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The identity of *Anopheles* (*Anopheles*) *barbirostris* species A3 of the Barbirostris Complex (Diptera: Culicidae)

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The *Anopheles barbirostris* species complex of the subgenus *Anopheles* Meigen, 1818 includes six formally named species: *An. barbirostris* van der Wulp, 1884, *An. campestris* Reid, 1962, *An. dissidens* Taai & Harbach, 2015, *An. saeungae* Taai & Harbach, 2015, *An. vanderwulpi* Townson & Harbach, 2013 (in Townson *et al*. 2013) and *An. wejchoochotei* Taai & Harbach, 2015. Members of the complex are widely distributed in the Oriental Region and are found on some Pacific islands. Recognition of most species of the complex has been based on the use of morphology, cytogenetics, cross-mating and molecular methods, summarized as follows.

An. barbirostris [= *An. barbirostris* form A (metaphase karyotypic form) in part of Baimai *et al*. (1995); *An. barbirostris* species A4 of Suwannamit *et al*. (2009); *An. barbirostris* clade I of Paredes-Esquivel *et al*. (2009); *An. barbirostris* of Towson *et al*. (2013)].

An. campestris [= *An. campestris* of Reid (1962, 1968); Reid *et al.* (1979); in part (?) of Harrison & Scanlon (1975) and of Baimai *et al.* (1995)].

An. dissidens [= *An. barbirostris* forms A and B in part and C of Baimai *et al*. (1995); *An. barbirostris* species A1 of Saeung *et al*. (2008); *An. barbirostris* clade III of Paredes-Esquivel *et al*. (2009)].

An. saeungae [= *An. barbirostris* forms A and B in part of Baimai *et al.* (1995); *An. barbirostris* forms A and B in part of Saeung *et al.* (2007); *An. barbirostris* species A2 of Saeung *et al*. (2008); *An. barbirostris* clade IV of Paredes-Esquivel *et al*. (2009)].

An. vanderwulpi [=*An. barbirostris* clade II of Paredes-Esquivel *et al.* (2009)].

An. wejchoochotei [= *An. barbirostris* form B in part of Baimai *et al.* (1995); *An. campestris*-like forms B and E of Saeung *et al.* (2007); *An. barbirostris* clade V of Paredes-Esquivel *et al*. (2009); *An. campestris*-like form E of Suwannamit *et al*. (2009); *An. campestris*-like forms B, E and F of Thongsahuan *et al*. (2009); *An. campestris* of Paredes-Esquivel & Townson (2014)].

A seventh species, informally designated *An. barbirostris* species A3, was recognized by Saeung *et al*. (2008) from isoline progeny of females collected in Kanchanaburi Province in west-central Thailand. Taai & Harbach (2015) did not formally name species A3 because molecularly identified specimens were not available at the time. Consequently, the specific status of this proposed species has remained uncertain. As noted by Paredes-Esquivel *et al*. (2009) and Otsuka (2011), species A3 has a much smaller ITS2 amplicon than the corresponding region of the other species of the Barbirostris Complex. Phylogenetic analyses of ITS2 and mitochondrial gene sequences (*COI* and/or *COII*) revealed that sequences of species A3 were clearly distinct from those of the other species of the complex, with high average genetic distances > 0.5 for ITS2 and > 0.03 for *COI* (Saeung *et al*. 2008; Suwannamit *et al*. 2009; Otsuka 2011; Taai & Harbach 2015), suggesting that it is not closely related to the five formally named species.

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Wilai *et al*. (2020) recently developed a multiplex PCR for identification of species of the complex based on *COI* sequences. The assay was developed using females collected from a cattle-baited trap in Tha Song Yang District, Tak Province (17.56496 N, 97.915571 E) located north of Kanchanaburi Province, about 300 km from the locality where *An. barbirostris* species A3 was originally found. The first batch of *Anopheles* specimens were collected in September 2020. They were kept in an ice box and transported to the Department of Parasitology, Faculty of Medicine, Chiang Mai University (PMCMU) for further study. The specimens arrived in rather poor condition. In particular, scales at the apex of the wings were mostly missing and pale scaling of the abdominal sterna of most specimens was partially denuded. Consequently, it was difficult to accurately identify the specimens to species based on morphology; hence, they were tentatively identified as belonging to the Barbirostris Subgroup, which includes four species in addition to those of the Barbirostris Complex. *COI* sequences were obtained from the specimens and compared with sequences in GenBank using BLAST. In addition to *An. dissidens* and *An. saeungae* that were found in Tak Province, the *COI* sequences of some specimens of the Barbirostris Subgroup were similar to those of *An. barbirostris* species A3 from Kanchanaburi Province registered in GenBank (accession numbers AB362238–AB362240), and phylogenetic analysis placed them in the same clade (Wilai *et al*. 2020: Fig. 1). At that time, the results led us to believe that the specimens from Tak Province corresponded to species A3. We then intended to conduct a taxonomic study of A3, for which we collected another batch of specimens from Tak Province in October 2020. Two isolines (TSY15, TSY143) were successfully acquired in the laboratory. Surprisingly, however, the morphology of larvae, pupae and adults of the two isolines (Figs 1 and 2) were found to be distinct from species of the Barbirostris Complex, and were identified as *An. hodgkini* Reid, 1962, a species of the Barbirostris Subgroup, based on the descriptions of Reid (1968), Harrison & Scanlon (1975) and Taai & Harbach (2015). *Anopheles hodgkini* differs from the species of the Barbirostris Complex as follows. Pupae: Trumpets without a secondary cleft (Fig. 1a), present in species of the complex (Fig. 1b); abdominal seta 9 highly pigmented and clearly contrasted with the lighter pigmentation of the segments (Fig. 1c), lighter or moderately pigmented in species of the complex (Fig. 1d). Larvae: Palmate seta 2-II usually without pigmentation (Fig. 1e), darkly pigmented in species of the complex (Fig. 1f). Adults: Apex of wing with 3 narrow pale fringe spots, middle spot at termination of vein R_2 (Fig. 2a) (if only 2 pale fringe spots, then anterior spot wide, extending to tip of vein R_2), wing with 2 narrow pale fringe spots and no spot at tip of vein R_2 in species of the complex (Fig. 2b); midtarsomeres 1 and 2 usually with apical pale bands; abdominal sterna II–VI usually with fewer median pale scales (0–20) (Fig. 2c) than species of the complex (Fig. 2d). However, in the F_1 progeny of the TSY15 and TSY143 isolines, the narrow pale scales at the apex of vein R_2 are more common in males (7 of 11) and infrequently present in females (1 of 8); midtarsomeres commonly with apical pale spots (10 of 11 males, 8 of 8 females).

Phylogenetic analysis generated a tree in which the ITS2 sequences of specimens of the two isolines of *An. hodgkini* (TSY15: GenBank OR290095, 974 bp; TSY143: GenBank OR290096, 974 bp) and eight specimens (Tak21, 28, 31, 33, 37, 38, 41, 45) of the Barbirostris Subgroup collected in Tak Province were recovered in a clade (Fig. 3) with sequences of *An. barbirostris* species A3 from Kanchanaburi (K2P genetic distances 0.003–0.004). This clade is clearly separated (bootstrap 100%) from the clade consisting of sequences of species of the Barbirostris Complex (K2P 0.679–0.719) and *An. donaldi* Reid, 1962 (K2P 0.682–0.696). The tree resulting from phylogenetic analysis of *COI* sequences (not shown here) agreed with the tree obtained from the analysis of ITS2 sequences shown in Fig. 3. The results of the present study agree with the results of Paredes-Esquivel *et al*. (2009) and Otsuka (2011), confirming that species A3 is not related to the complex.

While thoroughly sorting mosquito specimens held in PMCMU, we recently found 14 slide-mounted pupal and larval exuviae and 14 larvae and associated eggs on filter papers that were labelled as *An. barbirostris* species A3. The specimens were prepared by S. Thongsahuan, who used a colony of species A3 for a study of malarial susceptibility (Thongsahuan *et al.* 2011). The trumpets of the pupal exuviae lack a secondary cleft and abdominal seta 9 is darkly pigmented, and larval seta 1-II is unpigmented as shown in Fig. 1a, c and e, respectively. The eggs (not shown here) have the deck divided into a short area at each end. All of the specimens agree with Reid's (1962) original description of *An. hodgkini.* Unfortunately, adult specimens of *An. barbirostris* species A3 originally described by Saeung *et al*. (2008) and from a laboratory colony of A3 used in the study of Thongsahuan *et al.* (2011) were not available.

In conclusion, based on the available morphological and molecular information, it is obvious that *An. barbirostris* species A3 of Saeung *et al*. (2008) and subsequent authors is *An. hodgkini*. Harrison & Scanlon (1975) reported that in Thailand *An. hodgkini* was most common in the southern peninsular provinces but was found at localities to about 15° N latitude in central Thailand. But as the present study shows, this species occurs further northward into Kanchanaburi and Tak Provinces (to about 18° N). Harrison & Scanlon also noted that adults of *An. hodgkini* occasionally exhibit morphological variation which may cause them to be confused with species of the Barbirostris Complex, *e.g.* the lack of an accessory pale fringe spot at the tip of vein R_2 and the number of pale scales on the abdominal sterna. Considering there is variation in the branching of larval and pupal setae, the pupal trumpet without a secondary cleft is the best character for distinguishing *An. hodgkini* from species of the Barbirostris Complex. Larval seta 1-II of *An. hodgkini* is usually pale, as opposed to being darkly pigmented in members of the Barbirostris Complex. Additionally, it is interesting to note that the ITS2 sequences of *An. donaldi* were recovered in the clade consisting of sequences of species of the Barbirostris Complex, with 95% bootstrap value (Fig. 3). Consequently, we propose that *An. donaldi* should be included as a member of the Barbirostris Complex.

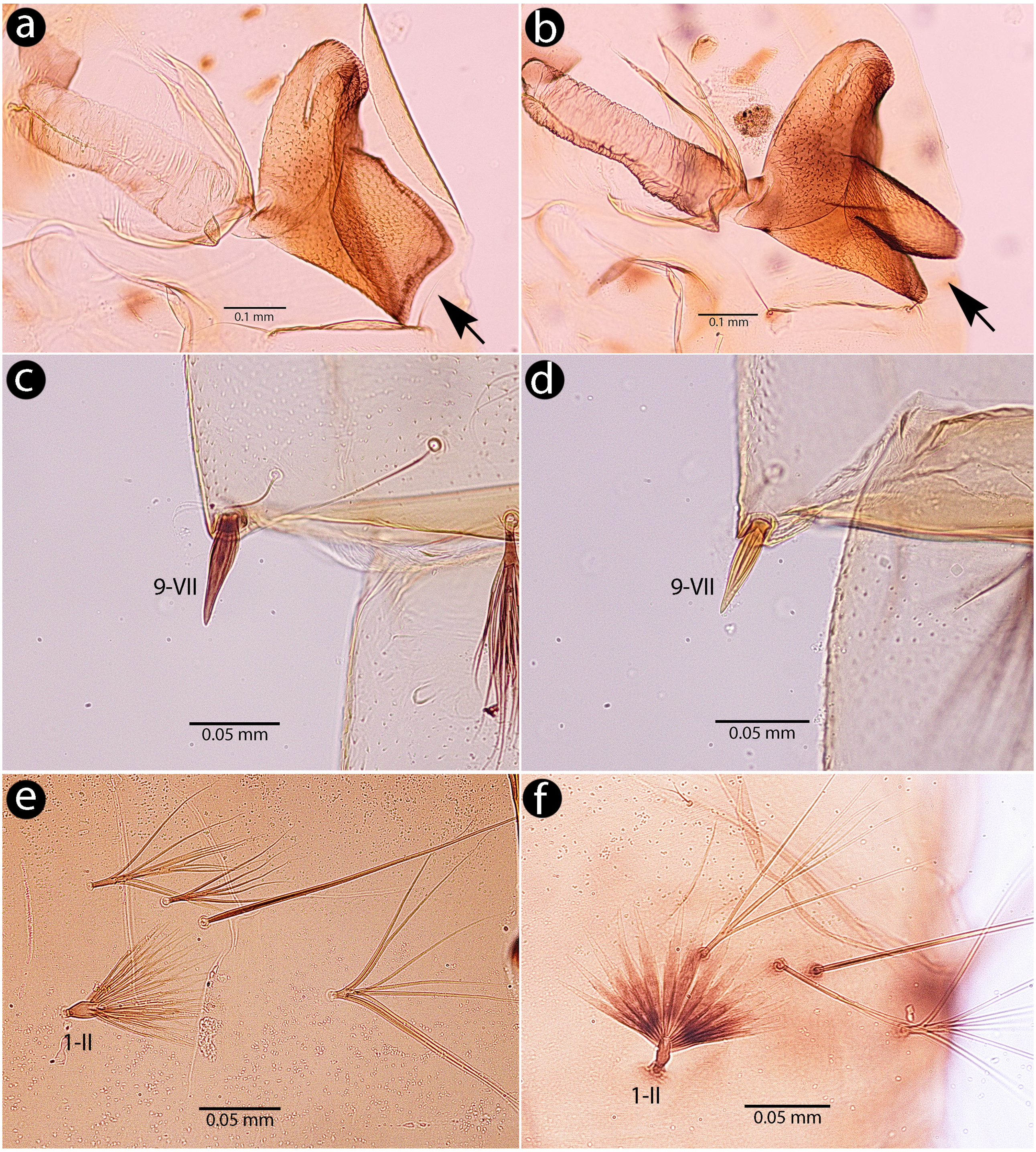

FIGURE 1. a, b, Pupal trumpets of (a) *An. hodgkini* male showing the absence of a secondary cleft (arrow) and (b) *An. dissidens* showing the secondary cleft (arrow). c, d, Pupal seta 9-VII of (c) *An. hodgkini*, darkly pigmented, and (d) *An. dissidens*, lightly pigmented. e, f, Larval palmate seta 1–II of (e) *An. hodgkini*, unpigmented, and (f) *An. dissidens*, pigmented.

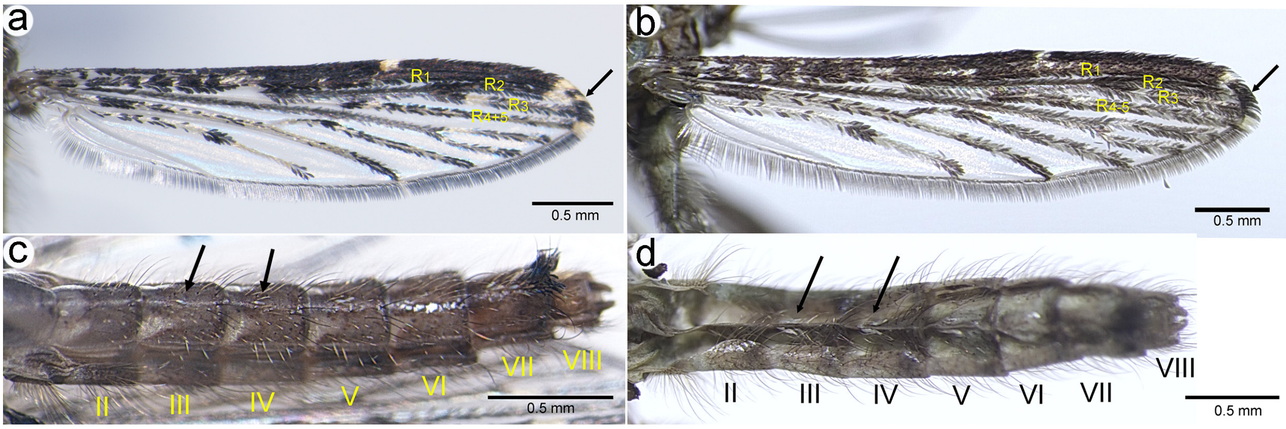

FIGURE 2. a, b, Wings of (a) *An. hodgkini* female with three apical fringe spots (arrow) and (b) *An. dissidens* with two apical fringe spots, without pale fringe spot at tip of vein R_2 (arrow). c, d, Abdominal sterna of (c) *An. hodgkini* female with one pale and (d) *An. dissidens* female with two short lines of pale scales on sterna III and IV (arrows).

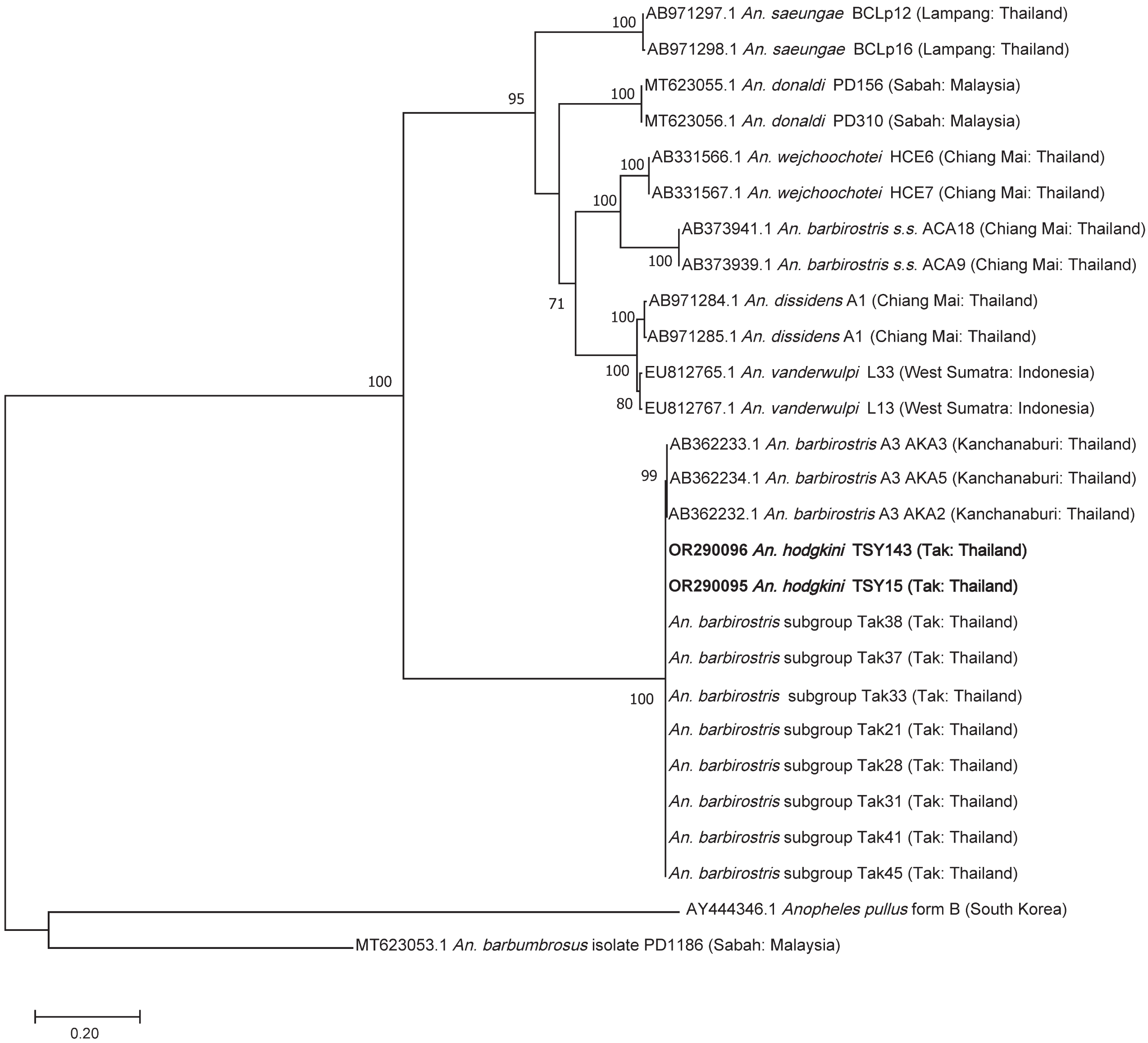

FIGURE 3. Maximum Likelihood tree of ITS2 sequences from specimens of *An. barbirostris* species A3 and other species of the Barbirostris Subgroup (which includes the Barbirostris Complex), with *An.* (*Ano.*) *barbumbrosus* Strickland & Chowdhury, 1927 and *An.* (*Ano.*) *pullus* Yamada, 1937 as outgroup taxa. Bootstrap values are shown at each node. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The final dataset included 1,913 positions. The best-fit model was GTR+G. Evolution analyses were conducted in MEGA11.

References

Baimai, V., Rattanarithikul, R. & Kijchalao, U. (1995) Metaphase karyotypes of *Anopheles* of Thailand and Southeast Asia: IV. The Barbirostris and Umbrosus species groups, subgenus *Anopheles* (Diptera: Culicidae). *Journal of the American Mosquito Control Association*, 11 (3), 323–328.

Harrison, B.A. & Scanlon, J.E. (1975) Medical entomology studies—II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). *Contributions of the American Entomological Institute*, 12 (1), iv + 1–307.

Meigen, J.W. (1818) *Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten. Vol. 1*. Gedrukt bei Beaufort Sohn, Aachen, xxxvi + 332 + 1 (errata), 11 pls.
https://doi.org/10.5962/bhl.title.12464

Otsuka, Y. (2011) Variation in number and formation of repeat sequences in the rDNA ITS2 region of five sibling species in the *Anopheles barbirostris* complex in Thailand. *Journal of Insect Science*, 11 (1), 137.
https://doi.org/10.1673/031.011.13701

Paredes-Esquivel, C. & Townson, H. (2014) Functional constraints and evolutionary dynamics of the repeats in the rDNA internal transcribed spacer 2 of members of the *Anopheles barbirostris* group. *Parasites and Vectors*, 7, 106.
https://doi.org/10.1186/1756-3305-7-106

Paredes-Esquivel, C., Donnelly, M.J., Harbach, R.E. & Townson, H. (2009) A molecular phylogeny of mosquitoes in the *Anopheles barbirostris* Subgroup reveals cryptic species: Implications for identification of disease vectors. *Molecular Phylogenetics and Evolution*, 50 (1), 141–151.
https://doi.org/10.1016/j.ympev.2008.10.011

Reid, J.A. (1962) The *Anopheles barbirostris* group (Diptera, Culicidae). *Bulletin of Entomological Research*, 53 (1), 1–57.
https://doi.org/10.1017/S0007485300047945

Reid, J.A. (1968) *Anopheline mosquitoes of Malaya and Borneo*. Studies from the Institute for Medical Research Malaya, No. 31. Government of Malaysia, Kuala Lumpur, xiii + 520.

Reid, J.A., Harrison, B.A. & Atmosoedjono, S. (1979) Variation and vector status in *Anopheles barbirostris*. *Mosquito Systematics*, 11 (3), 235–251.

Saeung, A., Baimai, V., Otsuka, Y., Rattanarithikul, R., Somboon, P., Junkum, A., Tuetun, B., Takaoka, H. & Choochote, W. (2008) Molecular and cytogenetic evidence of three sibling species of the *Anopheles barbirostris* form A (Diptera: Culicidae) in Thailand. *Parasitology Research*, 102, 499–507.
https://doi.org/10.1007/s00436-007-0788-0

Saeung, A., Otsuka, Y., Baimai, V., Somboom, P., Pitasawat, B., Tuetun, B., Junkum, A., Takaoka, H. & Choochote, W. (2007) Cytogenetic and molecular evidence for two species in the *Anopheles barbirostris* complex (Diptera: Culicidae) in Thailand. *Parasitology Research*, 101, 1337–1344.
https://doi.org/10.1007/s00436-007-0645-1

Strickland, C. & Chowdhury, K.L. (1927) *An illustrated key to the identification of the anopheline larvae of India, Ceylon, and Malaya, west of Wallace's Line with practical notes on their collection*. Thacker, Spink & Co., Calcutta and Simla, xi + 67 pp.

Suwannamit, S., Baimai, V., Otsuka, Y., Saeung, A., Thongsahuan, S., Tuetun, B., Apiwathnasorn, C., Jariyapan, N., Somboon, P., Takaoka, H. & Choochote, W. (2009) Cytogenetic and molecular evidence for an additional new species within the taxon *Anopheles barbirostris* (Diptera: Culicidae) in Thailand. *Parasitology Research*, 104, 905–918.
https://doi.org/10.1007/s00436-008-1272-1

Taai, K. & Harbach, R.E. (2015) Systematics of the *Anopheles barbirostris* species complex (Diptera: Culicidae: Anophelinae) in Thailand. *Zoological Journal of the Linnean Society*, 174 (2), 244–264.
https://doi.org/10.1111/zoj.12236

Thongsahuan, S., Baimai, V., Junkum, A., Saeung, A., Min, G-S., Joshi, D., Park, M-H., Somboon, P., Suwonkerd, W., Tippawangkosol, P., Jariyapan, N. & Choochote, W. (2011) Susceptibility of *Anopheles campestris*-like and *Anopheles barbirostris* species complexes to *Plasmodium falciparum* and *Plasmodium vivax* in Thailand. *Memórias do Instituto Oswaldo Cruz*, 106 (1), 105–112.
https://doi.org/10.1590/S0074-02762011000100017

Thongsahuan, S., Baimai, V., Otsuka, Y., Saeung, A., Tuetun, B., Jaryapan, N., Suwannamit, S., Somboon, P., Jitpakdi, A., Takaoka, H. & Choochote, W. (2009) Karyotypic variation and geographic distribution of *Anopheles campestris*-like (Diptera: Culicidae) in Thailand. *Memórias do Instituto Oswaldo Cruz*, 104 (4), 558–566.
https://doi.org/10.1590/S0074-02762009000400004

Townson, H., Dyer, N., McAlister, E., Satoto, T.B.T., Bangs, M.J. & Harbach, R.E. (2013) Systematics of *Anopheles barbirostris* van der Wulp and a sibling species of the Barbirostris Complex (Diptera: Culicidae) in eastern Java, Indonesia. *Systematic Entomology*, 38 (1), 180–191.
https://doi.org/10.1111/j.1365-3113.2012.00653.x

van der Wulp, F.M. (1884) Note XXXVIII. On exotic Diptera. Part 1. *Notes from the Leyden Museum*, 6 (4), 248–256.

Wilai, P., Namgay, R., Ali, R.S.M., Saingamsook, J., Saeung, A., Junkum, A., Walton, C., Harbach, R.E. & Somboon, P. (2020) A multiplex PCR based on mitochondrial *COI* sequences for identification of members of the *Anopheles barbirostris* Complex (Diptera: Culicidae) in Thailand and other countries in the region. *Insects*, 11, 409.
https://doi.org/10.3390/insects11070409

Yamada, S. (1927) An experimental study on twenty-four species of Japanese mosquitoes regarding their suitability as intermediate hosts for *Filaria bancrofti* Cobbold. *Scientific Reports from the Government Institute for Infectious Diseases, Tokyo Imperial University*, 6, 559–622, 3 pls.