sub>3</sub> with 2-5 ( $\bar{x}$ =3.4) pale spots excluding fork; cell R<sub>2</sub> fork noticeably proximal to cell M<sub>1</sub> fork, ratio of length of cell R<sub>2</sub> to R<sub>2+3</sub> 1.30-2.29 ( $\bar{x}$ =1.78), ratio of length of cell R<sub>2</sub> to cell M<sub>1</sub> 1.17-1.37 ( $\bar{x}$ =1.26); vein R<sub>4+5</sub> with 5-10 ( $\bar{x}$ =7.3) dark spots; ratio of length of cell M<sub>1</sub> to M<sub>1+2</sub> 0.96-1.64 ( $\bar{x}$ =1.29); vein M<sub>3+4</sub> with 3-7 ( $\bar{x}$ =5.5) dark spots; vein CuA with 6-12 ( $\bar{x}$ =9.7) dark spots, apex dark scaled; vein 1A with 4-8 ( $\bar{x}$ =5.9) dark spots, 1A pale fringe spot (PFS) absent, CuA-PFS usually absent, rarely present on one wing only (5/132), PFS usually present at  $M_{3+4}$  and  $M_2$ , rarely absent. Legs. Forelegs with pure-white spots and bands, foretarsomeres 1-4 with apical white bands as follows: 0.07-0.21 (x=0.11) mm, 0.06-0.34 (x=0.11) mm, 0.07-0.14 (x=0.09) mm and 0.01-0.08 $(\bar{x}=0.04)$  mm respectively, and basal white bands on tarsomeres 2-4 as follows: 0.08-0.45 ( $\bar{x}$ =0.13) mm, 0.07-0.21 ( $\bar{x}$ =0.11) mm and 0.06-0.14 ( $\bar{x}$ =0.09) mm respectively,

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bands complete ventrally though less prominent, tarsomere 1 with 4-9 ( $\overline{x}$ =6.5) white spots along length of posterodorsal aspect, occasionally some spots fused and appearing almost completely white along this aspect, tarsomere 2 with 0-2 ( $\bar{x}$ =0.70) pale spots or occasionally fused and cover most of length of tarsomere; midtarsomeres 1-5 with small apical bands of pale scales or occasionally very faint or absent, tarsomere 1 with 0-7 ( $\bar{x}$ =2.9) pale spots, tarsomere 2 with 0-3 ( $\bar{x}$ =0.73) pale spots; broad apical white band of hindtibia without narrow basal extention of dark scales on ventral aspect; hindtarsomere 1 with 6-13 ( $\bar{x}$ =8.9) pale spots mostly along posterior aspect with a few sometimes extending to dorsoanterior aspect, tarsomere 2 with 0-4 ( $\bar{x}$ =1.3) pale spots, tarsomere 4 with a prominent basal white band, occasionally without band on one or both legs of a small number of specimens from central Malaysia only. Abdomen. Terga VI, VII with a few scattered dark scales near posterior margin, VIII densely covered on apical 0.75 with creamy-yellow scales, a few median ones sometimes whitish, cercus covered with yellowish scales; sterna V, VI rarely with 2-4 dark scales posteriorly, VII with dense posteromedian patch of dark scales, VIII with a few yellowish scales laterally or sometimes also a few scattered medially, varying from 2 to 8 scales on each side.

MALE (Fig. 3). Like female except for obvious sexual differences and the following: Head. Proboscis longer than in female 2.33-2.68 ( $\bar{x}$ =2.48) mm, ratio of length to forefemur 1.31-1.48 ( $\bar{x}$ =1.39), occasionally (16/98) with few scattered pale scales or small patch, usually near basal 0.2-0.3 ventrally; palpomere 2 with a dorsal patch of white scales near middle, apex of 2 and base of 3 without scales, 3 with a long dorsal stripe of dull-white scales from about basal 0.2-0.6 and a broad apical band of creamy white scales, a small apical patch of dark scales on outer ventral aspect, 4,5 each with broad apical bands of creamy yellowish scales, darker than those on apical band of 3 and with narrow dark scaled basal bands of uniform width, without apical extension on ventral aspect. Thorax. Upper proepisternal setae 1:1-3:3, most commonly 2:2 (18/30). Wing. Vein scales fewer, pale scaling more extensive, pale costal spots usually more prominent, longer, number of dark and pale spots on posterior veins variable, often reduced, particularly on  $R_{3+4}$  and 1A; PSD spot on R usually not extending basally beyond level 1 (99/125) on both wings, or to the following levels on both wings for the remainder: 1:2 (14/125), 2:2 (3/125), 2:3 (5/125), 3:3 (3/125) and 3:4 (1/125); PSD spot of R with 0-2 (x=0.94) pale spots distributed on both wings as follows: 0:0 (9/125), 0:1 (28/125), 1:1 (63/125), 0:2 (1/125), 1:2 (18/125), and 2:2 (6/125); SD spot of R with 1-3 pale spots distributed on both wings as follows: 1:1 (39/125). 1:2 (46/125), 1:3 (3/125), 2:2 (29/125), 2:3 (7/125) and 3:3 (1/125); basal dark spot of SD spot of R occasionally reduced or absent; PD spot of R with 0-2 pale spots, usually with 1 on at least one wing (108/125); ASP spot rarely present on costa on both wings (7/125), usually present on subcosta on both wings (107/125) or on one wing only (12/125). Legs. Foretarsomeres 1-4 without basal pale bands, tarsomere 4 occasionally without apical pale band. Abdomen. Dark scale patch on sternum VII occasionally with few pale scales intermixed, sternum VIII and sternal surface of gonocoxites densely covered with creamy-yellow scales, a small patch of dark scales basolaterally on gonocoxites.

**PUPA** (Fig. 4). Modal number of branches, placement and character of setae as figured; range and modal number of branches listed in Table 5. Salient features are as follows: Integument light brown, metanotum occasionally slightly darker than abdominal segments, sterna II-V usually with narrow dark band near anterior margin,

otherwise without distinctive color patterns. Seta 9-II-VIII pigmented dark brown, occasionally lighter brown on II,III, darkest on VII,VIII. Abdomen. Seta 6-I usually double (1-3), 7-I usually 4 branched (2-6); 1-II brush-like with 20-40 fine branches arising from 2-6 stronger stem branches, 6-II single or double, 7-II usually 4 branched (2-6), 9-II very short, stout, length 0.009-0.013 ( $\bar{x}$ =0.012) mm; 1-III 4-11 ( $\bar{x}$ =7.1) branched, usually 4-9 (97/100), sum of branches of both sides 8-21, 5-III 4-10 branched, sum of branches of both sides 11-19, 6-III usually double (1-3), 9-III short, stout, length 0.011-0.033 ( $\bar{x}$ =0.021) mm; 1-IV 3-8 ( $\bar{x}$ =5.9) branched, 5-IV 4-9 ( $\bar{x}$ =6.6) branched, sum of branches of both sides 9-17, 6-IV single, 9-IV short type, length 0.028-0.098 ( $\bar{x}$ =0.049) mm, ratios of length of 9-IV/III 1.47-4.17 ( $\bar{x}$ =2.40) and 9-IV/V 0.24-0.79 ( $\bar{x}$ =0.42); 1-V 2-4 ( $\bar{x}$ =2.8) branched, 5-V 3-8 branched, 6-V single, 9-V long, length 0.079-0.142 ( $\bar{x}$ =0.117) mm; 1-VI 2,3 ( $\bar{x}$ =2.1) branched, 5-VI 4-8 ( $\bar{x}$ =5.7) branched, 6-VI single, 9-VI long, length 0.104-0.142 (x=0.121) mm; 1-VII single or double, 5-VII 4-8 ( $\bar{x}$ =5.6) branched, 6-VI single or double, 9-VII long, length 0.102-0.155 ( $\bar{x}=0.119$ ) mm; 9-VIII 11-17 branched. Paddle. Very lightly tanned, buttress slightly darker, midrib faint, inapparent on about apical 0.33; outer basolateral serrations prominent, filamentous spicules on outer apical margin and most of inner margin, usually prominent; seta 1-P single, 2-P single or double; toothed margin index (=ratio of lengths a/b, Fig. 4) 0.81-0.87 ( $\bar{x}=0.84$ ).

LARVA (Fig. 5). Modal number of branches, placement and character of setae as figured; range and modal number of branches listed in Table 6. All measurements taken from mounted exuviae. Salient features are as follows: pigmentation of sclerotized areas variable, light yellowish-brown to dark rust-brown, without distinctive color patterns, particularly on head capsule; without noticeable spiculation except on saddle of segment X. Head. Antenna length 0.31-0.44 ( $\bar{x}=0.36$ ) mm; seta 2-C single, often with 1-5 lateral aciculae on distal 0.5, occasionally one or more quite prominent and long, 3-C single, long, length 0.098-0.157 (x=0.116) mm, 4-C usually single, occasionally bi- or trifurcate, inserted posterolaterad of 2-C, length 0.095-0.200 ( $\bar{x}$ =0.116) mm. reaching forward beyond base of 2-C, distance between basal insertions of 4-C and 2-C 0.058-0.098 (x=0.074) mm, ratio of length of 4-C to distance between the insertions of 2-C and 4-C 0.120-2.83 ( $\bar{x}$ =1.59); 5-C long, usually reaching to or beyond anterior margin of head and considerably longer than antenna, 10-17 branched, 6-C 11-19 branched, 7-C 14-19 branched. Thorax. Tubercles of all large setae moderately tanned to dark rust-brown with at least the central stems of the setae concolorous with tubercles; seta 1-P 13-26 ( $\bar{x}$ =18.0) branched, sum of branches of both sides 26-47, stem stout, not noticeably expanded and flattened basally, inserted on a large sclerotized basal tubercle which is joined basally with an equally developed basal tubercle of 2,3-P, each tubercle with a strong apical tooth on posterodorsal side projecting forward over the bases of 1,2-P, teeth varied in shape and length but usually longer and apically pointed on base of 1-P, 14-P 6-13 ( $\bar{x}$ =8.3) branched, usually with 8 on at least one side (133/154) or with at least 7 on both sides (136/154); 4-M usually 2 (1-3) branched, 6-M 3-6 branched, 14-M 5-9 branched; 3-T with 3-8 weak, nearly transparent branches, rarely with weakly developed leaflets. Abdomen. Seta 1-I small, with 5-10 rigid branches, usually pigmented light brown, 2-I single or double, usually single on at least one side (149/160) or single on both sides (124/160), 3-I single to triple, usually single on at least one side (152/160) or single on both sides (123/160), 9-I 3-6 ( $\bar{x}$ =4.6) branched, with at least 4 branches on one side and usually at least 5 branches on one or both sides (103/158); 1-II with 9-19 moderately developed lanceolate leaflets, pigmented light to dark brown, basal stem usually noticeably expanded

and pigmented brown, differing significantly from 1-I; 2-IV 3-5 ( $\bar{x}$ =3.5) branched, usually 3 branched (166/300), 3-IV single to triple, 13-IV 3-4 branched, long, ratio of length to 10-IV 0.79-1.05 ( $\bar{x}$ =0.91); 2-V 3-5 ( $\bar{x}$ =3.4) branched, usually 3 branched (157/300); 1-VII smaller than 1-VI with 12-15 moderately broad lanceolate leaflets, without apicolateral serrations or apical filament; 1-X long, single, inserted on saddle; pecten teeth 12-16 with 3-6 longer than the others, usually about 5 longer ones, usually the shorter teeth each reaching to 0.5 or greater the length of the longer teeth.

**TYPE DATA.** Holotype female with slide of pupal and larval skins, with the following collection data: Thailand, *Phangnga*, Ban Bang Ra Ko, 23 May 1979, Kol Mongkolpanya and Sanit Nakngen, collectors; collection number 08108-2, collected as a larva from a partially shaded elephant footprint in secondary rain forest in mountainous terrain at an elevation of approximately 520 m. Paratypes: 6 females, 4 males (9 with larval and pupal skins and 1 with pupal skin) same data as holotype, collection numbers 08108-1, -3 (adult mounted on 2 slides), -4, -5, -6, -7, -8, -9, -11, -12 and 12 third instar larvae on slides; other paratypes with same data as holotype except as follows: 10 females, 13 males, (22 with larval and pupal skins and 1 with pupal skin), collection numbers 08103-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12 (adult on slide), -13 (adult on 2 slides), -14, -15, -16, -17, -18, -19, -20, -21, -22 (adult on 2 slides), -100.

The adults of the type series are generally in excellent condition but the mounted skins are in very poor condition due mostly to attached food particles, fungi and other debris, but also some torn, twisted and partially decomposed. This is almost certainly the consequence of leaving the skins in the water for several hours after ecdysis. However, diagnostic features can usually be seen on most specimens. Several additional collections and specimens are available from the same locality and date (see distribution).

The holotype and paratypes are deposited in the National Museum of Natural History, Smithsonian Institution. Paratypes of 2 females and 2 males with associated slides of larval and pupal skins will be deposited in the British Museum (Natural History). Examples of 1 female, 2 males, 2 with associated larval and pupal skins and 1 fourth instar larva on slides from collection number 3394, Fraser's Hill, Pahang, Malaysia and 4 females, 2 males with associated larval and pupal skins on slides from collection number 08105 and 08106, Ban Bang Ra Ko, Phangnga, Thailand were deposited earlier in the Department of Entomology, AFRIMS, Bangkok.

**DISTRIBUTION** (Fig. 1). Material examined (1,092 specimens): 153 , 172 , 268 L; 289 with slide-mounted associated skins (210 lp, 79 p), derived from 73 separate collections (5 adult, 68 immature).

MALAYSIA. Peninsular Malaysia: Pahang-Fraser's Hill ( $3^{\circ} 43^{\circ} N = 101^{\circ} 45^{\circ} E$ ); [395-13] 1  $\mathcal{Q}$ , 1 1p; [777-10] 27/8/67, 1  $\mathcal{Q}$ , 1 1p; Genting Heights (Fraser's Hill) ( $3^{\circ} 42^{\circ} N = 101^{\circ} 46^{\circ} E$ ); [3007] 3/9/69, 1  $\mathcal{Q}$ , 1  $\mathcal{Z}$ , 2 L; [3008] 3/9/69 3  $\mathcal{Q}$ , 3  $\mathcal{Z}$ , 2 L; [3394] 17/12/69, 13  $\mathcal{Q}$ , 9  $\mathcal{Z}$ , 9 1p, 2 L. Perak-Maxwell's Hill (Maxwell Bukit), Larut Hills ( $4^{\circ} 50^{\circ} N = 100^{\circ} 52^{\circ} E$ ) (Reid 1968, not seen); Negeri Sembilan-Kampong Inas [?] ( $2^{\circ} 37^{\circ} N = 102^{\circ} 15^{\circ} E$ ); (Colless 1956, not seen).

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THAILAND. Chanthaburi: Khao Hin Phoeng (Nr. Ban Chak Yai) (12° 31' N 102° 13´E); [0620] 5/11/65, 1 9, 1 1p, 1 L; [0629] 5/11/65, 1 L; [0631] 5/11/65, 2 9, 1 8, 1 lp, 2 p, 1 L; Ban Chak Yai (12° 31 N 102° 13 E); [0571] 29/10/65, 3 L; Khao Soi Dao Tai (12° 56´ N 102° 12´ E); [0854] 14/3/66, 1 L; [0886] 16/3/66, 1 Q, 1 &, 2 lp; [0887] 16/3/66, 9 9, 9 3, 18 p, 7 L; [0888] 16/3/66 1 L; [0889] 16/3/66 1 L; [0890] 16/3/66, 1 3, 1 L; [0928] 22/3/66, 1 3, 1 p; [0929] 22/3/66, 1 9, 1 lp; [0964] 26/3/66, 2 L; [0967] 26/3/66, 1 9, 1 p, 2 L; [0975] 29/3/66, 1 9, 2 8, 3 p. Chon Buri: Ban Huai Kum (13° 14' N 101° 00' E); [02211] 17/8/66, 2 ♀, 2 lp. Kanchanaburi: Huey Sai Yok (14° 25´ N 98° 53´ E); [08117] 16/11/79, (Progeny), 8 9, 6 8, 12 lp, 2 p, 6 L. Nakhon Nayok: Khao Yai, Sarika waterfalls (14° 25' N 101° 23´ E); [NY 220] 16/6/64, 1 L; [NY 227] 16/6/64, 4 L. Nakhon Si Thammarat: Thung Song (8° 11´ N 99° 42´ E); [01061] 6/6/66, 1 3, 1 lp, 2 L; Chong Khao (8° 12´ N 99° 44 E); [01074] 8/6/66, 2 Q, 3 S, 3 lp, 2 p; [01176] 17/6/66, 1 L; [01187] 17/6/66, 1 L; Ban Thuan Lek (8° 26' N 99° 46' E); [01075] 10/6/66, 3 Q, 7 &, 4 lp, 5 p, 1 L; [01076] 10/6/66, 6 L; [01077] 10/6/66, 1 J, 1 p; Khao Luang (8° 26' N 99° 46´ E); [01133] 14/6/66, 3 L; [01138] 14/6/66, 1 &, 1 lp. Phangnga: Nam Tai (8° 34´ N 98° 35' E); [01723] 18/10/66, 2 Q, 2 S, 2 lp, 2 p; Ban Bang Kaeo (8° 35' N 98° 32 E) [08029] 10/5/79, 2 L; [08093] 19/6/79, 2 9; [08191] 30/5/80, 2 9, 2 8, 3 lp, 1 p, 5 L; [08197] 30/5/80, 2 9; [08223] 4/6/80, 1 9; [TH 498] 28/5/87, 1 9, 5 8, 3 lp, 3 p, 4 L; [TH 499] 28/5/87, 1 d, 1 lp; [TH 500] 28/5/87, 2 9, 7 d, 9 lp; Ban Bang Ra Ko (8° 36′ N 98° 32′ E); [08051] 14/5/79, 4 L; [08052] 14/5/79, 1 9, 1 lp, 1 L; [08103] 23/5/66, 10 9, 13 8, 22 lp, 1 p; [08104] 23/5/79, 2 9, 3 8, 3 lp, 2 p, 8 L; [08105] 23/5/79, 5 9, 7 8, 12 lp, 8 L; [08106] 23/5/79, 13 9, 11 8, 17 lp, 6 p, 3 L; [08107] 23/5/79, 4 9, 6 3, 7 lp, 6 L; [08108] 23/5/79, 7 9, 4 3, 10 lp, 1 p, 12 L; [08110] 25/5/79, 7 9, 8 3, 8 lp, 7 P, 4 L; [08111] 25/5/79, 5 9, 3 3, 6 lp, 2 P, 2 lsk, 2 L; [08113] 25/5/79, 2 L; [08114] 25/5/79, 2 Q, 2 lp, 5 L; [08166] 26/5/80, 2 Q, 8 d, 8 lp, 2 p, 2 L; [08167] 26/5/80, 6 9, 5 8, 9 lp, 2 p, 8 L; [08168] 26/5/80, 8 9, 14 8, 19 lp, 2 p, 11 L; [08169] 26/5/80, 3 9, 3 lp, 3 L; [08185] 29/5/80, 2 3, 2 lp; [08186] 29/5/80, 1 9, 2 lp, 3 L; [08187] 29/5/80, 1 3, 1 lp, 16 L; [08188] 29/5/80, 4 9, 1 3, 5 lp, 1 L; [08220] 4/6/80, 1 Q, 4 &, 5 p, 44 L; [08221] 4/6/80, 2 Q, 2 p, 19 L. Ranong: Kraburi, Petkasam Rd. (10° 24' N 98° 47' E); [GP80] 14/9/64, 1 d, 1 lp, 1 L; Ban Chatri (9° 32' N 98° 32' E); [02085] 10/7/66, 5 9, 3 8, 5 lp, 2 p; [02086] 10/7/66, 1 9, 1 lp. Songkhla: Rattaphum, Boriphat Waterfalls (7° 05' N 100° 14' E); [SL33] 20/3/65, 1 &, 1 L; Had Yai (Satun Rd.), Ton Nga Chang Waterfalls (6° 56' N 100° 19' E); [SL49] 22/3/65, 2 3, 1 lp; [SL88] 27/3/65, 1 L; Sadao, Padang Besa (6° 40' N 100° 19' E); [PB] 12/12/86, 1 S, 1 lp, 1 L. Tak: Khao Salak Phra (Doi Sam Sao) (16° 50' N 98° 55' E);[0297] 31/7/65, 1 L; [0298] 31/7/65, 3 Q, 8 d, 9 lp, 38 L.

The latitude and longitude are approximate for the above named localities, which may in some cases differ considerably between the individual numbered collection sites following each locality, but would rarely exceed more than 2 kilometers in any direction. More precise information on maps and grid coordinates for most individual collections is available on the permanent collection records in the WRBU.

**ZOOGEOGRAPHY.** The known distribution of this species (Fig. 1) follows closely the distribution of the remaining tropical evergreen or monsoon forest of mountainous areas of central, southern, southeastern and western Thailand and Peninsular Malaysia, leaving some widely disjunct populations on a few isolated forested mountains, particularly in central and southeastern Thailand. Areas in Thailand such as Khao Yai National Park, Nakhon Nayok; Huai Kum, Chon Buri; Khao Soi Dao Tai and Khao Hin Phoeng of the Cardamon Mountains, Chanthaburi are well known for the considerably higher rainfall than the surrounding areas. The southeast corner of Thailand including much of the provinces of Chanthaburi and Trat, the offshore islands and in the adjoining area of Cambodia are known to have somewhat unique climatological features for the region which closely approximate that of the high rainfall, semi-ever-green tropical rain forest in the southern peninsular west-coastal provinces of Phangnga and Ranong (Whitmore 1984). Several species of *Anopheles* previously thought to be exclusively southern or Malaysian in distribution have been found in these areas (Harrison and Scanlon 1975, Harrison 1980).

Harrison and Scanlon (1975) discuss the zoogeography of a number of Anopheles (Anopheles) species in Thailand and in particular the similarity of rainfall patterns and mosquito fauna of the southeastern provinces of Chanthaburi and Trat compared with that of the southern peninsular provinces. They show at least four species (baezai Gater, hodgkini Reid, roperi Reid and whartoni Reid) which are considered primarily Malaysian to occur north to the Isthmus of Kra and then to reappear in these southeastern provinces or additionally in some of the same isolated, wetter central and western sites reported here for nemophilous. Whitmore (1984) defines and treats the distribution of these rain forests in more precise terms. In defining, "The transition from rain to monsoon forest north of Malaya: the Kra Ecotone" he states that "in the region of the Kra Isthmus north of the Thailand-Peninsular Malaysia border there is a change in forest type and also in flora, leading to the replacement of tropical lowland evergreen rain forest by tropical semi-evergreen rain forest. The change begins just north of the border between the two countries. It is due to decreasing rainfall and increasing seasonality in climate northwards." He further states that the boundary between the two forest types, which is floristic as well as structural, runs more or less west to east, from Kangar in Malaysia to Pattani in Thailand and that this "Kangar-Pattani line [Fig. 1] is believed to be a close approximation to the boundary between lowland evergreen forest to the south (the Malayan type) and tropical semi-evergreen rain forest to the north (the Thai type)". Distinct faunal and floral changes are known to occur in the vicinity of this boundary and presumably this includes the distribution of several species of mosquitoes (Knight and Harrison 1987). Several mosquito workers have noted a marked north-south discontinuance of a number of Malaysian-Thailand species but never attempted to establish a line to reflect this, though most often, references were made to somewhere near the Malaysian-Thailand border or in the vicinity of the Isthmus of Kra (Reid 1950, 1968; Scanlon et al. 1967, 1968; Reinert 1972; Harrison and Scanlon 1975; Harrison 1980). This is due in part to the paucity of collections from the region and particularly from northern Malaysia. Only in recent years have significant but limited study collections been made in southern peninsular Thailand.

Even though the north-south limits of several species of mosquitoes appear to closely approximate the Kangar-Pattani boundary, the distribution of the Leucosphyrus Group is one of the known exceptions. Based on confirmed collections, a second boundary between Malaysian and Thailand species of this group would appear to be farther north to the Isthmus of Kra to about 10° 45' N at least on the eastern side of Thailand. Of the seven known Peninsular Malaysian species of the Leucosphyrus Group (*dirus* B (Perlis form), Hii (1984), *hackeri* Edwards, *introlatus* Colless, *leucosphyrus* A, Baimai et al. 1988c, *macarthuri* Colless (Hii et al. 1988, to species status), *nemophilous* and *pujutensis* Colless), all are known to extend north beyond the border with Thailand and most are now known to extend north well beyond the Kangar-Pattani boundary, with *nemophilous* extending to the Isthmus of Kra and then reappearing north of the peninsula (Fig. 1). At least four of the seven species, *dirus* B (Perlis form), *leucosphyrus* A, *macarthuri*, and *nemophilous*, are known to extend to the Isthmus of Kra in Thailand. The farthest point north recorded for any of these in this region is Ban Chong Rakam, Amphoe Pathiu, Chumphon Province on the eastern side of the Isthmus ( $10^{\circ}$  43' N 99° 16' E). North of this line on the eastern side of the peninsula in Thailand, from Chumphon ( $11^{\circ}$  N) northwards, the natural forest has been replaced on the coastal plain by a low scrub (Whitmore 1984), but this changes again at the top of the peninsula at about  $13^{\circ}$  N.

We are not aware of many mosquito records from the southern peninsular tip of Burma (Tenasserim) and therefore are unable to comment on the distribution of the group west of the Burma-Thailand border across the very wide, lower Pakchan-Mae Nam Kra Buri river channel in the region of the Isthmus. However, there is no apparent reason to believe that it would differ significantly from the Thailand side except possibly in isolated pockets of true evergreen and semi-evergreen forest above  $10^{\circ}$  30' N on the west coast of the peninsula and the Mergui achipelago offshore, which are markedly wetter than the east coast according to Whitmore (1984).

The confirmed north-south distribution of *nemophilous* (16° 50' N to  $3^{\circ}$  42' N) follows the somewhat discontinuous forested mountain ranges from Fraser's Hill and Cameron Highlands of central Peninsular Malaysia and from the Larut Hills west of these, to the more continuous northern Bilauk Taung range of western Thailand to Tak in the north (Fig. 1). Based on the number of collections of *nemophilous* made from 1964 to 1987, it appears that the species is much more common in peninsular Thailand. The province of Tak might well represent the approximate northern limit of its distribution since the climate changes significantly further north. However, a narrow strip of tropical semi-evergreen rain forest extends further north (Scanlon and Sandhinand 1965 and Whitmore 1984) and the species may follow this forest beyond Tak Province. Colless (1956) lists "Fraser's Hill form" from N. Sembilan, Kg. Inas (2° 37' N) without reference to specific collections or specimens but it appears that this could have just as well been introlatus and Reid (1968) does not include the Malaysian state of Negeri Sembilan in the known distribution of the "Fraser's Hill form". In view of this we are reluctant to include the N. Sembilan locality in the current discussion of the distribution of *nemophilous*. The species is likely to occur in Burma north, along the Bilauk Taung and Dawna ranges though it may not cross the divide to the west.

Heavy logging operations, clearing for land resettlement projects and increased slash and burn for subsistance farming throughout the forested mountainous regions of Thailand during the past 25 years has significantly altered the environment of several sites of earlier collections of the Leucosphyrus Group. In fact, in 1980 Bruce A. Harrison and team (personal communication) from the Armed Forces Institute of Medical Sciences Bangkok, returned to the original collection site of Huai Kum, Chon Buri Province where 2 adult specimens of *nemophilous* were reared from larvae collected in a rock pool in 1966, only to discover that the small isolated forest no longer existed. Collections made in the area revealed no further evidence of members of the Leucosphyrus Group. Other unpublished reports from AFRIMS on the site of Khao Mai Kheo, Chon Buri Province, a previously hyperendemic malarious area, where the pioneering work of Scanlon and Sandhinand (1965) reported An. balabacensis Baisas (=dirus Peyton and Harrison) biting man in extremely high numbers and adult dissections revealed an 8% gland positive sporozoite rate during the period of peak abundance, now indicate that the species can no longer be found in this area. The entire area is now completely agricultural. Most species of the Leucosphyrus Group and particularly dirus (species A) can survive rather well in the wooded foothills or mountains with partially cleared secondary growth or fruit orchards skirting the forest, if it is not too sparse, but removal of most of the cover effectively eliminates these species. Based on the collections of nemophilous, it appears that it may be much more forest-cover sensitive than dirus (species A).

**BIONOMICS.** Anopheles nemophilous appears to be a true inhabitant of forested mountains and foothills, occurring at elevations of 100 to 1,500 m. It has been collected in the larval stage as high as 1,220 m in central Peninsular Malaysia and 1,500 m in southeastern Thailand, the highest ever reported for any member of the Leucosphyrus Group. Although it is commonly encountered at much lower elevations, it does not appear to occur in the forest fringe areas as commonly as *dirus* (species A). However, this needs further confirmation because of the strong probability of it being overlooked in some of the early adult studies.

The habitats of the immature stages are similar to those of other members of the Dirus Complex and particularly *dirus* (species A). Most of the habitats seem to share a number of essential characteristics such as: small to moderate size, shallow (rarely deeper than 30 cm), temporary pools, usually in clay soil (yellowish, reddish or black) or rock pools usually found in stream beds; usually with some organic matter such as fallen leaves and sticks and/or animal waste; water often colored or turbid, but always fresh due to frequent freshening or flooding by rains; always under partial to heavy shade: without grassy edges or middle. As with dirus (species A), the species was most commonly collected in all areas of Thailand from elephant footprints. This unusual habitat is especially common in forest where the elephant is still used in logging operations. Feral elephant herds still survive in several of the isolated forested mountains of the country and on one remote mountain top of Khao Soi Dao Tai, Chanthaburi Province, a collection was made from an elephant footprint in a seepage bog with many such footprints and wallows at about 1,500 m and less than 200 m below the peak. Collections of the immature stages have been recorded from the following: elephant footprint (32), elephant wallow in bog (2), flood pool in drying stream bed (1), stream pool at or near stream margin (5), rock pool (11), flood pool (4), seepage pool (4), wheel rut (2), puddle/ground pool (7). Within this somewhat narrow range of habitat, 40 mosquito species belonging to 5 different genera have been collected in association with nemophilous (see Table 4).

Little is known about the behavior of adults. The few confirmed outdoor adult collections were obtained using human bait during the time period 1830-2200 h. One of these man-biting collections was made in a village about 10 m from a house and one was made from a platform in the forest canopy. The number of collections are insufficient to speculate on the disease-vector potential for this species, but sufficient to confirm it as a man-biter and worthy of further studies.

**DISCUSSION.** The discovery of several undescribed, morphologically similar species in the Dirus Complex has made it nearly impossible to identify individual specimens, particularly in Thailand where the greatest number (5) are known to occur. This is of considerable concern to those involved in several ongoing studies on malaria transmission in Thailand since *dirus* (species A) is known to be a very significant vector of malaria in areas north of  $12^{\circ}$  N but is apparently absent in areas south of the Isthmus of Kra. Although most of the species can usually be confidently identified in at least one of the three life stages of adult, larva or pupa, this requires one to have reared adults with associated skins, a most impractical means for field studies and identifications. The stage most often collected and studied in respect to disease-vector behavior and relationships is of course, the adult female. Until now the adult of *nemophilous* could not be distinguished from those of *dirus* (species A) and it was also considered difficult to distinguish from *introlatus*.

Adults of all species of this complex show varying degrees of variation with some overlap of characters among them but there appears to be sufficient separation beyond the range of overlap of each character (see Tables 1-3 below), to be useful when used in combination with other characters to separate a high percentage of specimens. We attempt to present characters which will assist in distinguishing nemophilous from dirus (species A) and introlatus. We stress, however, that it is possible but not necessarily easy to identify each. This is because a few character differences are very subtle and require close examination and careful interpretation. This is particularly true when evaluating colors, which, at first glance, appear to be quite ambiguous or dubious and often confusing. It is difficult to define color and varying shades of color of scales as interpretation is sometimes influenced by type of light source, intensity and angle of reflected light. We have found that the colors and subtle differences in shades are much more obvious and easily interpreted at magnifications of 60X or higher; the higher the better. We also recommend a blue daylight filter for use with the light source. This softens the light and tends to reduce exaggerated reflections which are so common and often a nuisance with some unfiltered incandescent and fiberoptic light sources when studying adult mosquitoes. The filter is especially effective in seeing and interpreting white scales. We used a focused, halogen light source with blue daylight filter to define our colors, but other light sources should be just as effective. In the Dirus Complex there are varying shades of pale scales which prove to be of considerable value. Two basic colors of pale scales are involved, white and yellow, with all others expressed as lighter or darker shades of the two or a blending of the two as in the use of cream which is usually an expression of a very light yellow at the lower end of the spectrum and a creamy-white for instance, would be basic white with a tinge of yellow. Dull or bright colors are usually expressed in relation to the degree of reflected light and intensity of the basic color. Pure-white as used here is a very bright white with or without luster and dull of course is the opposite, a subdued flat or off-white without luster and dingy-white is an off-white without luster and with a tinge of gray. We use the bright white scales on the halters of all species of the complex as a standard for comparison with all other pale scales and on comparison, all off-whites and shades of yellow then become more readily apparent. Pale is used here when reference to the actual color is unimportant.

While *introlatus* has long been considered very similar to and difficult to distinguish from *nemophilous* in the adult stage, the two are not that difficult to separate. The adults of *introlatus* possess two characters which exclude it from the Dirus Complex

and which also readily distinguishes it from all but a very small number of specimens of *nemophilous* from Fraser's Hill. The species *introlatus* has an accessory sector pale (ASP) spot extending to the costa on at least one wing in 90% of specimens (Reid 1968) and it is without a basal pale band on hindtarsomere 4, though there is an occasional small inconspicuous patch. Adult females of *nemophilous* never have the ASP spot extending to the costa and also have a prominent basal pale band on hindtarsomere 4 on all but a very small number of specimens from Fraser's Hill. Wing spots on the radial vein are very similar (see Tables 1-3 below).

The separation of adults of *nemophilous* and *dirus* (species A) is slightly more difficult. The most critical character in the separation of the two species is in the color of pale spots on the wing and particularly those on the three anterior veins of the costa, subcosta and radius. The pale spots on the wing of dirus (species A) are primarily dull or dingy-white and these usually include the costal spots on the apical 0.5 of the wing and the apical fringe spots, but some apical spots occasionally show a yellowish tinge or distinct yellow. However, the HP, PSP and SP spots are usually all pure-white, sharply contrasting with all of the other spots on the wing and closely approximating the bright pure-white scales on the halters. Occasionally, the HP and PSP spots are not quite as bright but the SP spot is always bright and differing from the creamy-white or light yellowish spot of nemophilous. In nemophilous the end of vein CuA is dark and a CuA-PFS is absent (except rarely CuA-PFS present on one wing only). In dirus (species A) the end of vein CuA is usually pale and a CuA-PFS is almost always present. Male palpomeres 4,5 differ in that on dirus (species A) there is always a narrow ventrolateral line of dark scales extending apically from the basal dark band of each, varying in length from short to near the length of palpomere and on nemophilous the basal dark bands are of uniform width without ventrolateral extentions. The two species differ in the degree of basal extension of the PSD spot of vein R, the number of pale spots on PSD spot of vein R and the number of pale spots on SD spot of vein R. These are shown in the following tables (1-3). The number of specimens indicated in the tables represent samples from 42 separate collections of nemophilous and 84 collections of dirus (species A) from all areas of Malaysia and Thailand. The number of specimens of introlatus represent all available specimens.

Levels		1:1	2:1	2:2	3:1	3:2	3:3	4:1	4:2	4:3	4:4	5:3	5:4	5:5	5:6
nemophilous	f	96/n	13/n	6/n	3/n	8/n	6/n	0/n	0/n	0/n	0/n	0/n	0/n	0/n	0/n
(n=132)	%	72.6	9.9	4.6	2.3	6.1	4.6	-	-	-	-	-	-	-	-
introlatus	f	27/n	9/n	0/n	3/n	2/n	1/n	0/n	0/n	0/n	0/n	0/n	0/n	0/n	0/n
(n=42)	%	64.3	21.4	-	7.1	4.8	2.4	-	-	-	-	-	-	-	-
dirus (A)	f	0/n	1/n	0/n	3/n	5/n	6/n	3/n	9/n	<b>2</b> 9/n	83/n	3/n	<b>2</b> 3/n	34/n	1/n
(n=200)	%	-	0.5	-	1.5	2.5	3.0	1.5	4.5	14.5	41.5	1.5	11.5	17.0	0.5

**TABLE 1.** Frequency distribution (f) and percentage (%) of levels of basal extension of the PSD spot of vein R in females.

A word of caution is called for in the interpretation of the basal extention of the PSD spot of R (Fig. 2). The level of the basal extention is determined by any dark scales occurring beyond the PSD spot of the costa. It does not have to be a continuous uninterrupted extention of dark scales as it is often interrupted by one or more pale spots and these can occur beyond the PSP spot or, the most basal dark scales can be a small isolated spot of a few scales or rarely a few scattered dark scales. These conditions are only rarely a problem since the majority of specimens are easily interpreted at least on one wing. On occasional specimens the PSP spot of the costa is greatly reduced or rarely absent and the degree of extention must be judged accordingly. Note in Table 1 that the basal extention of the PSD spot on specimens of nemophilous does not extend beyond level 3 and on 87.1% of specimens it does not extend beyond level 2 on either wing. On dirus (species A) 92.5% extend beyond level 3 on at least one wing, which would suggest that any specimen with a basal extention to level 4 or beyond would not be *nemophilous* and conversely any specimen with basal extention of less than level 3 on both wings would not be *dirus* (species A).

The interpretation of the number of pale spots on the PSD and SD spots of vein R in the following tables 2 and 3 can be made in the same manner.

Spots		0:0	0:1	1:1	0: <b>2</b>	1: <b>2</b>	2:2	0:3	1:3	2:3	3:3	2:4	3:4	Mean
nemophilous	f	15/n	<b>3</b> 1/n	<b>4</b> 6/n	<b>4</b> /n	<b>2</b> 9/n	6/n	0	0	1/n	0	0	0	0.94
(n=132)	%	11.4	23.5	34.9	<b>3</b> .0	<b>22</b> .0	4.6	-	-	0.8	-	-	-	-
introlatus	f	0	5/n	16/n	2/n	<b>13</b> /n	1/n	0	0	0	0	0	0	1.14
(n=37)	%	-	13.5	<b>4</b> 3.2	5.4	35.1	2.7	-	-	-	-	-	-	-
dirus (A)	f-	0	0	8/n	0	<b>2</b> 8/n	83/n	1/n	3/n	56/n	13/n	4/n	<b>4</b> /n	2.1
(n=200)	%	-	-	4.0	-	14.0	41.5	0.5	1.5	<b>28</b> .0	6.5	2.0	2.0	-

**TABLE 2.** Frequency distribution (f) and percentage (%) of pale spots on PSD spot of vein R in females.

It is readily seen in this table that *dirus* (species A) tends to have a higher number of pale spots on the PSD spot of R with 40.5% of specimens with 3 or more pale spots on the PSD spot at least on one wing and *nemophilous* with only 0.8% in this range, with the probability that any specimen with 3 or more pale spots on at least one wing would not be *nemophilous*. Just as significant is the sum of 4 or more pale spots of both wings (1:3, 2:2 to 3:4) with 81.5% of *dirus* (species A) in this range with only 5.4% of *nemophilous* in this range. On the lower end of the range, *nemophilous* has 72.8% of *dirus* (species A) fall within this range and 37.9% of *nemophilous* have no pale spot on one or both wings with no more than 2 pale spots on the opposite wing (sum of 2 of both wings) and no *dirus* (species A) fall within this range.

Spots		1:1	1:2	2:2	1:3	2:3	3:3	2:4	3:4	4:4	Mean
nemophilous	f	57/n	35/n	31/n	2/n	7/n	0	0	0	0	1.46
(n=132)	%	43.2	26.5	23.5	1.5	5.3	-	-	-	-	-
introlatus	f	1/n	5/n	20/n	0	8/n	3/n	0	0	0	2.09
(n=37)	%	2.7	13.5	54.0	-	21.6	8.1	-	-	-	-
dirus (A)	f	0	1/n	16/n	1/n	56/n	69/n	11/n	35/n	11/n	2.91
(n=200)	%	-	0.5	8.0	0.5	28.0	34.5	5.5	17.5	5.5	-
·····											

**TABLE 3.** Frequency distribution (f) and percentage (%) of pale spots on SD spot of vein R in females.

The separations are obvious here also, there is separation on both ends of the range of pale spots, both significant. As with the pale spots on the PSD spot of R, *dirus* (species A) also tends to have a higher number of spots on the SD spot of R with 91.5% of the specimens with 3 or more pale spots on at least one wing and *nemophilous* with only 6.8% in this range. Within the range 3:3 to 4:4, 63.0% of *dirus* (species A) show no overlap with *nemophilous* and within the range 1:1, 1:2 and 1:3, with no more than 1 pale spot (the ASP) on one of the wings, 71.2% of *nemophilous* show an insignificant overlap of 1.0% with *dirus* (species A) with the probability that any specimen with only the ASP spot present on SD spot of R would not be *dirus* (species A).

The pupa of *nemophilous* cannot be distinguished from that of *dirus* (species A), but the pupa of *introlatus* is easily distinguished from those of *nemophilous* and *dirus* (species A). The pupa of *introlatus* has a long seta 9-IV with ratio of length of 9-IV/V 0.65 or more and seta 9-IV-VII is uniformly pigmented pale yellowish or bright golden, usually appearing clear or transparent. Seta 6-IV-VI is double. In the other two species seta 9-IV is the short type with ratio of length of 9-IV/V usually considerably less than 0.65 and seta 9-IV-VII pigmented light to dark brown with at least 9-VII dark brown. Seta 6-IV-VI is single.

The larva of *nemophilous* differs from that of *dirus* (species A) by the following: seta 4-C is longer in *nemophilous* and always extends well beyond the base of 2-C; seta 14-P has 6-13 branches with 8 branches on at least one side in a majority of specimens; setae 2,3-I are usually single; seta 9-I is usually 5 or more branched on at least one side; seta 1-II is usually moderately to well developed with distinct moderately broad, light to darkly pigmented, lanceolate leaflets with basal stem usually stout and distinctly inflated; seta 13-IV long, ratio of length of 13/10-IV 0.8-1.2. The larva of *dirus* (species A) possesses the following: seta 4-C usually extending to near or only slightly beyond the base of 2-C; seta 14-P has 4-8 branches, usually 6 or less and very rarely with 8 branches on one side only; setae 2,3-I usually bifid or trifid; 9-I is usually 3,4 branched and only rarely 5 branched; seta 1-II is weakly developed without clearly differentiated, pigmented, lanceolate leaflets or expanded basal stem; seta 13-IV is

short, ratio of length of 13/10-IV considerably less than 0.8 and usually about 0.5. The larva of *introlatus* is quite similar to that of *nemophilous* and differs only in the following: setae 2,3-I bifid or trifid; seta 1-II as *dirus* (species A); seta 13-IV short, usually about 0.4-0.6 the length of 10-IV, rarely exceeding 0.7; usually at least some of the leaflets of 1-VII with weak apicolateral serrations and poorly defined filaments (uniformly, narrowly lanceolate on *nemophilous*).

ACKNOWLEDGEMENTS. We wish to thank the following individuals for reviewing the manuscript: Ronald A. Ward, Richard C. Wilkerson of the Walter Reed Biosystematics Unit (WRBU), Museum Support Center, Smithsonian Institution and Bruce A. Harrison of the U.S. Army Medical Research Unit, Institute for Medical Research, Kuala Lumpur, Malaysia. We also thank Taina Litwak (WRBU), Ann L. Hoskins Dery and Vichai Malikul, formerly of the Medical Entomology Project, Smithsonian Institution for the preparation of the illustrations and James Pecor (WRBU) for preparing the manuscript for photoreproduction.

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SPECIES	Elephant Footprint	Elephant Wallow in Bog	Flood Pool in Drying Stream Bed	Stream Pool at or Near Stream Margin	Rock Pool	Flood Pool	Seepage Pool	Wheel Rut	Puddle/Ground Pool
Ae. (Adm.) alboscutellatus Ae. (Adm.) caecus Ae. (Adm.) lowisii Ae. (Adm.) orbitae Ae. (Fin.) orbitae Ae. (Fin.) chrysolineatus Ae. (Fin.) macfarlanei Ae. (Fin.) saxicola Ae. (Stg.) albopictus An (Ano) aberrans	X X X	x			X X X X X X	x			
An. (Ano.) barbumbrosus		v					Х		
An. (Ano.) bengalensis	x	Λ		x			v		
An. (Ano.) fragilis	x			~~			Л		
An. (Ano.) insulaeflorum				Х					
An. (Ano.) montanus			Х						
An. (Ano.) roperi			Х						
An. (Ano.) dirus A An. (Col.) dirus D	X	Х		X	Х				X
An. (Cel.) alrus $\mathbf{D}$	Х			X					
An (Cel) maculatus	v			X	Х				X
An. (Cel.) tessellatus	Л			Λ		v			
Cx. (Cux.) mimeticus	x			x		А			
Cx. (Cux.) mimulus				<i>.</i>				x	v
Cx. (Cux.) murrelli								<b>7</b>	X
Cx. (Cui.) bailyi	Х						X		
Cx. (Cui.) fragilis	X								
Cx. (Cui.) nigropunctatus	X								
Cx. (Cui.) pallidothorax	37				Х		Х		
$C_{X}$ . (Cui.) papuensis $C_{X}$ . (Cui.) scanloni	X								
$C_X$ (Eur.) scantoni $C_X$ (Eur.) sp	А				X	37			
$C_{x}$ (Eum.) sp.		x			Х	X	v		
Cx. (Lop.) bicornutus		Л				А	Х		X
Cx. (Lop.) brevipalpis	х								X
Cx. (Lop.) minor					x				
Cx. (Lut.) halifaxi	Х	Х			x		x	x	
Ho. malaya						x		2 <b>x</b>	
Ur. (Ura.) annandalei							Х		İ
Ur. (Ura.) hebes	_						Х		
Ur. (Ura.) macfarlanei	X	Х			Х	Х	X		
Ur. (FJC.) DICOLOR	Х				Х				

TABLE 4. Species and habitat associations of the immature stages of Anopheles nemophilous.

nemophilous. <sup>a</sup>
(Cellia)
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Number
Table 5.

Seta <u>Number</u>	CT	Ι	II	Abdc			IV	ΝI	VIII	raddie IX	А
0	ı	ı	I	I	-	I	Π	-	Ι	ı	I
I	2,3(2)	NCb	20-40(25)	4-11(7)	3-8(6)	2-4(3)	1-3(2)	1,2(1)	ı	-	I
2	2,3(2)	6-11(7)	3-7(5-6)	5-8(6)	2-4(3)	2-4(3)	2-4(3)	2,3(3)	ı	1	1,2(1)
3	2-5(3)	1,2(1)	2-5(4)	2-5(3)	4-8(6)	2-4(3)	1-3(2)	1-5(4)	I	I	ı
4	1-3(2)	2-6(6)	1-7(5)	3-6(4)	3-6(4)	4-6(5)	1-3(2)	1,2(2)	1,2(2)	ł	ı
S	3-6(4)	2-4(3)	3,4(3)	4-10(7)	4-9(6)	3-8(6)	4-8(6)	4-8(5)	ı	1 1	11
6	1-6(2)	1-3(2)	1,2(2)	I-3(2)	l	Ι	Ι	1,2(1)	ı	ı	L.
7	2-4(3)	2-6(4)	2-7(4)	2-4(3)	2,3(3)	2-4(3)	1,2(1)	1,2(2)	ı	ı	ı
∞	I-4(2)	I	ł	2-4(3)	2-4(3)	2-4(3)	1-4(2)	1-3(2)	I	ı	ı
6	I-4(3)	I	I	I	1	I	-	1	11-17(14)	ı	۱
10	2-5(3)	ı	ı	2,3(3)	1,2(1)	1,2(2)	1-3(2)	1,2(2)	I	I	ı
11	1-6(4)	ı	ı	-	1,2(1)	1,2(1)	1	1,2(2)	ı	I	ı
12	I-3(3)	ı	ı	8	ı	ı	I	ı	ı	ı	ı
13	ı	ı	ŀ	ı	ı	I	ı	I	ı	ı	ı
14	ŧ	I	ı	I	I	Ι	1	1	I	ı	I

Mosquito Systematics

	X	<b>I</b>	1		15-16	(16)	6-10	(7)	NCp		ı		4-6	(4)	5-8	(9)	<b>–</b>		-		2-4	(3)	<u>1-2</u>		2-4	(3)	2-4	(3)	) 1		ı
	VIII	1	I	1	5-9	(9)	4-8	(5)	-		3-6	(4)	1-S		2-S		4-S		5-S		6-S		7-S		8-S		9-S		I		I.
	ΝI	1	12-15	(14) 7	5-8	(9)	2-5	(4)	1		3-6	(4)	4-7	(4)	3-5	(3)	4-6	(4)	4-6	(2)	5-7	(9)	I-3	(2)	Π		2,3	(3)	-		1
	ΙΛ		16-19	(17)	C-2	(4)	Π		I		4-6	(2)	2-4	(3)	2,3	(3)	1-3	(2)	5-8	(9)	1-3	(3)	2,3	(3)	T		5-7	(9)	-		I
	V	-	17-20	(20) 0 č	3-5	(3)	1		2-4	(3)	3-5	(3)	2,3	(2)	3-5	(4)	2,3	(2)	4-8	(9)	-		I-3	(3)	1-3	(2)	3,4	(3)	1		I
nents	IV	1	15-22	(20) 0	<b>C-</b> E	(3)	1-3	(3)	3,4	(3)	2-4	(3)	2,3	(2)	3-5	(4)	2,3	(2)	5-10	(8)	1		2,3	(3)	2-4	(2)	3,4	(3)	Π		ł
minal Segr	III	-	18-23	(19) 2	4-7	(2)	-		3-5	(3)	3-5	(3)	15-17	(15)	4-6	(4)	1-3	(2)	6-9	(2)			2-4	(3)	2-3	(2)	3-6	(4)	1		I
Abdo	Ш	-	9-19	(14)	4-8	(9)	I		2-7	(4)	4-5	(4)	17-26	(24)	16-23	(17)	1-3	(2)	6-11	(6)	2-4	(3)	-		I		4-6	(4)	ł		ł
	I	I	5-10	6	1,2	Ξ	1-3	(1)	4-6	(5)	ς		17-24	(23)	12-20	(14)	I		3-6	(5)	1		3,4	(3)	3-5	(3)	3-6	(3)	I		I
	F	ı	1-3	Ξ.	1,2	(1)	3-8	(9)	2-4	(3)	28-36	(35)	2-4	(3)	28-38	(29)	26-35	(28)	1		1		I		2,3	(2)	2-4	(3)	ı		I
Thorax	W	I	21-32	(26) . 7	I-/	(3)	-		1-3	(2)	-		3-6	(4)	2,3	(3)	21-27	(25)	I		I		-		1,2	(1)	4-5	(2)	5-9	(1)	I
	Р		13-26	(18)	CI-8	(11)	-		14-23	(16)	NCo		I		23-31	(28)	21-33	(29)	1		-		-				3-6	(4)	6-13	(8)	I
Head	C	I	1	-	-		-		1-3	(1)	10-17	(12)	11-19	(14)	14-19	(16)	1,2	(1)	3-5	(4)	1-4	(2)	18-30	(29)	1,2	(2)	5-7	(5)	6-16	(6) (	و-ر (8)
Seta	Number	0		ſ	7		ŝ		4		5		6		7		~		6		10		11	(	12		13		14	t	SI

 $\frac{a}{b}$  Minimum of 24 checked for each seta and up to 300 were checked for selected setae.

Table 6. Number of branches for setae of the fouth-instar larva of Anopheles (Cellia) nemophilous.<sup>a</sup>

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Basal extention of Presector Dark spot (PSD) on vein R as compared to the Humeral Dark (HD), Presector Pale (PSP) and Presector Dark (PSD) spots on Costa.





Level 1. Not extending basally beyond PSD on Costa or barely so.

- Level 2. Extending basally but not beyond 0.5 of PSP.
- Level 3. Extending basally beyond 0.5 of PSP but not into level of HD.
- Level 4. Extending basally into level of HD but not beyond 0.5 of HD.
- Level 5. Extending basally beyond 0.5 of HD but not beyond HD.

Level 6. Extending basally beyond HD.



nemophilous

Vichai Malikul



