

***Reinertia*, a New Subgenus of the Genus *Aedes* Meigen and Its Type Species *Aedes (Reinertia) suffusus* (Diptera: Culicidae), Newly Recorded From Bhutan**

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Abstract

A new subgenus, *Reinertia* Somboon, Namgay & Harbach, of the genus *Aedes* Meigen and its type species, *Ae. suffusus* Edwards, are described from specimens reared from larvae and pupae found in a tree hole in Bhutan. The scutum of the adults is mostly covered with narrow pale falcate scales. The proboscis, maxillary palpus, tibiae, and tarsi are dark-scaled. The gonocoxite of the male genitalia bears a unique setose basomesal sclerite. The larva closely resembles larvae of the subgenus *Downsiomyia* Vargus in having setae 4–6-C with numerous branches and inserted more or less on level with seta 7-C, abdominal seta 12-1 is present and the comb is composed of 6–10 spine-like scales arranged in an irregular row. Surprisingly, *Reinertia* shares features of the adult habitus, male genitalia, and larva with the Palearctic subgenus *Dahlia* Reinert, Harbach & Kitching. However, in phylogenetic analyses of the mitochondrial *COI* gene of species representing 38 subgenera of *Aedes* and six other genera of the tribe Aedini Neveu-Lemaire, *Reinertia* was not associated with *Dahlia* or *Downsiomyia*. In both maximum likelihood and Bayesian analyses of the data, *Ae. suffusus* was recovered as the weakly supported sister of a clade composed of five species of the subgenus *Protomacleaya* Theobald. In the absence of strong support, and because *Protomacleaya* is an unnatural group of species that resemble each other phenetically by virtue of what they lack, *Ae. suffusus* cannot be placed in the subgenus *Protomacleaya*. Thus, the morphological and molecular data attest the uniqueness of *Ae. suffusus* and its recognition as a monobasic subgeneric lineage.

Key words: *Aedes suffusus*, Bhutan, *COI*, mosquito, new subgenus

Bhutan is a small land-locked country of about 700,000 people situated in the eastern Himalayan Mountains between India to the south and Tibet to the north. Most of the country is mountainous, with well-conserved forests covering about 60% of the territory. The climate varies from subtropical in the south to temperate in the highlands, with polar-like conditions in the north. The geographical features and climate are favorable for tropical and montane mosquito species that develop in a variety of ground-water habitats and in natural containers. Studies of mosquitoes in Bhutan conducted during recent years have revealed a great diversity of species in the country (Namgay et al. 2018). Morphological and molecular studies have led to the recognition of several new species and species complexes of mosquitoes, including *Aedes (Hulecoeteomyia) bhutanensis* Somboon & Harbach (Somboon et al. 2020a), the Lindesayi Complex of *Anopheles* Meigen consisting of five species

(Namgay et al. 2020), the Baileyi Complex of *Anopheles* consisting of three species (Somboon et al. 2020b), and a new *Anopheles* species of the Barbirostris Complex (Wilai et al. 2020).

During surveys conducted in 2018, we collected a number of larvae and pupae of *Aedes* Meigen from a tree hole in high-altitude forest north of Thimphu, the capital city of Bhutan. Some of the specimens were reared to adults. Attempts to identify the adults and larvae to subgenus and species using the keys of Rattanarithikul et al. (2010) and Tanaka (2018) for members of the tribe Aedini Neveu-Lemaire, and comparison with morphological characteristics of the subgenera of *Aedes* provided in the online Mosquito Taxonomic Inventory (<http://mosquito-taxonomic-inventory.info/simpletaxonomy/term/8577>), were unsuccessful. Later, with more critical examination of earlier published descriptions and key characters (Edwards 1922, 1932; Barraud 1934; Knight and Marks 1952), we were able

to identify the species as *Aedes suffusus* Edwards, which is currently without subgeneric placement (Reinert et al. 2009; Wilkerson et al. 2015). Careful examination of the specimens, especially the previously unknown male and incompletely described larva, as well as phylogenetic analyses based on *COI* sequence data, revealed that *Ae. suffusus* is dearly distinct from species of the currently recognized subgenera of *Aedes*; hence, herein we establish a new subgenus, *Reinertia* Somboon, Namgay & Harbach, with *Ae. suffusus* as its type species.

Materials and Methods

Morphological Study

Larvae and pupae were collected from a small tree hole in mountain forest about 14 km north of Thimphu city along the walkway up to the Tango Monastery. The specimens were individually reared in the field with water, sediment, and debris from the tree hole. However, due to difficulties in transporting a large number of rearing containers and limited facilities, some larvae died and some exuviae were damaged. Four adult females and one male were successfully reared. The females were killed with chloroform vapor, mounted on triangular points, and dried in an oven at about 42°C; the single male was preserved in 80% ethanol. The specimens were examined under artificial light using a stereomicroscope (Olympus SZX7, Japan) equipped with a calibrated eyepiece micrometer. Some larvae were killed by briefly placing them in hot water (about 60°C) and preserved in 80% ethanol. The larvae and the larval and pupal exuviae were mounted on microscope slides with Hoyer's medium (Neo-shigaral, Shiga Konchu Fukuyusha, Tokyo, Japan). Larval and pupal chaetotaxy was studied with a bright-field microscope (Olympus CX31, Japan) using 10× and 40× objective lenses with a calibrated eyepiece micrometer. Photographs were taken with a digital camera (Olympus E-330, Japan).

The new subgenus is placed in the modified traditional concept of *Aedes* established by Wilkerson et al. (2015). The morphological terminology and abbreviations used in the descriptions follow the Anatomical Glossary of the online Mosquito Taxonomic Inventory (<http://mosquito-taxonomic-inventory.info/node/11027>). The symbols ♀, ♂, L, Le, and Pe used in the Specimens Examined section represent female, male, larva, and larval and pupal exuviae, respectively.

Phylogenetic Study

Genomic DNA was extracted from the three left legs of a female *Ae. suffusus* (DNA extracted from a second specimen failed to amplify) using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's protocol. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene was amplified using the universal barcode forward primer LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and reverse primer HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') of Folmer et al. (1994). PCR were carried out in a 20- μ l volume containing 2 μ l (10 ng) of DNA, 0.4 U of PlatinumTaq DNA Polymerase (Invitrogen, Carlsbad, CA), 1 \times of PCR buffer (Invitrogen), 3.0 mM of MgCl₂ (Invitrogen), 0.2 mM of dNTP (Invitrogen), and 0.2 μ M of each primer. The amplification profile comprised initial denaturation at 95°C for 2 min, 40 cycles at 95°C for 30 s, 45°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 5 min. Amplified products were electrophoresed on 2% agarose gels and stained with ethidium bromide. PCR products were purified using Illustra ExoProStar 1-Step (Cytiva, Little Chalfont, United Kingdom) and sequenced using the BigDye Terminator v3.1 cycle sequencing kit chemistry (First BASE, Salangor, Malaysia). Specimens of *Ae. (Finlaya) poicilius* (Theobald) from Thailand and *Ae. (Gilesius) pulchriverter* (Giles) and *Ae.* (subgenus uncertain) *oreophilus* (Edwards) from Bhutan

were also sequenced as above. The *COI* sequences generated in this study are deposited in the GenBank database under accession numbers MW021558–MW021561.

The *COI* sequence of *Ae. suffusus* was compared with the species of 38 subgenera of *Aedes* and six other genera in the tribe Aedini (a total of 67 species, including *Ae. suffusus*) available in GenBank using the Basic Local Alignment Search Tool (BLAST, available at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) under default parameters. Sequences that were shorter than 559 bp or from different *COI* regions were excluded from the analyses. Available *COI* sequences for species of *Anopheles* Meigen, *Culex* Linnaeus, *Culiseta* Felt, *Mansonia* Blanchard, and *Toxorhynchites* Lahille precluded the use of those species as outgroup taxa because they were recovered within the tribe Aedini. Therefore, the *COI* sequence of the blackfly *Simulium nigrogilvum* Summers (Diptera: Simuliidae) was used instead. Sequences were aligned using CLUSTAL W under default parameters (Larkin et al. 2007), and ragged ends were trimmed using MEGA v. 10.0.5 (Kumar et al. 2018). The best-fit model GTR+I+G was implemented using the Akaike information criterion in jModelTest v. 2.1 (Darrriba et al. 2012). The phylogenetic analyses were conducted using maximum likelihood (ML) in MEGA v. 10.0.5 (Kumar et al. 2018) and Bayesian inference (BI) in MrBayes v. 3.2.7 (Ronquist et al. 2012). Robustness of the ML tree was tested with a 1,000 bootstrapped data set with bootstrap support values shown at each node. For BI, the Markov chain Monte Carlo simulation was run for 5 million generations (which resulted in an average standard deviation of split frequencies below 0.05), using three heated chains and one cold chain, and sampling every 100 generations with a burnin of 25%. The BI tree was generated using Figtree software v. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bayesian posterior probability (PP) support values ≥ 0.95 and ML bootstrap values of 70% were taken as being highly supportive of a node (Hillis and Bull 1993, Lemoine et al. 2018).

Nomenclature

This article and the nomenclatural act(s) it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:7FCC7990-FFCB-48E5-96DE-FA4B5804DA66.

Descriptions of the New Subgenus and the Type Species

Aedes Subgenus *Reinertia* Somboon, Namgay & Harbach, n. subg.

(urn:lsid:zoobank.org:act:5306F80D-8FA8-417E-BEF6-A7BE35770D9D)

The description of *Reinertia* is based on the type species *Aedes suffusus* Edwards, 1922. With the exception of a unique feature of the male genitalia, the subgenus, like all other genus-group taxa of the tribe Aedini, is diagnosed and distinguished by combinations of characters in each life stage. The most important differential and diagnostic characters for the recognition of *Reinertia* are as follows.

Females

Small blackish brown mosquitoes; vertex of head with dark upright forked scales covering most of dorsal surface, recumbent scales on dorsal midline largely narrow and white; proboscis and maxillary

palpus dark-scaled; scutum mostly covered with narrow pale falcate scales; postspiracular area with pale scales and setae; anterior surfaces of fore- and midfemora mostly dark-scaled with pale scaling posteroventrally, approximately proximal 0.5 of hindfemur pale-scaled with narrow anteroventral line of pale scales connected to apical pale patch on anterior surface, tibiae and tarsi of all legs dark-scaled; abdominal terga III–V and all sterna with conspicuous basal pale bands; segment VIII broad, not completely retracted into segment VII, cercus moderately long, somewhat cylindrical in dorsal view. Tergum IX short, bilobed, lobes widely separated by narrow basal strip.

Males

Gonocoxite without dorsomesal apical and dorsomesal basal lobes, ventromesal margin without group of prominent scales; mesal membrane with a setose basomesal sclerite (homologous with dorsomesal basal lobe).

Larvae

Antenna very long, narrow and bowed; seta 1-A very long and branched; seta 4-C inserted mesad and slightly posterior to seta 6-C; seta 5-C inserted slightly posterior to 7-C; seta 6-C inserted slightly anterior to 7-C; seta 12-C present; seta 13-C branched, seta 14-C single; comb scales spine-like, in a single irregular row; pecten spines evenly spaced; seta 1-S inserted distal to pecten; ventral brush (seta 4-X) with precratal setae.

Pupae

Seta 11-CT well developed, single; seta 6-VII inserted posterior to level of seta 9-VII; paddle long, essentially symmetrical.

Etymology

The subgeneric name is a Latinized diminutive patronym to recognize Dr. John F. Reinert for his extensive knowledge of aedine mosquitoes and his numerous contributions to mosquito taxonomy and systematics. The three-letter abbreviation *Rei.* is recommended for this subgenus.

Aedes (Reinertia) suffusus Edwards, 1922

Aedes (Finlaya) suffusus Edwards, 1922: 270. Holotype ♀: Simla, Himachal Pradesh State, India (Natural History Museum, London).

Aedes (Finlaya) suffusus of Edwards 1932: 155 (classification); Knight and Marks 1952: 539, 541 (key, classification, morphology, taxonomy, bionomics); Stone et al. 1959: 171 (India, type info.); Bhat 1975: ? (India, West Bengal State, collection record); Knight and Stone 1977: 105 (India, type info.); Darsie et al. 1996: 132 (Nepal, collection records, bionomics).

Aedes suffusus of Lien 1967: 177, 179 (Morphology)

The description is based on four females (one with associated larval and pupal exuviae), one male, four fourth-instar larvae, and five pupal exuviae.

Female

Small blackish brown mosquito, wing length 2.96–3.12 mm ($n = 4$). **Head:** Vertex with dark upright forked scales covering most of dorsal surface (Fig. 1a); decumbent scales on dorsal midline largely narrow and white, broad scales laterally (Fig. 1b and c); eyes above antennal pedicels contiguous; maxillary palpus entirely dark-scaled, 0.20 length of proboscis (Fig. 1d); proboscis entirely dark-scaled, 1.25 length of forefemur (Fig. 1d); antenna brown, 0.8 length of proboscis; clypeus bare. **Thorax:** Scutum, except prescutellar area, mostly covered with narrow white falcate scales (Fig. 2a); acrostichal and dorsocentral setae in complete rows; scutellum (Fig. 2b) with narrow pale scales on central area, lateral lobes with few narrow pale scales, each lobe with 4–6 long dark setae; paratergite bare; pleura with noticeably broad white scale-patches (Fig. 2c); mesopostnotum bare; anteprepronotum with narrow white scales; postpronotum with narrow pale crescent-shaped scales with a few broad white scales posteriorly; postspiracular area with a few broad white scales and setae posteriorly; subspiracular and prealar areas with patch of broad white scales, patch on prealar area distinctly separated from upper mesokatepisternal scale-patch; lower mesokatepisternal scale-patch well separated from upper patch; large patch of broad white scales on mesepisternum extending from midanterior area to upper mesepisternal setae; lower anterior

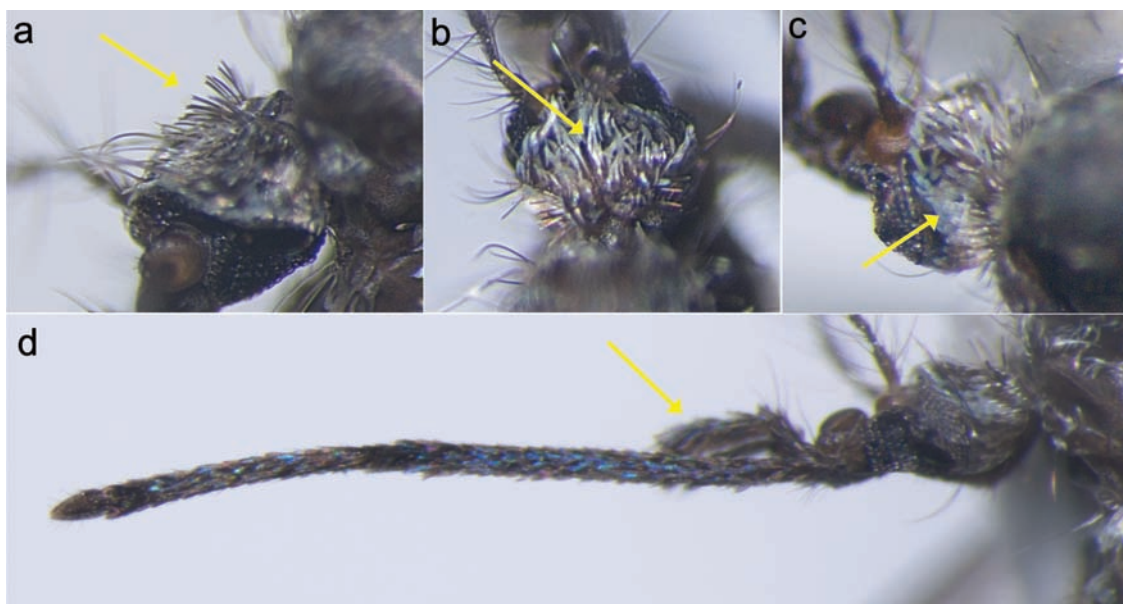


Fig. 1. *Aedes (Reinertia) suffusus* female: arrows indicate the positions of (a) erect scales of the head, lateral aspect; (b) recumbent falcate scales on dorsal midline of the vertex; (c) decumbent spatulate ocular scales; (d) the maxillary palpus (arrow) and proboscis.

mesepimeral seta absent. *Wing*: Covered with a mix of narrow and moderately broad dark scales, vein R_2 2.1–2.4 times longer than vein R_{2+3} (Fig. 2d), alula fringed with moderately broad dark scales (Fig. 2e), remigium with a few dark setae. *Legs*: Forefemur largely dark-scaled, proximal 0.5 of ventral surface with pale scales (Fig. 3a), posterior surface with some scattered pale scales toward apex (Fig. 3b); midfemur dark-scaled with ventral pale scaling expanding onto anterior surface before apex (Fig. 3c and d); about proximal 0.5 of hindfemur pale-scaled, with narrow anteroventral line of pale scales connected to apical pale patch on anterior surface (Fig. 3e and f), all tibiae and tarsi dark-scaled (Fig. 3g–i); tarsal claws (ungues) equal, both fore- and midungues with a tooth, hindungues simple. *Abdomen*: Terga III–V with narrow basal pale bands (Fig. 2f), terga I–VIII each with basolateral white spots, visible from dorsal aspect on segments VI and VII; sterna I–VI with white basal bands (Fig. 2g). *Genitalia* (Fig. 4a–c): Tergum and sternum of segment VIII with numerous broad scales, tergum VIII broadly rounded posteriorly, sternum VIII

slightly broadly emarginate posteriorly; tergum IX short, bilobed, lobes widely separated by narrow basal strip; cercus conspicuous, moderately long, somewhat cylindrical, width about 0.4 of length, scales present, dorsal surface with short stout setae; postgenital lobe more or less rectangular in dorsal view, posterior margin slightly but distinctly emarginate.

Male

Based on one specimen preserved in ethanol; some characters not apparent. Generally similar to the female but smaller, wing length 2.72 mm. *Head*: Maxillary palpus dark scaled, 0.85 length of proboscis, composed of five palpomeres, palpomeres 2 and 3 ankylosed and long, 4 and 5 and apex of three setose; proboscis 1.28 length of forefemur; antenna 0.6 length of maxillary palpus, two distal flagellomeres (12 and 13) equally long, combined length 1.5 times longer than combined length of flagellomeres 1–11. *Thorax*: Scutum

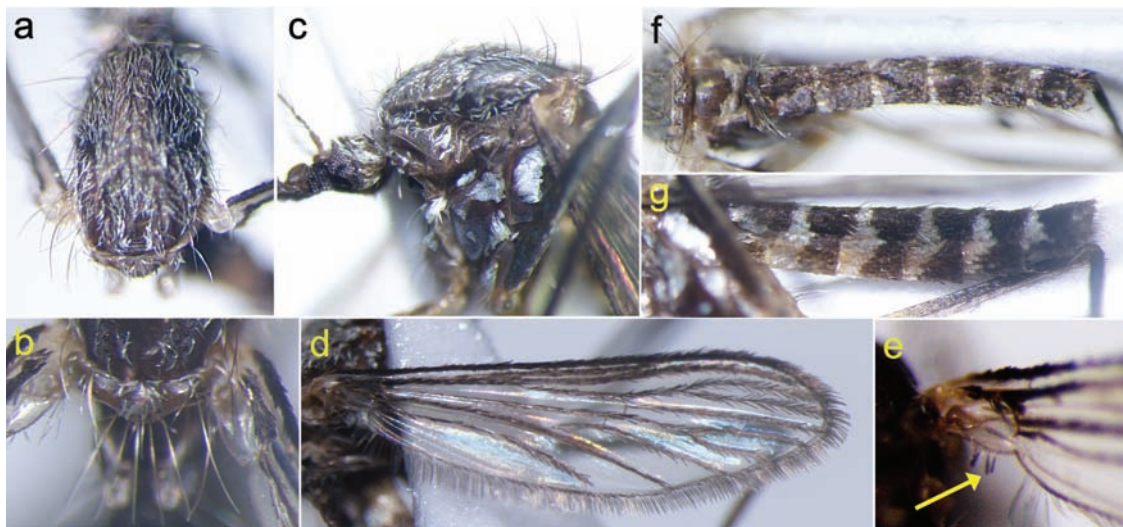


Fig. 2. *Aedes (Reinertia) suffusus* female: (a) scutum with narrow white falcate scales; (b) scutellum; (c) lateral aspect of the thoracic pleura; (d) wing, length of vein R_2 in relation to the length of vein R_{2+3} ; (e) wing, alula fringed with moderately broad dark scales (arrow); (f) abdominal terga; (g) lateral aspect of the abdomen.

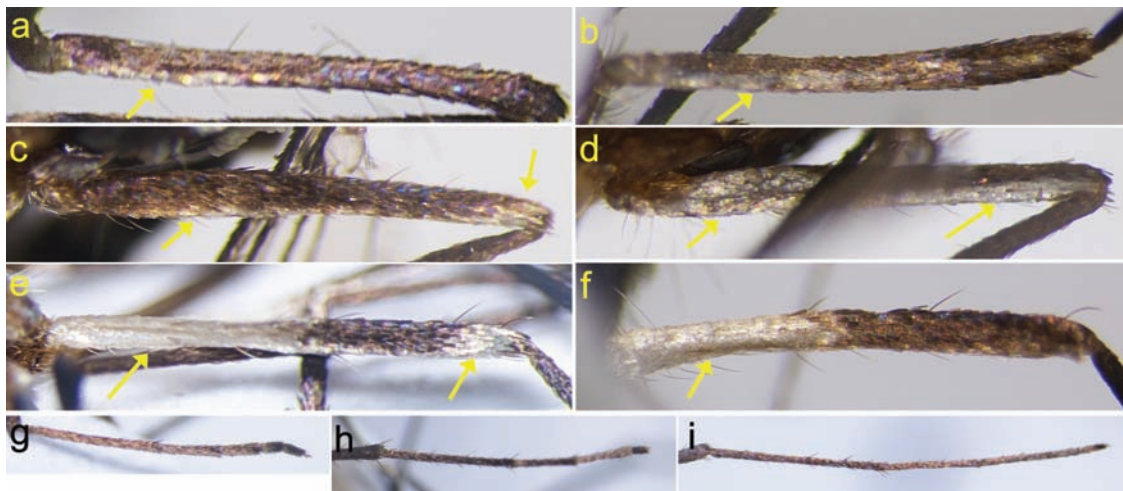


Fig. 3. *Aedes (Reinertia) suffusus* female: forefemur, (a) anterior aspect showing the pale-scaled proximal 0.5 of the ventral surface (arrow), and (b) posterior aspect showing some scattered pale scales toward apex (arrow); midfemur, anterior (c) and posterior (d) aspects showing the ventral pale scaling (arrows); hindfemur, (e) anterior aspect showing the proximal pale scaling continuous with a narrow anteroventral line of pale scales connected to the apical pale patch (arrows), (f) posterior aspect showing the proximal pale scaling (arrow), (g) foretarsus, (h) midtarsus, (i) hindtarsus.

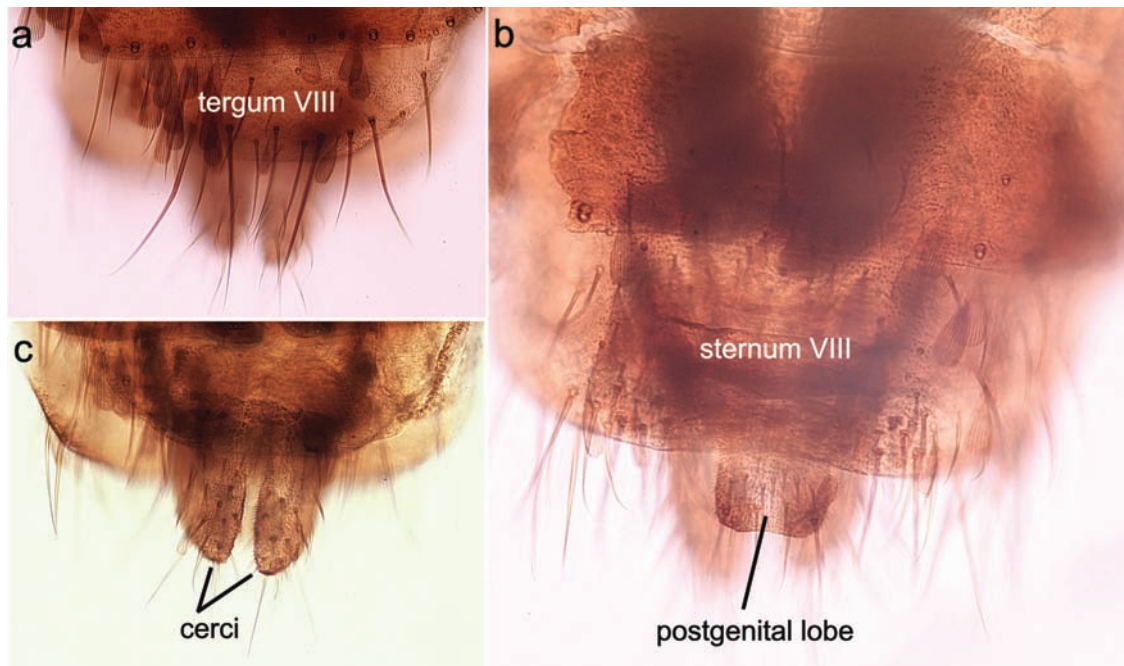


Fig. 4. *Aedes (Reinertia) suffusus* female genitalia: (a) dorsal aspect; (b) ventral aspect; (c) cerci.

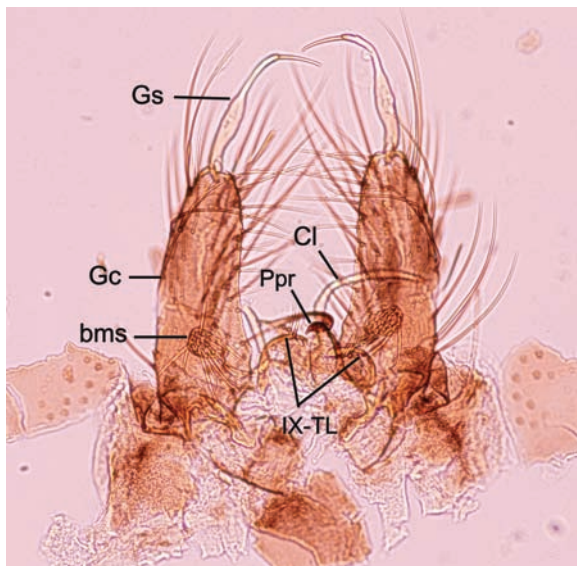


Fig. 5. *Aedes (Reinertia) suffusus* male genitalia: dorsal aspect. Cl, claspette; Gc, gonocoxite; Gs, gonostylus; bms, basomesal sclerite; Ppr, paraproct; IX-TL, ninth tergal lobes.

covered with narrow pale falcate scales as in female. *Legs*: As in female; fore- and midungues unequal, each with a tooth, hindungues equal and simple. *Abdomen*: As in female. *Genitalia* (Fig. 5): Ninth tergal lobes small, each bearing four or five short simple setae; gonocoxite long, relatively narrow, numerous scales on lateral and ventral surfaces, without dorsomesal apical lobe, ventromesal margin without group of prominent specialized scales, mesal surface largely membranous, bearing a unique setose basomesal sclerite; gonostylus attached at apex of gonocoxite, long, swollen in proximal 0.6, distal part narrow and gently curved mesad; gonostylar claw long, narrow, attached at apex of gonostylus; claspette with long narrow columnar

stem covered with minute setae, claspette filament attached apically, very long, about same length as stem, slightly flattened and slightly curved to acute apex; aedeagus damaged but apparently simple and tube-like, lateral sclerites outwardly bowed; proctiger with few to several cercal setae, paraproct strongly sclerotized, ending in a short curved beak-like point.

Egg

Unknown.

Larva (Fourth Instar)

Description based on four larvae and one larval exuvia. *Chaetotaxy* in Table 1. *Head* (Fig. 6a): Brownish yellow; median labral plate narrow, distinctly separated from dorsal apotome; seta 1-C long, single, slender, curved mesad; setae 4–6-C with numerous aciculate branches; seta 4-C weaker than setae 5,6-C, inserted mesad and slightly posterior to seta 6-C, distance between seta 4-C and 6-C slightly greater than distance between seta 4-C and 5-C; seta 6-C inserted slightly anterior to seta 7-C; seta 7-C inserted near base of antenna slightly anterior to 5-C, with numerous branches; seta 13-C with four to six branches. *Antenna*: Long, about 0.8 length of dorsal apotome, narrow, gently tapered and curved mesad, moderately spiculate, articulation with head weakly sclerotized; seta 1-A inserted slightly distal to midlength of shaft, long, reaching apex of shaft, with four or five aciculate branches; dorsosentum more or less triangular in ventral view (Fig. 6b), with 10–12 teeth on each side of large median tooth, median tooth about 2.5 times larger than immediately franking teeth, which are smaller than five or six most lateral teeth on each side. *Thorax* (Fig. 6c): Integument smooth; seta 0-P with 7–11 long branches; setae 1–3-P inserted on a common setal support plate, 1-P with 5–7 and 3-P with 4–7 strong aciculate branches; seta 4-P with two to four simple or weakly aciculate branches, weaker than 1-P; setae 5–10, 12-P long, aciculate; seta 1-M stellate, with short basal stem and five to seven stiff aciculate branches, not reaching insertion of seta 0-P; setae 2,3-M single, long,

Table 1. Number of branches (mode) for larval setae of *Aedes (Reinertia) suffusus* (four specimens).

| Seta no. | Head | Thorax Abdomen | | | | | | | | | | | | | | | |
|----------|-----------|----------------|---------|----------|-----------|-----------|-----------|-----------|-----------|--------|----------|---------|-------------|---|---|---|---|
| | | Pro- | Meso- | Meta- | I | II | III | IV | V | VI | VII | VIII | X | | | | |
| 0 | - | 7-11(8) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 1 | 5-7(6) | 5-7(7) | 6-10(9) | 13-17(14) | 10-16(11) | 11-15(12) | 10,11(11) | 10-14(12) | 8-16 | 7-16(10) | 6-7(6) | 3-5(4) | | | | |
| 2 | - | 1 | 1 | 1 | 1,2(1) | 1,2(1) | 2 | 1,2(2) | 1,2(1) | 1,2(1) | 1-2(1) | 1 | 5,6(5) | | | | |
| 3 | 1 | 4-7(6) | 1 | 2-5(3) | 1,2(1) | 1,2(1) | 1 | 1,2(1) | 2,3(2) | 1,2(1) | 1-3(1) | 1 | 1 | | | | |
| 4 | 7-10(8) | 2-4(4) | 2 | 1-3(2) | 5-7(5) | 3-6(5) | 2 | 1,2(2) | 1 | 1 | 1 | 1 | 5-8(5) | | | | |
| 5 | 7-9(8) | 4-6(5) | 1 | 1 | 3-5(3) | 5,6(5) | 5,6(5) | 4,5(5) | 4,5(5) | 3-5(4) | 4-7(5) | 7-15(8) | - | | | | |
| 6 | 5-8(6) | 1 | 4,5(4) | 1 | 2 | 2,3(2) | 2 | 2 | 2 | 2 | 5-8(8) | - | - | | | | |
| 7 | 10,11(11) | 3-5(4) | 1 | 6-9(8) | 1-3(2) | 4,5(4) | 3-5(5) | 3,4(4) | 3,4(4) | 1 | 1 | - | 1-S, 1-3(3) | | | | |
| 8 | 2 | 3-5(4) | 6 | 5-7(7) | - | 2 | 1,2(2) | 1 | 1 | 3 | 5-9(8) | - | - | | | | |
| 9 | 4-6(4) | 1,2(2) | 6,7(6) | 5-7(7) | 2,3(2) | 1 | 1,2(1) | 2 | 2 | 2 | 2,3(2) | - | - | | | | |
| 10 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - | - | | | | |
| 11 | 9-10(9) | 2,3(2) | - | 2-4(2) | 1,2(1) | 1 | 1 | 1 | 1 | 1 | 1 | - | - | | | | |
| 12 | 2,3(2) | 1 | 1 | 1 | 2 | 2 | 2 | 1,2(2) | 1 | 1 | 1 | - | - | | | | |
| 13 | 4-6(5) | - | 7-11(8) | 7-13(11) | 5-7(6) | 8-14(11) | 8-15(11) | 9-12(12) | 10-12(10) | 5-9(8) | 5-8(8) | - | - | | | | |
| 14 | 1 | 2,3(2) | 7-12(8) | - | - | - | 1 | 1 | 1 | 1 | 1,2(1) | 1,2(1) | - | | | | |
| 15 | 10-11(11) | - | - | - | - | - | - | - | - | - | - | - | - | | | | |

Table 2. Number of branches (mode) for pupal setae of *Aedes (Reinertia) suffusus* (five exuviae)

| Seta no. | Cephalothorax | | Abdominal segments | | | | | | | | | | Paddle | |
|----------|---------------|----------------|--------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---|
| | CT | I | II | III | IV | V | VI | VII | VIII | Pa | | | | |
| 0 | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 1 | 2-4(4) | N ^a | 6-17(9) | 3-7(3) | 1-5(2) | 2,3(2) | 1-3(2) | 1 | 1,2(1) | 1-3(2) | 1 | 1,2(1) | 1 | 1 |
| 2 | 1-3(2) | 1,2(1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 3 | 1 | 1 | 1,2(1) | 1 | 2,3(2) | 1,2(1) | 1,2(1) | 1,2(1) | 1,2(1) | 1,2(1) | 1,2(1) | 1,2(1) | 1,2(1) | - |
| 4 | 2,3(3) | 2-4(3) | 2-4(3) | 2-4(2) | 2,3(2) | 1,2(1) | 1,2(1) | 2 | 2 | 1,2(1) | 1 | 1 | 1,2 | - |
| 5 | 1,2(2) | 1,2(2) | 1,2(1) | 1-4(1) | 1,2(2) | 1,2(1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 6 | 1,2(1) | 1,2(1) | 1,2(1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 7 | 2 | 2 | 2,3(2) | 2-4(3) | 2-4(2) | 2,4(3) | 1 | 1 | 1 | 1 | 1,2(1) | 1,2(1) | 1,2(1) | - |
| 8 | 2,3(2) | - | - | 1,2(2) | 1-3(1) | 1,2(1) | 1,2(2) | 1,2(1) | 1,2(1) | 1,2(2) | 1,2(1) | 1,2(1) | 1,2(1) | - |
| 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2(1) | 1,2(1) | 1,2(1) | - |
| 10 | 3-6(4) | - | - | 1-3(1) | 1,2(1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 11 | 1 | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 12 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |

^aNumerous.

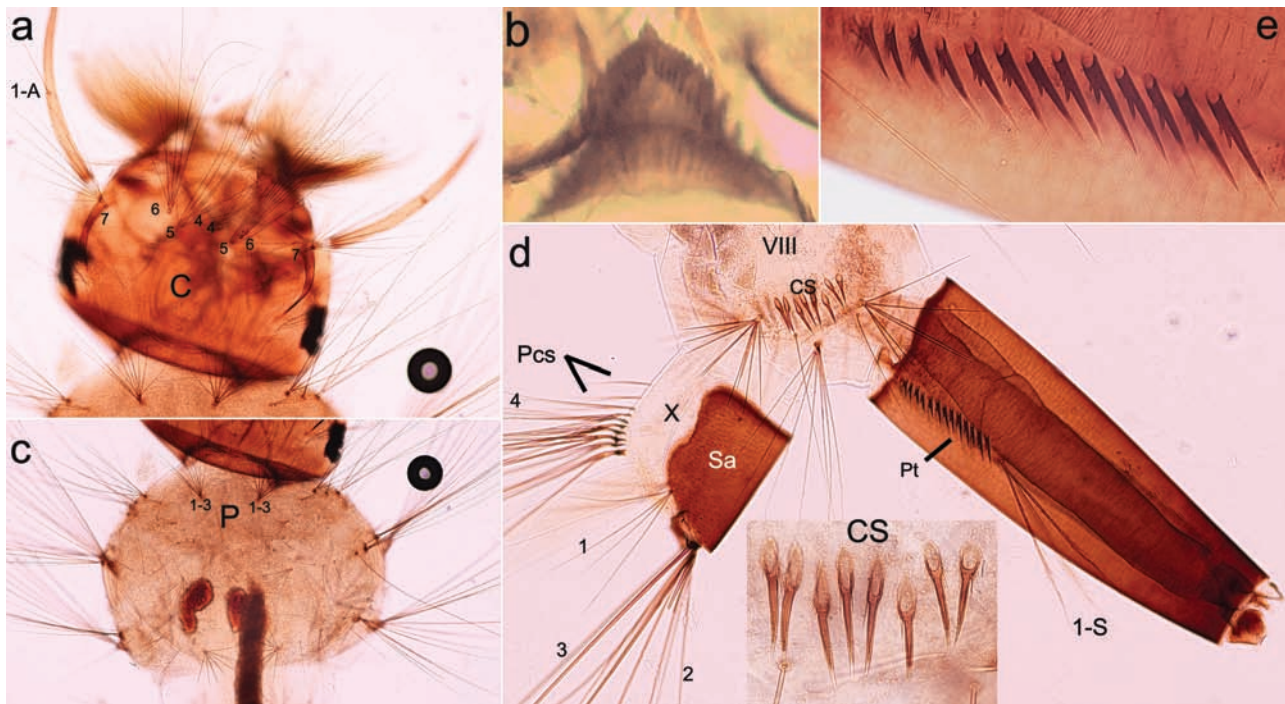


Fig. 6. *Aedes (Reinertia) suffusus* larva: (a) dorsal aspect of the head; (b) dorsomentum; (c) dorsal aspect of the thorax; (d) posterior abdominal segments and siphon, comb scales in high magnification below; (e) pecten spines. CS, comb scales, Pcs, precratal setae, Pt, pecten, Sa, saddle.

weakly aciculate; setae 6–10,12,13-M long, aciculate; seta 11-M apparently absent; seta 1,13-T similar to 1-M, stellate with multiple stiff aciculae branches; setae 7,9,10-T with long branches; seta 11-T present. **Abdomen:** Integument smooth with minute spicules dorsad of comb; setae 1,13-I–VII, 4-I and 5-II–VIII stellate, with short basal stems and multiple stiff aciculate branches; seta 1-VIII with strong aciculate branches; comb of segment VIII with 6–10 spine-like scales arranged in a single irregular row, each scale with large, long, lateral denticles at base (Fig. 6d). **Siphon (Fig. 6d):** Length 3.0–4.5 times width at base, acus present; pecten with 14–17 evenly spaced spines, spines increase in size from base of siphon, spines at middle of pecten with one or two strong denticles and one to four minute denticles along basal 0.25 of lower side (Fig. 6e); seta 1-S long, with one to three (usually three) aciculate branches, inserted <0.5 length of siphon from base beyond distal pecten spine directly in line with pecten, not reaching posterolateral spiracular lobe. **Segment X:** Saddle incomplete, covering dorsal 0.67 of segment, posterior margin without strong spicules; seta 1-X with three to five aciculate branches, inserted on caudolateral margin of saddle, longer than length of saddle; seta 2-X with five or six long aciculate branches; seta 3-X single, long, aciculate; ventral brush (seta 4-X) comprised 10 setae, with 8 setae inserted on grid with both lateral and transverse grid bars and 2 precratal setae inserted on ventral midline of segment; anal papillae tapered, slightly longer than length of segment X.

Pupa

Description based on exuviae of five females. Chaetotaxy in Table 2. **Cephalothorax:** Light brown; seta 1-CT with two to four branches; seta 2-CT with one to three branches; setae 3,9,11-CT single; setae 5,6-CT with one or two branches; setae 4,8-CT with two or three branches; setae 7,12-CT double, seta 10-CT with three to six branches; 11-CT single, strong, darkly pigmented.

Trumpet (Fig. 7a): Elongate funnel-shaped; length 0.41–0.44 mm, pinna 0.23–0.32 length of trumpet; tracheoid area weakly developed. **Abdomen (Fig. 7b):** Seta 1-I well developed, with numerous aciculate dendritic branches; seta 1-II well developed, with 6–17 branches, stronger than seta 1-III,IV with fewer branches; seta 1-V,VI usually with two or three branches, weaker than seta 1-III,IV; seta 2-II inserted lateral to seta 1; seta 5-IV,V usually double, strong and longer than abdominal segments IV–VI, respectively; seta 5-VI single, shorter than abdominal segment VII; seta 6-II inserted posterior to seta 9-II; seta 9-VII well developed, with 1 or 2 aciculate branches; seta 9-VIII well developed, with three or four aciculate branches. **Paddle (Fig. 7c):** Long, length 0.78–0.84 mm, width 0.49–0.63 mm, index 0.56–0.81; inner and outer parts essentially symmetrical; midrib distinct from base to apex; about proximal 0.6 of outer margin with small saw-like serration along refractile border, distal 0.25 of inner and outer margins with short hyaline spicules; seta 1-Pa single, long, about 0.2 length of paddle; seta 2-Pa absent.

Bionomics and Medical Importance

Little is known about the bionomics of *Ae. suffusus*, and whether or not it plays a role in the transmission of pathogenic agents. The immature stages are found in tree holes at high altitude in mountain forest (Edwards 1922, Barraud 1934, Darsie et al. 1996, present study). Darsie et al. (1996) found females resting on walls and attracted to humans in a guest house in northwestern Nepal, and Bhat (1975) captured a female attracted to humans in West Bengal State in eastern India.

Distribution

Aedes suffusus is known only from the Himalaya Mountains of Bhutan, India, and Nepal. It is probably more widespread in other high-altitude areas of these countries, Pakistan and the Tibetan

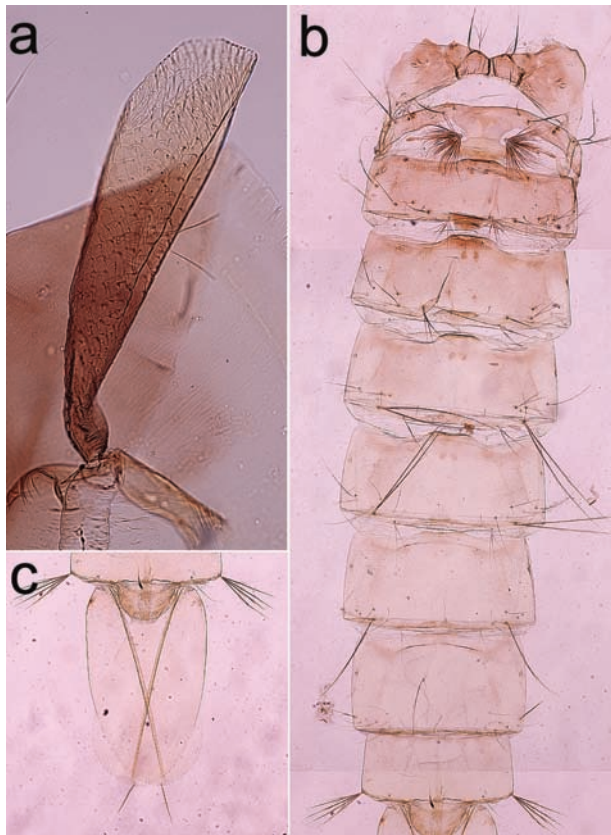


Fig. 7. *Aedes (Reinertia) suffusus* pupa: (a) trumpet; (b) abdominal segments; (c) genital lobe and paddles.

Plateau on the northern side of the Himalayas with mountain forest, but further surveys are needed to establish this.

Specimens Examined

Fifteen specimens, 4♀ with larval and pupal exuviae on a microscopic slide, 1♂ and 4 fourth-instar larvae, mounted on separate microscope slides, with following collection data. BHUTAN: Thimphu District, collected at 27°35'32"N, 89°38'23"E, 2890 m AMSL) about 14 km north of Thimphu city, on the way to Tango Monastery, 11 September 2018, coll. P. Somboon. One ♀ (BT-TP 2018-1) with associated larval and pupal exuviae and one larva are deposited in the collection of the Entomology Section, Queen Sirikit Botanic Garden (QSBG), Chiang Mai, Thailand. The other specimens (3♀, ♂, larvae and pupae exuviae) are retained in the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Phylogenetic Analysis

The results of the phylogenetic analyses based on the 559-bp fragment of the *COI* gene are shown in Fig. 8 (ML) and Fig. 9 (BI), which show the relationships of the 38 subgenera of *Aedes* and the other six genera of Aedini. It is interesting to note that species of the same subgenera or groups, i.e., *Bothaella* Reinert, *Downsiomyia*, *Hulecoeteomyia* Theobald, *Jarnellius* Reinert, Harbach & Kitching, *Ochlerotatus* Lynch Arribálzaga (Serratus Group), and *Ochlerotatus* (Woodius Group), and the genus *Psorophora* Robineau-Desvoidy subgenus *Grabhamia* Theobald, often share the nodes with bootstrap values > 70% and Bayesian

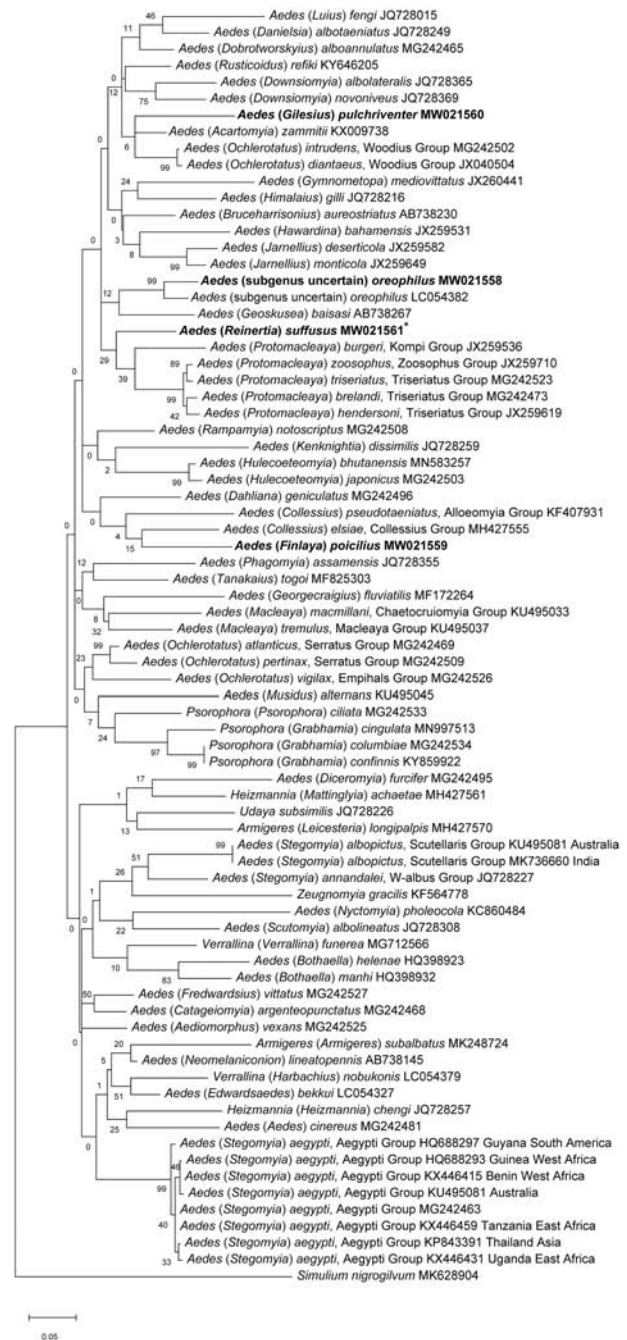


Fig. 8. The maximum likelihood phylogeny of *COI* sequences of mosquitoes of the genus *Aedes* and six other genera of the tribe Aedini. Specimens sequenced in the present study are in boldface.

PP of 0.95–1.00. In contrast, species of different genera or subgenera, or species of the same subgenus but different groups, i.e., the Collessius and Alloemyia Groups of *Aedes* subgenus *Collessius* Reinert, Harbach & Kitching, the Chaetocruimyia and Macleaya Groups of *Aedes* subgenus *Macleaya* Theobald, the Empihals, Serratus and Woodius Groups of *Ae. (Ochlerotatus)*, the Aegypti, Scutellaris and W-albus Groups of *Aedes* subgenus *Stegomyia* Theobald, *Armigeres* Theobald subgenera *Armigeres* and *Leicesteria* Theobald, *Heizmannia* Ludlow subgenera *Heizmannia* and *Mattinglyia* Lien, *Psorophora* subgenera

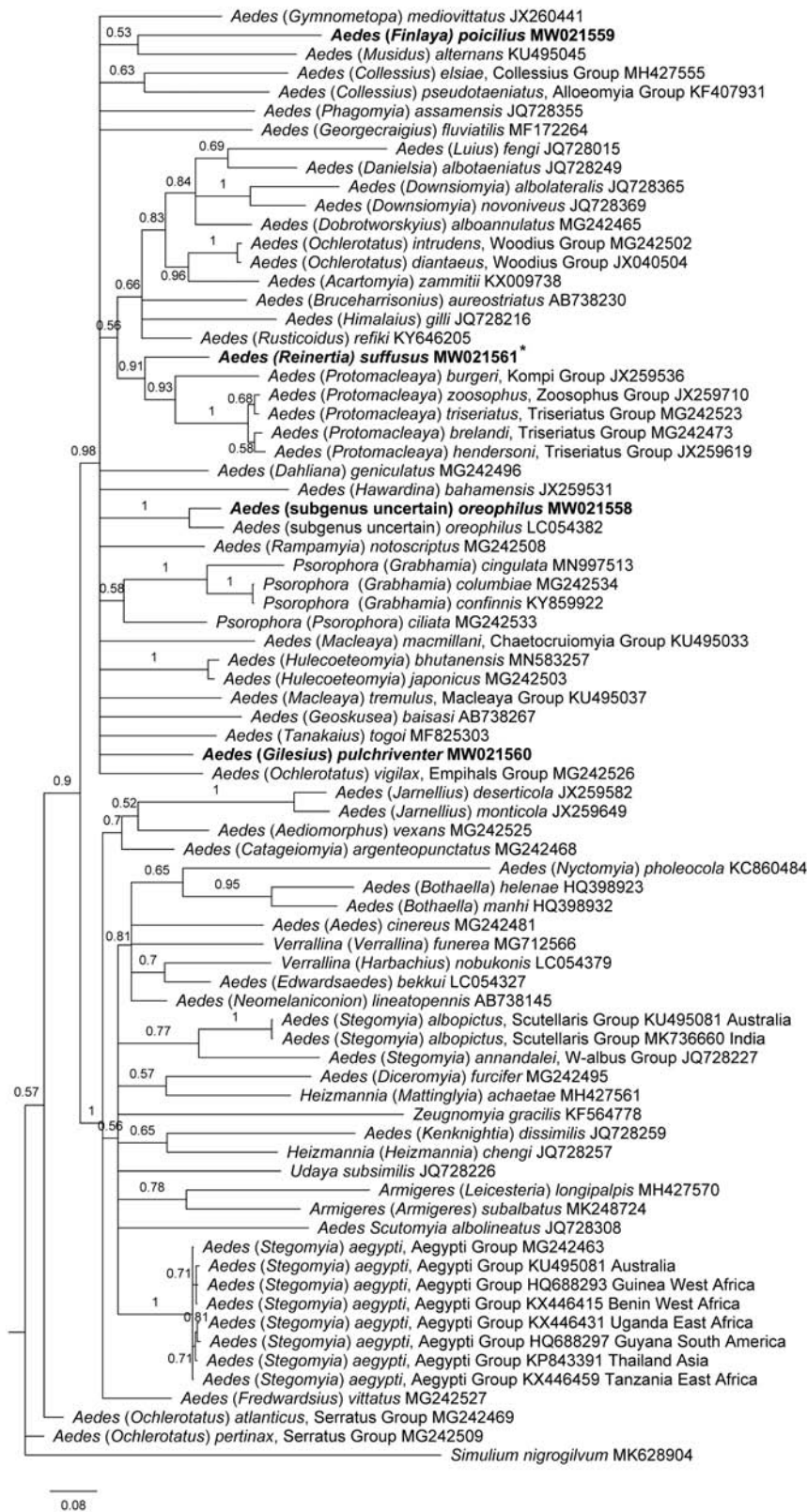


Fig. 9. The Bayesian inference phylogeny of *COI* sequences of mosquitoes of the genus *Aedes* and six other genera of the tribe Aedini. Specimens sequenced in the present study are in boldface.

Grabhamia and *Psorophora* and *Verrallina* Theobald subgenera *Harbachius* Reinert and *Verrallina*, are often placed in different clades and/or in the same clade with weakly supportive bootstrap

values (<70%) and PP < 0.95. The new subgenus *Reinertia* appears to have some affinity with species of the subgenus *Protomacleaya*, but the relationship is weakly supported (bootstrap 29%; PP

0.91). These results, along with the morphological distinctions, indicate that *Reinertia* cannot be placed in any currently recognized subgenus of *Aedes* included in the analyses.

Discussion

Prior to the study of Reinert et al. (2006), *Ae. suffusus* was placed in the polyphyletic subgenus *Finlaya* Theobald (Knight and Stone 1977). Based on morphological features of the adults, larvae, and pupae, *Ae. suffusus* appears to be most closely allied to species of the subgenera *Bruceharrisonius* Reinert, *Collessius*, *Dabliana* Reinert, Harbach & Kitching, *Danielsia* Theobald, *Downsiomyia*, *Gilesius* Reinert, Harbach & Kitching, *Himalaius* Reinert, Harbach & Kitching, *Hulecoeteomyia*, *Jiblienius* Reinert, Harbach & Kitching, and *Phagomyia* Theobald, as currently defined.

The unique basomesal sclerite borne proximally on the mesal membrane of the gonocoxite is apparently homologous with the basal tergal lobe of Belkin (1962), the basal ridge of Knight and Harrison (1988), and the dorsomesal basal lobe of Reinert et al. (2009). The dorsomesal basal lobe is absent in *Collessius*, *Dabliana*, *Gilesius*, *Himalaius*, *Hulecoeteomyia*, *Jiblienius*, and *Phagomyia*; it is present in *Bruceharrisonius*, *Danielsia*, and *Downsiomyia*. However, the species of *Jiblienius*, and some species of the other subgenera, have a small dorsomesal area at the base of the gonocoxite that bears a dense collection of long setae, which bears some resemblance to the detached setose sclerite of *Reinertia*. Such a lobe is also present in some other species of *Aedes* without subgeneric assignment, e.g., *Ae. crossi* Lien and *Ae. oreophilus*. Lien (1967) surmized that *Ae. crossi* was closely related to both *Ae. oreophilus* and *Ae. suffusus*, but in addition to lacking an independent basomesal sclerite on the mesal membrane of the gonocoxite, *Ae. crossi* and *Ae. oreophilus* both differ from *Ae. suffusus* in several invariable features of the larvae, including a shorter antenna, differently developed seta 6-C and dorsomentum, lack of stellate setae, and the presence of seta 12-I and squamiform comb scales arranged in a patch. The results of the ML and BI analyses also do not support a relationship with *Ae. oreophilus*.

The pupa of *Reinertia* differs from the pupae of *Bruceharrisonius* and *Himalaius* in having seta 6-VII inserted posterior to seta 9-VII, which is the condition in pupae of the other related subgenera. Seta 13-C is branched and 14-C is single in larvae of *Reinertia*. As far as known, this combination of developmental states does not occur in species of the apparently allied subgenera. Of species previously included in the polyphyletic concept of *Finlaya*, this combination is known only in species of the Afrotropical subgenera *Hopkinsius* Reinert, Harbach & Kitching and *Vansomerensis* Reinert, Harbach & Kitching, and is recognized in three species whose subgeneric placement is uncertain: the Australian *Ae. biocellatus* (Taylor), *Ae. crossi* of Taiwan, and *Ae. keefeii* King & Hoogstraal known only from the island of New Guinea (based on data recorded in the study of Reinert et al. 2009).

Edwards (1932) established informal groups for species that were recognized at the time as members of the subgenus *Finlaya*. He recognized *Ae. oreophilus* and *Ae. suffusus* as species of Group H. Knight and Marks (1952) refined the classification by dividing Edwards' groups into subgroups. They established the monobasic Subgroup V for *Ae. suffusus*. Based on significant differences in the vestiture of females and features of the larvae, they established a separate subgroup for *Ae. oreophilus*, Subgroup IV. *Aedes oreophilus* was originally described as a species *Ochlerotatus* by Edwards (1916). Our findings confirm that *Ae. oreophilus*, and also the

more recently described *Ae. crossi*, do not belong in a group with *Ae. suffusus*. Oddly, however, the larva of *Ae. crossi* shares an uncommon setal combination with *Ae. suffusus*, i.e., both have seta 13-C branched and 14-C single (see below).

Surprisingly, *Ae. suffusus* seems to share some morphological features with species of the Palearctic subgenus *Dabliana* in addition to those of the Oriental subgenera. They share the following characteristics: the proboscis, maxillary palpi, tibiae, and tarsi of the adults are dark-scaled; the gonostylus, claspette, and paraproct of the male genitalia are similarly developed; and the larvae have a single row of spine-like comb scales, seta 1-S is inserted at the distal end of the pecten, the ventral brush has precratal setae, and they inhabit tree holes. The mesal membrane of the gonocoxite of species of *Dabliana*, however, does not bear a basomesal sclerite and a dorsomesal basal lobe is absent.

The phylogenetic analyses conducted during this study clearly show that *Reinertia* does not belong to any currently recognized subgenus or species group of *Aedes*. It is recovered in a weakly supported sister-group relationship with species of the New World subgenus *Protomacleaya*, which, despite the morphological similarities with *Dabliana*, precludes a close affinity with that subgenus. Although we only used COI sequences to explore phylogenetic relationships among aedine taxa, the analyses generated relationships that are mostly in accord with the relationships recovered in the study of Soghigian et al. (2017), who used seven molecular markers in their phylogenetic analyses of aedine taxa. The results of the present study support the results of previous studies which show that *Aedes* is not a monophyletic taxon (e.g., Reidenbach et al. 2009; Reinert et al. 2009; Soghigian et al. 2017); in particular, groups of the subgenus *Ochlerotatus* are recovered as the sister taxa of different subgenera of *Aedes*, supporting the elevation of *Ochlerotatus* to generic status by Reinert (2000), which was later returned to subgeneric status by Wilkerson et al. (2015). Similarly, mosquitoes of the subgenera of *Armigeres*, *Heizmannia*, and *Verrallina* are recovered in relationships to various subgenera of *Aedes*. Moreover, the recovery of the Aegypti Group of the subgenus *Stegomyia* separated from the Scutellaris and W-albus Groups raises a question about its status as a monophyletic taxon.

Acknowledgments

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