



Reconsideration of the status of subspecies in the Japonicus Group of the subgenus *Hulecoeteomyia* Theobald of *Aedes* Meigen (Diptera: Culicidae)

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The current concept of *Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901) (Diptera: Culicidae) includes four taxonomic entities: subspecies *amamiensis* Tanaka, Mizusawa & Saugstad, 1979, subspecies *shintienensis* Tsai & Lien, 1950, subspecies *yaeyamensis* Tanaka, Mizusawa & Saugstad, 1979 and the nominotypical form, i.e. subspecies *japonicus* (Theobald, 1901). The four forms, plus *Aedes* (*Hul.*) *koreicus* (Edwards, 1917), comprise the Japonicus Group (as “group”) of Tanaka *et al.* (1979). A sixth member from Bhutan, *Ae.* (*Hul.*) *bhutanensis* Somboon & Harbach, 2020 (in Somboon *et al.* 2020), was recently added.

Tanaka *et al.* (1979) defined the Japonicus Group as follows: “Aedeagus broadly open in tergobasal 0.67 and sternoapical 0.75, without apical denticle, slightly constricted in apical 0.33. Basistyle [gonocoxite] without distinct basal tergomesal lobe. Larval setae 4–6-C anterior of 7-C; 4-C small; 5,6-C subequal, multibranching. Antenna spiculate. Mandibular ventral tooth with VT₃. Maxillary mesostipes [maxillary body] with mesal area detached; palpostipes [maxillary palpus] mesobasally fused with mesostipes; stipital sensoria [seta 1-Mx] distad of middle, without basal ring. Abdominal seta 7-II strong. Comb scales paddle shaped. Siphon with acus attached; pecten teeth [spines] unilaterally denticulate. Immatures in a wide variety of containers, but apparently essentially rock-hole inhabitants.” *Aedes bhutanensis* can only be partially compared with the above since the male is unknown and characters of the larval mouthparts were not included in the original description.

The nominotypical subspecies was described from Japan but occurrence in the Soviet [Russian] Far East, Korea, China, Hong Kong, the Ryukyu Islands, Taiwan and Cheju [Jeju] Island was also listed by Knight & Stone (1977). Since then, *japonicus s.s.* became established, due to multiple introductions, in temperate North America and Europe (Fonseca *et al.* 2010; Medlock *et al.* 2012; reviewed by Kaufman & Fonseca 2014 and Ibáñez-Justicia 2020). Of note, even though it is a temperate species, it was successfully established in the tropical Hawaiian Islands by occupying niches at higher elevations (Egizi & Fonseca 2014). Subspecies *shintienensis* is known only from Taiwan, subspecies *amamiensis* only from the northern Ryukyu Islands, Japan and subspecies *yaeyamensis* only from the southern Ryukyu Islands, Japan. *Aedes koreicus* was described from South Korea and listed by Knight & Stone (1977) as also present in China, Japan and Russia.

Tanaka *et al.* (1979) provided keys to separate adult females, male genitalia and larvae of *Ae. japonicus s.l.* and *Ae. koreicus*, but also made extensive comparisons of *Ae. koreicus* with the subspecies of *Ae. japonicus* and found significant differences between all of them. They identified two species groups in *Ae. japonicus s.l.*, one (subspecies *shintienensis* and *yaeyamensis*) in the Oriental Region (south China, Taiwan and Yaeyama Guntô, a southern island of the Ryukyu Archipelago) and the other (subspecies *amamiensis* and *japonicus*) in the Palaearctic Region (palaearctic Japan and South Korea). They found a rather wide gap between the two groups because of the absence of members of the group in northern China, Okinawa and the central Ryukyu Archipelago. They stated that the differences between three southern “populations” (Taiwan, Yaeyama and Amami) are especially remarkable and not clinal. We assume their “populations” correspond to subspecies *shintienensis*, *yaeyamensis* and *amamiensis*, respectively. They further stated that *Ae. japonicus* can be divided into four subspecies.

The discussion and comparisons of *Ae. koreicus* with *Ae. japonicus s.l.* by Tanaka *et al.* (1979) are somewhat artificial since, in spite of significant subequal morphological differences between the taxa, they retained *Ae. japonicus* as a group of four subspecies but kept *Ae. koreicus* as a separate species. We believe that the morphological data they presented are sufficient to treat the Japonicus Group as having a total of five species, with no subspecies, i.e. there is adequate morphological and geographic separation to assume genetic separation typical of separately evolving species. The addition of *Ae. bhutanensis* brings the total number of species to six.

In another study, Cameron *et al.* (2010) found genetic evidence that clearly separated the five taxa of the Japonicus Group (*sensu* Tanaka *et al.* 1979, as the *Ae. japonicus* complex). They used two mitochondrial genes, *ND4* and *COII*, and a nuclear locus, 28S-D2 spacer, for a total of 1,337 bp. A combined Bayesian analysis showed a fully resolved monophyletic clade corresponding to the Japonicus Group of Tanaka *et al.* (1979), with *Ae. koreicus* unambiguously included in it. They carried out a separate distance analysis of the mitochondrial data and stated that “The minimum percent difference among subspecies in the *Ae. japonicus* complex at the mitochondrial loci was 6.3%, comparable to that between any of the subspecies and *Ae. koreicus*”. They also noted that “The primary conclusion of this study is that the four subspecies in the *Ae. japonicus* complex are genetically quite distinct, averaging $\approx 8\%$ nucleotide differences at the two mitochondrial loci.” Cameron *et al.* (2010), however, followed Tanaka *et al.* (1979) and retained four subspecies of *Ae. japonicus s.l.*, with *Ae. koreicus* as a separate species, but suggested the need for a taxonomic reconsideration of the group. In comparison to *Ae. bhutanensis*, Somboon *et al.* (2020) found that the *COI* mtDNA sequences of *amamiensis* and *yaeyamensis* were distinct from those of *japonicus s.s.*, differing by 43 (7.4%) and 49 (8.4%) fixed sites, respectively. In the same study, *Ae. koreicus* was unambiguously recovered as a member of the Japonicus Group, closely related to *yaeyamensis*. Somboon *et al.* suggested that *amamiensis* and *yaeyamensis* “should probably be treated as separate species rather than subspecies,” but they did not formally elevate them to specific rank. The *COI* sequence comparisons of the Japonicus Group (*sensu* Tanaka *et al.* 1979) to *Ae. bhutanensis* (K2P genetic distances) were also significant: between *bhutanensis* and *japonicus s.s.*, 2.09–2.81%; between *bhutanensis* and *amamiensis* and *yaeyamensis*, $> 9\%$.

Hereby, in view of the substantial morphological and molecular divergence and apparent allopatric distributions described above, we formally elevate the three forms currently recognized as subspecies of *Ae. japonicus* to species rank: *Ae. (Hul.) amamiensis* Tanaka, Mizusawa & Saugstad, 1979, *Ae. (Hul.) shintienensis* Tsai & Lien, 1950 and *Ae. (Hul.) yaeyamensis* Tanaka, Mizusawa & Saugstad, 1979, together with nominotypical *Ae. (Hul.) japonicus* (Theobald, 1901).

Aedes japonicus has a single synonym, *Ae. eucleptes* Dyar, 1921 (Guangdong Province, romanized as Canton, China) and *Ae. shintienensis* has two synonyms, *Ae. japonicus* var. *tokushimaensis* Tanimura, 1952 and *Ae. bisanensis* Suzuki, Tanimura, Miyagawa & Murata, 1953 (both Tokushima, Shikoku, Japan). These nominal forms remain in synonymy with their senior synonyms. *Aedes amamiensis* and *Ae. yaeyamensis* are without synonyms.

Before now, the subgenus *Hulecoeteomyia* included 15 species in the Oriental and eastern Palearctic Regions (Wilkerson *et al.* 2021; Harbach, 2022). It now includes 18 species.

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