



Systematics of *Anopheles (Cellia) yaeyamaensis* sp. n., alias species E of the *An. minimus* complex in southeastern Asia (Diptera: Culicidae)

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Abstract

Species E of the *Anopheles minimus* sibling species complex is diagnosed and formally named *An. yaeyamaensis* Somboon & Harbach, **sp. n.** Information is provided on the morphology, genetics and bionomics of the species, which has a restricted distribution in the Yaeyama and Miyako Islands located at the southern end of the Ryukyu Archipelago of Japan.

Key words: *Anopheles harrisoni*, *Anopheles minimus*, *Anopheles minimus* E, Minimus Complex, mosquito, new species

Introduction

The Minimus Complex of *Anopheles* subgenus *Cellia* occupies a geographical range in the Oriental Region that extends northward to about 32° 30' N in China, westward to Uttar Pradesh in India, southward to the Thai-Malay Peninsula (Manguin *et al.*, 2008), possibly reaching south to the northwestern corner of Malaysia (Reid, 1968), and eastward to the Ryukyu Archipelago of Japan. The complex may include five or six species, but only three have been fully documented. Green *et al.* (1990) showed that *An. minimus* Theobald consisted of two species (denoted as species A and C) in western Thailand based on the sympatric occurrence of homozygotes of two enzyme loci in the absence of heterozygotes. Green and colleagues (see Baimai, 1989) also recognised a third species (denoted as species D) in sympatry with species A and C based on electrophoretic data. Sharpe *et al.* (1999, 2000) confirmed the presence of species A and C in western Thailand and suggested the possible presence of another species (specimen #157). Chen *et al.* (2002) concluded that forms A and B of Yu & Li (1984) and Yu (1987) in China were morphological variants of species A, and that subspecies X of Baba (1950) in southern China probably referred to *An. aconitus* Dönitz. Recently, Somboon *et al.* (2001, 2005a) provided morphological, cytogenetic, molecular and hybridisation evidence for the recognition of another sibling species of the complex on Ishigaki Island in the Ryukyu Archipelago of Japan, which they informally designated as species E.

Anopheles minimus was named and described by Theobald (1901) from a single female that became non-extant after 1907 (Harrison, 1980). To fix the identity of this species and provide a foundation for further taxonomic studies of the Minimus Complex, Harbach *et al.* (2006) designated a neotype for *An. minimus* from specimens collected near the original type locality in Hong Kong. Sequences for the D3 domain of the 28S locus of ribosomal DNA (rDNA) and the cytochrome oxidase subunit II locus (COII) of mitochondrial DNA

(mtDNA) obtained from a hindleg of the neotype confirmed its identity as species A. Fixing the identity of *An. minimus s.s.* made it possible for Harbach *et al.* (2007) to resolve the taxonomy of species C and formally name it *An. harrisoni* Harbach & Manguin based on unique sequences for the ITS2 and D3 loci of rDNA and the COI, COII and cyt b loci of mtDNA (Garros *et al.*, 2005a,b; Harbach *et al.*, 2007). Based on available genetic, molecular and morphological distinctions, species E is formally described and named *An. yaeyamaensis* **sp. n.** in this report.

Material and methods

This study is based on specimens of *An. minimus* species E (Somboon *et al.*, 2001), with associated larval and pupal exuviae, that were reared from fourth-instar larvae collected on Ishigaki Island within the Yaeyama Island group of the Ryukyu Archipelago of Japan. Observations of adults were made under simulated natural light. Larval and pupal chaetotaxy were studied using bright-field and differential-interference microscopy. Setal branching counts were obtained from 10 specimens (20 setae) of each life stage. Unless indicated otherwise, numbers in parentheses represent modes of the reported ranges. The morphological terminology and abbreviations contained in the Anatomical Glossary of the online Mosquito Taxonomic Inventory (<http://mosquito-taxonomic-inventory.info/anatomical-glossary-overview>) are used in the descriptions and illustrations, respectively. The symbols A, ♀, ♂, ♂G, E, Le, Pe, L and P used in the literature summary and the **Type series** represent adult, female(s), male(s), male genitalia, egg(s), larval exuviae, pupal exuviae, fourth-instar larvae and pupae, respectively. An asterisk (*) following these symbols in literature citations indicates that at least part of the life stage is illustrated in the publication. The specimens are deposited in the Natural History Museum (NHM), London.

Systematics

Anopheles (Cellia) yaeyamaensis Somboon & Harbach, **sp. n.**

Anopheles formosaensis I of Miyasaki, 1902: 66 (Ishigaki Island).

Anopheles minimus of Bohart, 1959: 195 (Ishigaki and Iriomote Islands; L bionomics); Tanaka *et al.*, 1975: 210 (Ishigaki and Miyako Islands; distribution, bionomics); Harrison, 1980: 84 (in part, Miyako and Yaeyama Guntō Islands); Miyagi & Toma, 1980: 83–89, 91 (Iriomote Island; A L bionomics); Toma & Miyagi, 1986: 11, 28, 33, 41, 95, 96, 102 (Ishigaki, Iriomote and Miyako Islands; A* L* keys, bionomics, distribution); Toma *et al.*, 1996a: 63–71 (Ishigaki Island; L bionomics); Toma *et al.*, 1996b: 167–169 (Miyako Island; L distribution, bionomics); Tsuda *et al.*, 1999: 601–603 (Ishigaki Island; A bionomics); Somboon *et al.*, 2001: 98–101, 102 (figs 4–6, 8), 103–111 (Ishigaki Island; A, ♀ cibarial armature* E L, metaphase karyotype*, crossmating, D3 of 28S rDNA; designation as *An. minimus* species E); Toma *et al.*, 2002a: 29–40 (Ishigaki Island; L bionomics); Toma *et al.*, 2002b: 146–149 (Ishigaki Island; susceptibility to *Plasmodium yoelii nigeriense*); Higa *et al.*, 2003: 257, 258, 260–264 (Ishigaki Island; bionomics); Sawabe *et al.*, 2003: 772–778 (Ishigaki Island; ITS1, ITS2 and D3 of 28S rDNA); Toma *et al.*, 2003: 267–273 (Iriomote and Kohama Islands; L distribution, bionomics); Toma, 2006: 125–127 (Miyako Island; A L bionomics); Nakagawa *et al.*, 2009: 97–99, 105, 106 (Ishigaki Island; bionomics).

Anopheles minimus minimus of Miyagi & Toma, 1978: 245–247, 250 (Ishigaki and Iriomote Islands; A L bionomics).

Anopheles minimus species E of Somboon *et al.*, 2000a: 136 (summary); Somboon *et al.*, 2000b: 476–478 (Ishigaki Island; crossmating, ♀ cibarial armature*); Harbach, 2004: 540, 545 (classification); Somboon *et al.*, 2005a: 5–9 (crossmating); Somboon *et al.*, 2005b: 605–608 (crossmating); Garros *et al.*, 2006: 103–106, 108 (distribution); Sungvornyothin *et al.*, 2006: 185 (A morphology); Harbach *et al.*, 2007: 41, 45, 47, 49 (DNA sequence: ITS2 and D3 of 28S rDNA, COII mt DNA); Manguin *et al.*, 2008: 490, 491 (taxonomy, distribution); Garros *et al.*, 2008: print p. 2 (distribution).

Anopheles (Cellia) minimus of Tanaka *et al.*, 1979: 31, 47–51, 554, figs 11, 12 (Miyako and Yaeyama Guntō Islands; ♀* ♂ ♂G* L* P, keys, distribution, bionomics).

Diagnosis. Sequences for the ITS1, ITS2 and the D3 domain of the 28S rDNA and COII mt DNA (Somboon *et al.*, 2001; Sawabe *et al.*, 2003; Harbach *et al.*, 2007: figs 2–6) distinguish *An. yaeyamaensis* from the other

members of the Minimus Complex, i.e. *An. minimus* and *An. harrisoni*. Comparative anatomical studies indicate that the adult and pupal stages of the three species are essentially isomorphic in all life stages, however the number of branches of larval seta 7-C will distinguish most larvae of *An. yaeyamaensis* from those of *An. minimus*. The metaphase karyotype of *An. yaeyamaensis* is distinct from that of *An. harrisoni* (see below).

Description. ADULT (female)—As described and illustrated by Tanaka *et al.* (1979, available at <http://www.mosquitocatalog.org/files/pdfs/WR159.pdf>), with following additional details. *Head*: Antennal length about 1.1 mm, flagellomeres 1–3 with pale scales on mesal surfaces. Proboscis length about 1.5 mm. Maxillary palpus 1.4–1.6 mm long, with 3 pale bands (in dorsal view), apical pale band about length of preapical dark band and slightly longer than preapical pale band. *Cibarial armature*: Cone filaments thorn-like, relatively narrow and gradually tapered to pointed apex [filaments lancet-like in *An. minimus* and *An. harrisoni* (see Somboon *et al.*, 2001: figs 1–8)]. *Thorax*: Integument brown, pleura with darker areas; scutum with broad median pale pruinose area confluent with scutellum of similar appearance; anterior promontory with long erect white falcate scales that transition into semi-erect pale golden piliform scales on acrostichal and dorsocentral areas that extend posteriorly to and then on lateral margin of prescutellar area to scutellum; long golden-brown to brown setae on acrostichal, dorsocentral and prescutellar areas, dark setae on fossal, antealar and supraalar areas. *Wing*: Length 2.1–3.4 mm; dark scaling very black on costa, subcosta and R–R1, subdued on posterior veins, pale scaling pale yellow, not white; humeral pale spot rarely absent and presector pale spot occasionally absent on one or both wings (in 1 of 87 and 5 of 87 feral females, respectively), vein R3 often (about 50%) with median pale spot and 1A pale fringe spot frequently (about 80%) present (Somboon, unpublished observations). *Legs*: Coxae and trochanters without scales; femora, tibiae and tarsi dark-scaled, apices of tibiae indistinctly pale, tarsomeres 1–4 with minute faint dorsoapical pale spots. *Abdomen*: Integument dark with uniform covering of golden setae. ADULT (male)—As described and illustrated by Tanaka *et al.* (1979), with following additional details. *Head*: Eyes more widely separated, decumbent falcate scales of interocular space slightly more numerous. Proboscis longer and more slender, approximately 1.4 length of forefemur. *Wing*: Generally paler and scaling reduced, fringe spots less distinct. PUPA—Habitus and chaetotaxy as described and illustrated for *An. minimus* by Harbach *et al.* (2006), setal branching compared with that of *An. minimus* in Table 1, differing as follows. *Trumpet*: Length 0.34–0.39 mm, meatus 0.03–0.09 mm, pinna 0.27–0.33 mm. *Abdomen*: Length 1.97–2.36 mm. *Genital lobe*: Length 0.13–0.17 mm in female; 0.34–36 mm in male. *Paddle*: Length 0.61–0.64 mm, width 0.41–0.47 mm, index 1.30–1.54. LARVA (fourth-instar)—Habitus and chaetotaxy as described and illustrated by Tanaka *et al.* (1979), setal branching compared with that of *An. minimus* in Table 2, with following additional details. *Head*: Slightly wider than long, width 0.57–0.62 mm, length 0.54–0.60 mm; integument with variable pattern of moderately to darkly pigmented areas; collar and mentum darkly pigmented. Seta 7-C with 17–26(21) branches [14–18(16) in type specimens of *An. minimus* (see Harbach *et al.*, 2006) and 19/15 [left/right sides] in holotype of *An. harrisoni* (see Harbach *et al.*, 2007)]. *Thorax*: Integument hyaline, smooth. Mesothorax with conjoined pair of median notal plates, sometimes also with pair of submedian notal plates; metathorax usually with separated pair of notal plates, apparently without submedian notal plates. Setae 1,2-P inserted on narrowly separated tubercles; support plate of pleural setal groups 9–12-P,M,T with short spine. *Abdomen*: Integument hyaline, smooth. Seta 0-III–VII well developed, normally branched, not noticeably larger on segments IV and V; seta 1-I–VII fully palmate with moderately pigmented leaflets, leaflets with distinct shoulders and long slender filaments (blades and shoulders narrower on segment I), blades usually with distal patch of darker pigment near shoulder. Pecten with long spines usually at each end and several interspersed among short spines. Saddle moderately to darkly pigmented, length about 0.22 mm. Seta 1-X single, simple, inserted on saddle; 4-X (ventral brush) with 9 offset pairs of setae, longest branches on anterior side of main stems. Dorsal and ventral anal papillae equal in length, shorter to slightly longer than saddle. EGG—In general as described for *An. minimus* by Reid (1968); length 0.39–0.55 mm (mean 0.45 mm); deck usually complete (Somboon *et al.*, 2001).

TABLE 1. Range (mode) of branches for pupal setae of *Anopheles yaeyamaensis* (in boldface type) and the neotype series of *An. minimus* from Hong Kong (below boldface entries) taken from Harbach *et al.* (2006).

| Setae no. | Cephalothorax | | Abdominal segments | | | | | | | | | | Paddle | |
|-----------|---------------|-----------------|--------------------|------------------|----------------|---------------|--------|---------------|---------|---------------|----------|----------|--------|--|
| | CT | I | II | III | IV | V | VI | VII | VIII | IX | IX | Pa | | |
| 0 | - | - | 1 | 1-4(1) | 1-5(2) | 1-4(2) | 1,2(1) | 1,2(1) | 1 | - | - | - | | |
| 1 | 2-5(4) | nc [†] | 1 | 1-4(2) | 2-4(3) | 1-3(2) | 1-3 | 1-4(2) | 1 | - | - | - | | |
| 2 | 3-5(4) | nc | 16-26(26) | 11-15(12) | 7-12(9) | 1,2(1) | 1 | 1,2(1) | - | 2,3(2) | 1 | 1 | | |
| 3 | 3-5(4) | 2-7(3) | 16-32(22) | 12-21 | 7-13(9) | 1-3(1) | 1,2(1) | 1 | - | 2-4(3) | 1 | 1 | | |
| 4 | 2-5(4) | 5-9(5) | 3-9(5) | 6-10(7) | 5-8(5) | 4-6(5) | 3-6(5) | 2-5(4) | - | - | 3-5(4) | 3-5(4) | | |
| 5 | 4-6(5) | 1-3(2) | 4-8(6) | 6-11(7) | 5-9(7) | 4-7(6) | 3-6(5) | 3-5(4) | - | - | 2-5(3) | 2-5(3) | | |
| 6 | 4-6(5) | 1-3(2) | 4-6(5) | 5-8(6) | 5-8(6) | 2,3(3) | 1-3(2) | 1-4(3) | - | - | - | - | | |
| 7 | 1-6(3) | 5-9(5) | 5-8(5) | 5-10(8) | 6-8(7) | 1-5(3) | 1-3(1) | 2-4(3) | - | - | - | - | | |
| 8 | 1-3(3) | 5-9 | 2-7 | 3-5(4) | 2-5(3) | 1-5(4) | 1-3(1) | 1,2(1) | 1-4(2) | - | - | - | | |
| 9 | 6-11(9) | 2-4(2) | 3-7(4) | 3-5(5) | 2,3(2) | 2-5(3) | 1-3(3) | 1-3(2) | 1-3(2) | - | - | - | | |
| 10 | 6-11(8) | 2-4(3) | 2-6(5) | 6-11 | 4-10 | 4-6(5) | 4-6(5) | 3-6(5) | - | - | - | - | | |
| 11 | 2-5 | 2,3(2) | 4-8(7) | 3-7(5) | 3-9(7) | 3-7 | 3-7(5) | 3-5(4) | - | - | - | - | | |
| 12 | 2-6(3) | 1-6(2) | 1-3(1) | 2-7(5) | 3-6(4) | 2,3(2) | 1-3(2) | 1,2(1) | - | - | - | - | | |
| 13 | 2,3(2) | 2-6 | 5-7(7) | 2-6(4) | 3-6(4) | 2-4(2) | 1,2(1) | 1 | - | - | - | - | | |
| 14 | 1,2(1) | - | 3-8(4) | 2-6(5) | 1-5 | 2-6(4) | 1 | 1 | - | - | - | - | | |
| 15 | 1 | - | 0,1(0)* | 2-4(3) | 2,3(2) | 1,2(2) | 1,2(2) | 1-3(2) | - | - | - | - | | |
| 16 | 3-5(3) | 2-5(3) | 0-2(0) | 2-5(4) | 2,3(3) | 1-3(2) | 2-4(2) | 1-6(3) | - | - | - | - | | |
| 17 | 3-6(4) | 2-5(3) | 1 | 1 | 1 | 1 | 1 | 1 | 7-10(9) | - | - | - | | |
| 18 | 1,2(1) | - | 1 | 1 | 1 | 1 | 1 | 1 | 8-12(9) | - | - | - | | |
| 19 | 1-3(2) | - | 0 | 2-5(3) | 1-3(2) | 1,2(1) | 1-3(2) | 1-3(2) | - | - | - | - | | |
| 20 | 3-6(4) | - | 0-2 | 2-4(4) | 1-3(2) | 1-3(1) | 1-3(2) | 1-4(2) | - | - | - | - | | |
| 21 | 3-6(5) | - | 0 | 1 | 1 | 1 | 1,2(1) | 1-3(1) | - | - | - | - | | |
| 22 | 3-7(5) | - | 0,1(0) | 1 | 1 | 1 | 1 | 1,2(2) | - | - | - | - | | |
| 23 | 3-7(4) | - | - | - | - | - | - | - | - | - | - | - | | |
| 24 | - | - | - | 1 | 1 | 1 | 1 | 1 | - | - | - | - | | |
| 25 | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | - | - | - | | |

[†] nc— not counted.

* Minute single seta present on both sides in only 1 of 10 specimens.

TABLE 2. Range of branches for fourth-instar larval setae of *Anopheles yaeyamaensis* observed during the present study and by Tanaka *et al.* (1979) (modes from the present study) compared to data for *An. minimus* (neotype series from Hong Kong) taken from Harbach *et al.* (2006). nc = not counted.

| Setae no. | Head | | Thorax | | Abdominal segments | | | | | | | | | |
|-----------|-----------|-----------|-----------|-----------|--------------------|-----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|--|
| | C | P | M | T | I | II | III | IV | V | VI | VII | VIII | X | |
| 0 | 1 | 1 | - | - | - | 1,2(1) | 1-3(2) | 1-3(2) | 1-4(3) | 1-3(2) | 1-4(2) | 1,2(1) | - | |
| 1 | 1 | 1 | - | - | - | 1 | 1,2(2) | 2,3(2) | 2,3(2) | 1,2(2) | 1,2(2) | 1,2(1) | - | |
| 1 | 1 | 19-25(23) | 25-36(30) | 1,2(2) | 12-19(16) | 14-22(18) | 17-23(18) | 14-24(20) | 17-24(18) | 15-22 | 15-21(17) | 1-3(2) | 1 | |
| 1 | 1 | 19-29 | 25-37(28) | 1,2(2) | 11-16(13) | 13-20(18) | 16-22(21) | 17-23 | 16-21(18) | 15-21(17) | 15-19(16) | 1-4 | 1 | |
| 2 | 1 | 13-21(16) | 1,2(1) | 1 | 4-6(4) | 3-6(4) | 2-5(3) | 1 | 1 | 1 | 2-5(3) | 9-12(10) | 18-27(20) | |
| 1 | 1 | 13-17(14) | 1,2(1) | 1 | 4-7(5) | 3-6(5) | 2-4 | 1 | 1 | 1,2(1) | 3-5(3) | 7-14(13) | 19-22(19) | |
| 3 | 1 | 1 | 1 | 12-18(15) | 1,2(1) | 1 | 1 | 2-5(3) | 1-3(1) | 1 | 1-4(2) | 6-13(13) | 9-13(10) | |
| 1 | 1 | 1,2(1) | 1,2(1) | 11-18(16) | 1 | 1 | 1 | 2-4(2) | 1 | 1 | 3 | 8-13(11) | 8-13 | |
| 4 | 1 | 10-15(11) | 4-6(5) | 3,4(3) | 5-9(7) | 4-8(6) | 3-5(4) | 2-5(4) | 1-4(3) | 1 | 1,2(1) | 4-6(5) | 4-15 | |
| 1 | 1 | 8-16 | 3-6 | 3-5(4) | 4-7(6) | 4-7(5) | 2-5(3) | 2-5(4) | 2,3(3) | 1 | 1 | 3-5(3) | 4-13 | |
| 5 | 11-17(14) | 28-46(35) | 1 | 29-38(36) | 3-8(5) | 4-8(5) | 4-8(6) | 5-7(6) | 7-10(7) | 7-12(10) | 7-12(10) | 3-7 | - | |
| 1 | 11-16(15) | 27-42(28) | 1 | 32-41(35) | 5-8(6) | 5-8(5) | 6-10(6) | 6-10 | 8-13(10) | 9-13(11) | 10-13(11) | 4-6(5) | - | |
| 6 | 11-17(15) | 1 | 3-5(4) | 3-5(4) | 25-31 | 23-31(27) | 15-23(16) | 2-5(3) | 3 | 2-3 | 3-6(4) | - | - | |
| 1 | 12-17(14) | 1 | 3-6(4) | 3-5(4) | 25-31(29) | 23-31(25) | 16-24 | 3 | 3 | 3,4(3) | 3-6(3) | - | - | |
| 7 | 18-26(21) | 23-26(26) | 2-4(3) | 28-37(32) | 24-32 | 26-36(33) | 4-7(5) | 4-7(6) | 4-6(5) | 4-6(5) | 3-5(4) | 1-S, | 7-13(8) | |
| 1 | 14-18(16) | 24-30(26) | 3,4(3) | 29-39 | 22-3(28) | 24-32 | 4-8(5) | 5-7(6) | 4-8(5) | 3-5(4) | 3-7(5) | 2-S, | 5-8(7) | |
| 4-7(6) | 28-37 | 13-24(20) | 27-36 | - | - | 2-5(3) | 2-4(3) | 2,3(3) | 2-4(3) | 1-4(3) | 3-6(4) | 7-10(8) | - | |
| 4-7(6) | 31-34(34) | 16-26(23) | 27-39(34) | - | - | 2,3(3) | 2,3(2) | 2-4(2) | 1-3(2) | 2-4(2) | 3-8(5) | 6-S, | 2-4(3) | |
| 3-8(7) | 10-14(14) | 1 | 5-11(8) | 4-7(5) | 5,6(6) | 6-10(8) | 6-8(7) | 5-8(6) | 6-9(7) | 7-10(8) | 6-12(8) | 7-S, | 2-4(3) | |
| 3-7 | 9-13(10) | 1 | 7-9(7) | 5,6(6) | 5,6(6) | 7-9(7) | 5-8(7) | 4-7(6) | 5-8(7) | 3-10(8) | 3-10(8) | 2 | - | |
| 2,3(3) | 1 | 1 | 1 | 2-4(3) | 2-4(3) | 3-5(3) | 2-4(3) | 2-5 | 2-4(3) | 2-5(3) | 6-8(7) | 1,2(2) | - | |
| 2,3(3) | 1 | 1 | 1 | 2-5(3) | 2-5(3) | 2-4(3) | 2-4(3) | 2,3(3) | 2,3(3) | 3 | 4-7(5) | 8-S, | 6-9(6) | |
| 30-44 | 2-5(3) | 1 | 1,2 | 3-5(3) | 3-5(3) | 2-4(3) | 2-5(3) | 2-5 | 2-4(3) | 2-5(4) | 2-4(2) | 9-S, | 4-7 | |
| 30-44 | 2-5(3) | 1 | 1 | 3-5(4) | 3-5(4) | 2,3(3) | 2-4(3) | 3-5(3) | 2-4(3) | 2-5(4) | 2-5(3) | - | - | |
| 4-7(5) | 1 | 1,2(2) | 2-5(4) | 2-5(4) | 3-6(4) | 2-5(4) | 2-4(3) | 3,4(4) | 3-5 | 2-5(4) | 2-5(3) | - | - | |
| 4-7(6) | 1 | 1,2 | 3-5(3) | 3-5(4) | 3-5(4) | 2-5(3) | 1-4(3) | 2-4 | 2-4(3) | 2-4(3) | 1-3(3) | - | - | |
| 4-9 | 3-6 | 5-10 | 3,4(3) | 5-10(7) | 4-9(8) | 6-11(8) | 4-9(5) | 3-6(5) | 3-5 | 7-10 | 3,4(3) | - | - | |
| 5-9(7) | 4-7(5) | 5-9 | 3,4(3) | 4-9(8) | 5-11 | 6-10(8) | 5-11 | 4-6(6) | 3-5 | 6-11(8) | 3-5(3) | - | - | |
| nc* | 4-6(4) | 9-12(11) | - | - | - | - | 1-4(3) | 1-4(3) | 2-5(3) | 3-5(4) | 2-4 | 1-3(2) | - | |
| 5-7 | 3-5(5) | 8-12 | - | - | - | - | 1-4(2) | 1-4(3) | 2-4(2) | 2-5(2) | 1-3(1) | - | - | |
| 7-11 | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| 6-9 | - | - | - | - | - | - | - | - | - | - | - | - | - | |

*Obscured by cranial pigmentation.

Metaphase karyotype. The metaphase karyotype of *An. yaeyamaensis* (Somboon *et al.*, 2001) is similar to that of *An. minimus* but very different from that of *An. harrisoni* (Baimai *et al.*, 1996). *Anopheles harrisoni* has a prominent landmark of pericentric heterochromatin in the autosomes and the short arm of the submetacentric X chromosome. The ratio of the short to long arms of the submetacentric Y chromosome is about 1:1.5 in *An. harrisoni*, whereas it is 1:3–1:4 in *An. yaeyamaensis* and *An. minimus*.

Crossing experiments. Crossmating carried out between *An. yaeyamaensis* and *An. minimus* (Somboon *et al.*, 2001; Somboon *et al.*, 2005b) and between *An. yaeyamaensis* and *An. harrisoni* (Somboon *et al.*, 2005a) revealed postzygotic incompatibility. The former yielded hybrid progeny only when *An. minimus* females were mated with *An. yaeyamaensis* males, but the hybrid (F₁) males were sterile or almost sterile with atrophied testes. No asynapsis was observed in the salivary gland polytene chromosomes of the hybrid larvae. The latter cross yielded hybrid (F₁) progeny from reciprocal crosses, but the hybrid males were sterile with atrophied testes and accessory glands or were partially sterile with abnormal and inactive spermatozoa. The polytene chromosomes of hybrid larvae exhibited partial asynapsis or a fixed heterozygous inversion. Backcrosses revealed more severe incompatibility.

Molecular characterization. Three PCR-based assays have been developed that distinguish *An. minimus* and *An. harrisoni* of the Minimus Complex: SSCP-PCR of D3 rDNA (Sharpe *et al.*, 1999), RFLP-PCR of ITS2 rDNA using the restriction endonuclease BsIZI (Van Bortel *et al.*, 2000; Garros *et al.*, 2004b), allele-specific PCR based on SCAR markers (Kengne *et al.*, 2001) and ITS2 nucleotide variations (Phuc *et al.*, 2003; Garros *et al.*, 2004a). None of these methods included *An. yaeyamaensis*, but nucleotide variation observed in the D3 and ITS2 regions of rDNA and the COII locus of mt DNA (Harbach *et al.*, 2007: figs 2, 3, 5) readily distinguish this species from *An. minimus* and *An. harrisoni*.

Bionomics. *Anopheles yaeyamaensis* (identified as *An. minimus*) played a major role in the transmission of *falciparum* malaria in the Yaeyama and Miyako Islands before the disease was eradicated from the islands between 1957 and 1962 (Kuroshima, 1960; Farid *et al.*, 1966; Sakihara *et al.*, 1994). The immature stages typically inhabit unpolluted streams, springs and ground pools near springs. Larvae and adults are found throughout the year, but densities decrease significantly during the cold season from December to February, during which individuals are larger and darker in colour (Miyagi & Toma, 1980; Toma *et al.*, 1996a,b; Toma *et al.*, 2002a; Toma *et al.*, 2003; Somboon *et al.*, 2001).

Anopheles yaeyamaensis is stenogamous (insemination rate about 50%) and females mate readily with a stenogamous strain of *An. minimus* in 30 cm cages (insemination rates of about 80%). *Anopheles harrisoni* is not stenogamous but females (about 20%) are able to mate with *An. yaeyamaensis* males (Somboon *et al.*, 2001, 2005a).

Distribution. *Anopheles yaeyamaensis* has only been found on Iriomote, Ishigaki and Kohama Islands of the Yaeyama Island Group and Miyako Island of the Miyako Island Group in the Okinawa Prefecture, Japan. Ishigaki and Iriomote are the largest two islands of the Yaeyama Group and Miyako is the largest and most populous island among the Miyako Islands. The two island groups form the southern part of the volcanic Ryukyu Islands. The Yaeyamas are closer to Taiwan, about 125 km, than Miyako Island, which lies approximately 400 km east of Taipei, Taiwan.

Etymology. The specific name, *yaeyamaensis*, is a Latinised geographical name (yaeyama and the Latin suffix *-ensis*) denoting the type locality of the species in the Yaeyama Islands.

Type series. Eighty-nine specimens (13 ♀, 13 ♂, 26 Le, 26 Pe, 11 L). *Holotype*, ♀ (no. 5), with LePe on microscope slide, JAPAN: Ryukyu Archipelago, Ishigaki Island, Ishigaki City, Nosoko, Nishihama stream, 6 July 2006 (Somboon *et al.*) (BMNH). *Paratypes*, same data as holotype, 12 ♀LePe (nos. 2, 3, 8, 10, 11, 13, 15, 19–22, 24), 1 ♂ (no. 18), 12 ♂LePe (nos. 1, 4, 6, 7, 9, 12, 14, 16, 17, 23, 27, 32), 1 LePe (no. 25) and 11 L (nos. 1–11). All specimens are deposited in the Natural History Museum, London (BMNH).

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