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Review

Review of the Mimeticus Subgroup of *Culex* (*Culex*) (Diptera: Culicidae), With an Assessment of the Specific Status of Three Nominal Species Described From India

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Abstract

The Mimeticus Subgroup of the subgenus *Culex*, genus *Culex* Linnaeus, is reviewed and revised. Mitochondrial *COI* sequences of species of the subgroup available in GenBank were analysed to verify the identity of voucher specimens. The specific status of three species described from India, *Cx. gaugleri* Suman, *Cx. katezari* Karlekar, Andrew & Deshpande, and *Cx. kodaikanalensis* Suman, Veer & Chandra, was examined and found to be unsupportable. *Culex gaugleri* and *Cx. kodaikanalensis* are synonyms of *Cx. jacksoni* based on the similarity of DNA sequences and dubious evidence of morphological differentiation. *Culex katezari* is recognized as a *nomen dubium* because the morphological description and molecular information provided by the authors are insufficient for its identification. A number of specimens registered in GenBank were found to be misidentified. In addition, available data indicate that the Mimeticus Subgroup may include at least three unrecognized species, referred to informally as *Cx. mimeticus* form B, *Cx. mimulus* form X, and *Cx. mimulus* form Y.

Key words: DNA, morphology, mosquito, systematics, taxonomy

Mosquitoes of the subgenus *Culex*, genus *Culex* Linnaeus, are widely distributed throughout the world, consisting of 203 currently recognized species (Harbach 2022), and about 25% of all known species of *Culex*. The internal classification of the subgenus was first developed by Edwards (1932), who divided the mosquitoes of the subgenus into two groups, Group A (Sitiens Group) and Group B (Pipiens Group). Three additional groups were established by subsequent authors, the Atriceps Group (Belkin 1962), the Duttoni Group (Harbach 1988), and the Guiarti Group (Edwards 1941). The Sitiens Group includes five subgroups, the Barraudi Subgroup (Sirivanakarn 1976), the Gelidus Subgroup (Sirivanakarn 1976), the Mimeticus Subgroup (Mimeticus Series of Edwards 1932), the Sitiens Subgroup (Bram 1967), and the Vishnui Subgroup (Bram 1967). Adult mosquitoes of the Mimeticus Subgroup are easily distinguished morphologically from the adults of other species of the Sitiens Group, as well as all other species of the genus *Culex*, by having spotted wings, which resemble the spotted wings of many species of the subgenus *Cellia* Theobald of *Anopheles* Meigen. Also, the larvae of most species of the Mimeticus Subgroup are distinguished by having an unbranched seta 4-P.

Sirivanakarn (1976) recognized two complexes in the Mimeticus Subgroup, the Mimeticus and Mimulus Complexes, based on the pattern of pale spots on the wings of adults, and features of the male genitalia, larvae, and pupae. Species of the Mimeticus Complex bear the following morphological distinctions: Adults – first or median costal pale spot of the wings limited to the costa and/or subcosta. Male genitalia – 1) gonostylus without a distinct subapical crest of minute spicules, 2) foliform seta *g* of the subapical lobe relatively narrow, 3) outer division of the phallosome with a strong, prominent, laterally divergent sternal spine, and 4) basal sternal process of the proctiger present or absent, if present, it is usually very slender, thin, and pale. Pupae – 1) seta l-II usually single or double, rarely with more branches, 2) seta l-III,IV usually with 2–6 branches, rarely with more branches, 3) seta 6-III,IV usually single or double, rarely 3-branched, and 4) seta 4-VIII usually single, rarely double. Larvae – 1) comb scales relatively coarse and usually with the fringe of spicules terminating in a strong median apical spine, 2) siphon usually 1.4–l.6 mm in length, rarely exceeding 2.0 mm, 3) four proximal pairs of siphonal seta 1-S usually strongest, closely spaced,

forming a prominent row, the two distal pairs weaker and shorter, and 4) distal half of the siphon with or without prominent spines on the posterior surface. In comparison, species of the Mimulus Complex exhibit the following morphological distinctions: Adults – 1) wings with or without a basal costal pale spot in addition to three other costal spots on the distal half, 2) first costal pale spot located at about midlength, involving the costa, subcosta, and veins R_1 and R_s, sometimes also extending onto veins M and Cu. Male genitalia – 1) gonostylus with a distinct subapical crest of several minute spicules, 2) foliform seta *g* of the subapical lobe broad, 3) outer division of the phallosome with a poorly or well-developed sternal spine that is usually strongly curved with the apex projecting basad, and 4) basal sternal process of the proctiger present, very strong, stout, and dark. Pupae – 1) seta l-II usually more than 3-branched (2–9), 2) seta l-III,IV with 5–10 branches, 3) seta 6-III,IV usually triple, rarely double, and 4) seta 4-VIII double. Larvae – 1) comb scales relatively fine, with an even fringe of fine spicules or terminating in a weak median apical spine, 2) siphon 1.4–2.5 mm in length, 3) all siphonal setae relatively weak, short, subequal, widely spaced, not forming a prominent row, and 4) distal half of the siphon without prominent spines on the posterior surface.

Sirivanakarn (1976) included seven species in the Mimeticus Complex, *Cx. diengensis* Brug (Indonesia), *Cx. fasyi* Baisas (the Philippines), *Cx. jacksoni* Edwards (northeastern Oriental and southeastern Palaearctic Regions), *Cx. mimeticus* Noè (southwestern Palaearctic Region eastward to the Oriental Region), *Cx. mimuloides* Barraud (China and India), *Cx. solitarius* Bonne-Wepster (Indonesia and Papua New Guinea), and *Cx. tsengi* Lien (Taiwan); he included four species in the Mimulus Complex, *Cx. mimulus* Edwards (Oriental and northern Australasian Regions), *Cx. murrelli* Lien (Oriental Region), *Cx. orientalis* Edwards (southwestern Palaearctic Region eastward to the Oriental Region), and *Cx. propinquus* Colless (Singapore). In 1981, a twelfth species, *Cx. tianpingensis* Chen, was described from China. Although the wing spots place *Cx. tianpingensis* in the Mimulus Complex, the male genitalia and larva of this species exhibit characteristics of members of the Mimeticus Complex.

Knowledge about the Mimeticus Subgroup progressed slowly until 2016 when we initiated studies of mosquitoes in Bhutan, a small country situated in the eastern Himalayan Mountains bordering India in the south and Tibet in the north and west. Mosquitoes in Bhutan are highly divergent, especially the montane species. In recent years, the studies resulted in the recognition of new species of the genera *Aedes* Meigen, *Anopheles*, and *Uranotaenia* Lynch Arribálzaga; new species groups of *Anopheles*; and a new subgenus of *Aedes* (Namgay et al. 2020, Somboon et al. 2021a,b; Somboon et al. 2022a,b,c). With regard to the Mimeticus Subgroup, in addition to *Cx. jacksoni*, *Cx. mimeticus*, *Cx. mimulus*, *Cx. murrelli*, and a species near *Cx. tsengi*, two new species of the group, *Cx. bhutanensis* Somboon, Namgay & Harbach and *Cx. longitubus* Somboon, Namgay & Harbach, were described (Somboon et al. 2021a,b, respectively).

In addition to *Cx. bhutanensis* and *Cx. longitubus* in Bhutan, three new species of the Mimeticus Subgroup were recently described from India. *Culex katezari* Karlekar, Andrew & Deshpande was described as a species of the Mimulus Complex from Maharashtra (Karlekar et al. 2020), and *Cx. gaugleri* Suman and *Cx. kodaikanalensis* Suman, Veer & Chandra, implicit members of the Mimeticus Complex, were described from Tamil Nadu in publications by Suman et al. (2021, 2022, respectively). Regrettably, the specific status of these three nominal species is doubtful because they were described based on only minor differences in adult morphology and insufficient

molecular evidence was provided to support their identities (see *Cx. katezari*, *Cx. gaugleri*, and *Cx. kodaikanalensis* below).

DNA (mainly mitochondrial *COI*) sequences for some species of the Mimeticus Subgroup were available in the GenBank database at the start of our studies. However, the results of analyses using the sequences, verified and unverified, were problematic. Some sequences listed for the same species were largely different, and some listed for different species were identical. It seemed likely that several sequences were either derived from misidentified specimens or unknown species in the Mimeticus Subgroup. Those findings prompted the present review, the objective of which is to provide updated information about species of the Mimeticus Subgroup, with an assessment of morphological and molecular data and the recognition of the species.

Material and Methods

Morphology

This review is based principally on information extracted and integrated from the works of Edwards (1915, 1932), Barraud (1924, 1934), Baisas (1938), Delfinado (1966), Lien (1968), Gutsevich et al. (1974), Sirivanakarn (1976), Tanaka et al. (1979), Chen (1981), Harbach (1988), Lu et al. (1997), Rattanarithikul et al. (2005), Karlekar et al. (2020), Somboon et al. (2021a,b), and Suman et al. (2021, 2022), and from websites: https://mosquito-taxonomic-inventory.myspecies.info/ and http://www.mosquitocatalog.org/default.aspx. The anatomical terminology used herein is defined in the Anatomical Glossary of the Mosquito Taxonomic Inventory (https://mosquito-taxonomic-inventory.myspecies.info/node/11027).

The costa and anterior veins of the wings of species of the Mimeticus Subgroup have three main areas of pale scaling traditionally referred to as the first (or middle), second, and third costal pale spots, respectively (see e.g., Sirivanakarn 1976 and Lien 1968). We use the traditional names, which more clearly define the positions of the spots than the more technical names, i.e., the 'sector pale + accessory sector pale spots', the 'subcostal pale spot', and the 'preapical pale spot', respectively, which were developed principally for *Anopheles* mosquitoes (Wilkerson and Peyton 1990).

Sequences, Alignment, and Phylogenetic Analysis

Sequences of the mitochondrial *COI* (or *COX1*) gene of species of the Mimeticus Subgroup deposited in GenBank (available at https://www.ncbi.nlm.nih.gov/nucleotide/) were used in the present study. Sequences of the *COII* and *cytochrome b* mitochondrial genes and rRNA, which are not available for most species, were not used. The similarity of sequences and the recorded identity of species of the Mimeticus Subgroup available in GenBank were compared using the Basic Local Alignment Search Tool (BLAST, available at http://blast.ncbi.nlm.nih.gov/Blast.cgi), under default parameters. Sequences were aligned using CLUSTAL W under default parameters (Larkin et al. 2007). Ragged ends were trimmed using MEGA v. 10.0.5 (Kumar et al. 2018). The best evolutionary model of nucleotide substitution that fits the data was obtained using jModelTest v. 2.1.10 (Darriba et al. 2012). Phylogenetic analyses were conducted using Maximum Likelihood in MEGA v. 10 (Kumar et al. 2018). Maximum Likelihood bootstrap (BS) values of 70% were taken as being highly supportive of a node (Hillis and Bull 1993). Genetic distances were estimated by using the Kimura two-parameter (K2P) method (Kimura 1980). Sequences that could not be reliably aligned were omitted from the analysis. *Culex quinquefasciatus* Say and *Cx. tritaeniorhynchus* Giles were used as the outgroup taxa because they

Table 1. Nucleotide sequences of the *COI* gene of species of the Mimeticus Subgroup deposited in GenBank (as of 16 August 2022)

Species (no. of sequences)	Accession no. Voucher no./ No. of base pairs	Country	Corrected identification
Cx. bhutanensis (6)	MW476147 658 bp	Bhutan: Mongar, below Korila	*Cx. bhutanensis*
	MW476148 658 bp	Bhutan: Mongar, Chompa	*Cx. bhutanensis*
	MW476149 658 bp	Bhutan: Mongar, Chompa	*Cx. bhutanensis*
	MW476152 658 bp	Bhutan: Thimphu, Namseling	*Cx. bhutanensis*
	MW476153 658 bp	Bhutan: Thimphu, Namseling	*Cx. bhutanensis*
	MW476155 658 bp	Bhutan: Sarpang, Asina	*Cx. bhutanensis*
*Cx. gaugleri** (1)	MW309109 21927/H6 678 bp	India: Tamil Nadu, Kodaikanal hills	*Cx. jacksoni**
Cx. jacksoni (3)	MW476157 658 bp	Bhutan: Paro, Shaba river	*Cx. jacksoni*
	MW476158 658 bp	Bhutan: Bumthang, Kurji	*Cx. jacksoni*
	MT578860 21940/H6 630 bp	India: Tamil Nadu, Kodaikanal hills	*Cx. jacksoni*
Cx. kodaikanalensis (*Culex* sp. n. 1 DS-2021)* (1)	MT578858 21920/H6 526 bp	India: Tamil Nadu, Kodaikanal hills	*Cx. jacksoni**
Cx. longitubus (5)	MW476150 658 bp	Bhutan: Mongar, Chompa	*Cx. longitubus*
	MW476151 658 bp	Bhutan: Mongar, Chompa	*Cx. longitubus*
	MW476156 658 bp	Bhutan: Sarpang, Asina	*Cx. longitubus*
	MW549522 658 bp	Bhutan: Sarpang, Asina	*Cx. longitubus*
	MW549523 658 bp	Bhutan: Sarpang, Asina	*Cx. longitubus*
Cx. mimeticus (28)	MK402796 658 bp	Spain: La Rioja, Logrono, Rio Iregua	*Cx. mimeticus*
	MK402806 658 bp	Spain: La Rioja, Logrono, Pantano de La Grajera	*Cx. mimeticus*
	MZ027402 514 bp	Spain: Araba, Basque Country	*Cx. mimeticus*
	KJ012096 658 bp	Turkey: Hatay, Kalecik	*Cx. mimeticus*
	KJ012097 658 bp	Turkey: Hatay, Kalecik	*Cx. mimeticus*
	KJ012098 658 bp	Turkey: Adana, Pozanti	*Cx. mimeticus*
	KJ012099 658 bp	Turkey: Hatay, Kalecik	*Cx. mimeticus*
	KJ012100 658 bp	Turkey: Hatay, Kalecik	*Cx. mimeticus*
	KJ012101 658 bp	Turkey: Adana, Pozanti	*Cx. mimeticus*
	MW961279 658 bp	Portugal	*Cx. mimeticus*
	MW961280 658 bp	Portugal	*Cx. mimeticus*
	MW961281 658 bp	Portugal	*Cx. mimeticus*
	KF406801 658 bp	Pakistan: AJK, Banjosa	*Cx. mimeticus*
	LC054532 650 bp	Japan: Tsushima, Nagasaki	*Cx. mimeticus*

Table 1. Continued

Species (no. of sequences)	Accession no. Voucher no./ No. of base pairs	Country	Corrected identification
	AB738226 658 bp	Japan	*Cx. mimeticus*
	AB738235 658 bp	Japan	*Cx. mimeticus*
	MW476159 658 bp	Bhutan: Bumthang, Badbalathang	*Cx. mimeticus*
	OP208064 SPCx-1 658 bp	Bhutan: Sarpang, Gelephu	*Cx. mimeticus*
	OP208065 SP-10 658 bp	Bhutan: Sarpang, Nepaltar	*Cx. mimeticus*
	OP208066 MGMT-4 658 bp	Bhutan: Mongar, below Korila	*Cx. mimeticus*
	OP208067 PRMT-1 658 bp	Bhutan: Paro, Getena	*Cx. mimeticus* form B
	MF278819 625 bp	China	*Cx. mimeticus*
	JQ728078 673 bp	China: Zhejiang Province	*Cx. mimeticus*
	JQ728151 679 bp	China: northern Yunnan Province	*Cx. mimeticus* form B
*Cx. mimeticus**	KT358433 658 bp	S. Korea	*Cx. jacksoni**
*Cx. mimeticus**	KT358434 658 bp	S. Korea	*Cx. jacksoni**
*Cx. mimeticus**	JQ728150 675 bp	China: northern Yunnan Province	*Cx. jacksoni**
*Cx. mimeticus**	JQ728152 678 bp	China: northern Yunnan Province	*Cx. jacksoni**
Cx. mimulus (28)	MN540352 544 bp	Malaysia: Sarawak, Siburan	*Cx. mimulus*
	KF564743 683 bp	Singapore	*Cx. mimulus*
	KF564744 672 bp	Singapore	*Cx. mimulus*
	KF564745 683 bp	Singapore	*Cx. mimulus*
	KF564746 677 bp	Singapore	*Cx. mimulus*
	KF564747 683 bp	Singapore	*Cx. mimulus*
	KF564748 644 bp	Singapore	*Cx. mimulus*
	KF564749 643 bp	Singapore	*Cx. mimulus*
	KF564750 643 bp	Singapore	*Cx. mimulus*
	KF564751 633 bp	Singapore	*Cx. mimulus*
	MG709138 454 bp	India	*Cx. mimulus*
	MG736656 459 bp	India	*Cx. mimulus*
	MG774467 465 bp	India	*Cx. mimulus*
	OP051157 CxMU1 658 bp	Bhutan: Phuntsholing, Pakharling,	*Cx. mimulus* form Y

Table 1. Continued

Species (no. of sequences)	Accession no. Voucher no./ No. of base pairs	Country	Corrected identification
	JQ728079 657 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728080 657 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728081 655 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728082 659 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728083 659 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728084 661 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728085 660 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728086 660 bp	China: Zhejiang	*Cx. mimulus* form X
	JQ728244 649 bp	China: northern Yunnan	*Cx. mimulus* form X
	JQ728245 663 bp	China: northern Yunnan	*Cx. mimulus* form X
	JQ728246 654 bp	China: northern Yunnan	n.d.
	MF278820 625 bp	China	n.d.
*Cx. mimulus**	JQ728247 678 bp	China: southern Yunnan, Mengla,	*Cx. murrelli**
*Cx. mimulus**	KU530127 539 bp	China	Not matched with any species*
Cx. mimulus complex (2)	MH427577 595 bp	Laos	*Cx. murrelli**
	MH427582 595 bp	Laos	*Cx. murrelli**
Cx. mimuloides (2)	EU259294 669 bp	India: Tamil Nadu, Doddabetta	*Cx. mimuloides*
	MT578859 21922/H6 531 bp	India: Tamil Nadu	*Cx. mimuloides*
Cx. murrelli (6)	MW476161 658 bp	Thailand: Lampang	*Cx. murrelli*
	MW476162 658 bp	Thailand: Chiang Mai	*Cx. murrelli*
	OP051158 658 bp	Bhutan: Panbang, Manus	*Cx. murrelli*
*Cx. murrelli**	MF278821 625 bp	China	n.d.
*Cx. murrelli**	AY729968 494 bp	India: Pondicherry	n.d.
*Cx. murrelli**	JQ728017 631 bp	China: Zhejiang Province	n.d.
Sp. near *Cx. tsengi* (3)	MW476154 658 bp	Bhutan: Thimphu, Namseling	Sp. near *Cx. tsengi*
	MW476160 658 bp	Bhutan: Haa, Jabalingzhi	Sp. near *Cx. tsengi*
	OP208068 658 bp	Bhutan: Haa, Jitshephu	Sp. near *Cx. tsengi*
Cx. orientalis (18)	AB690841 658 bp	Japan: Miyagi, Numata City	*Cx. orientalis*
	LC054467 650 bp	Japan: Wakayama, Kinokawa	*Cx. orientalis*
	LC054468 650 bp	Japan: Wakayama, Kinokawa	*Cx. orientalis*

Table 1. Continued

Species (no. of sequences)	Accession no. Voucher no./ No. of base pairs	Country	Corrected identification
	LC054469 650 bp	Japan: Fukushima, Kitakata	*Cx. orientalis*
	LC054470 650 bp	Japan: Hokkaido, Yubari	*Cx. orientalis*
	LC054471 650 bp	Japan: Gifu, Takayama	*Cx. orientalis*
	LC646375 658 bp	Japan: Niigata, Sado	*Cx. orientalis*
	LC646376 658 bp	Japan: Niigata, Sado	*Cx. orientalis*
	LC646377 658 bp	Japan: Niigata, Sado	*Cx. orientalis*
	LC646378 658 bp	Japan: Niigata, Sado	*Cx. orientalis*
	KT358427 658 bp	S. Korea	*Cx. orientalis*
	KT358428 658 bp	S. Korea	*Cx. orientalis*
	KT358429 658 bp	S. Korea	*Cx. orientalis*
	MW476163 658 bp	S. Korea: Seoul, Deokso	*Cx. orientalis*
*Cx. orientalis**	MW228487 658 bp	China: Xizang, Linzhi	*Cx. bhutanensis**
*Cx. orientalis**	MW228488 658 bp	China: Xizang, Linzhi	*Cx. bhutanensis**
*Cx. orientalis**	MW228489 658 bp	China: Xizang, Linzhi	*Cx. bhutanensis**
*Cx. orientalis**	MF278822 625 bp	China	*Cx. bhutanensis**

Originally misidentified specimens in the left column are marked with an asterisk (*) and the corrected identification is indicated in the right column and marked with an asterisk (*).

n.d. = not determined.

Results and Systematics

In total, 103 *COI* sequences (including 'unverified' sequences) of the following species of the Mimeticus Subgroup deposited in GenBank (as of 16 August 2022) were assessed and analyzed in the present study: *Cx. bhutanensis* (6 sequences), *Cx. gaugleri* (1), *Cx. jacksoni* (3), *Cx. kodaikanalensis* (as *Culex* sp. n. 1 DS-2021) (1), *Cx. longitubus* (5), *Cx. mimeticus* (28), *Cx. mimuloides* (2), *Cx. mimulus/mimulus* complex (30), *Cx. murrelli* (6), *Cx. orientalis* (18), and species near *Cx. tsengi* (3) (Table 1). The *COI* sequences varied in length from 454 to 683 bp. All sequences, except GenBank MN540352, MT578859, and KU530127, were included in the phylogenetic analysis – MN540352 and MT578859 were excluded because they only overlapped about 50% with most of the sequences and BLAST placed KU530127 outside species of the Mimeticus Subgroup (see Table 1 above and *Cx. mimulus* below). Phylogenetic analysis of 100 *COI* sequences, based on 348 positions, is shown in Fig. 1. Estimated K2P genetic distances are summarized in Table 2. No DNA sequences were available for *Cx. diengensis*, *Cx. fasyi*, *Cx. propinquus*, *Cx. solitarius*, *Cx. tianpingensis*, and *Cx. tsengi*. It is noteworthy that none of the sequences for *Cx. jacksoni*, *Cx. mimeticus*, and *Cx. murrelli* were for specimens from or in the vicinity of their type localities. Alignments of the sequences are shown in Suppl Figs. S1 and S2 (online only). Details of K2P genetic distances are shown in Supp Table S1 (online only).

To facilitate clear assessment and interpretation, the results of the morphological and molecular analyses are integrated here with the taxonomy and systematics of the species. The species, with relevant results, are treated in chronological order below, from the first, the nominotypical member of the Mimeticus Subgroup, to the most recently described species.

Culex mimeticus Noè, 1899

Culex mimeticus, originally described from an unspecified number of females collected at Grassano in the southern region of Basilicata, Italy (Noè 1899), has been recorded in many countries in the southern Palaearctic and Oriental Regions, from the Mediterranean Subregion to eastern and southeastern Asia (Harbach 1988). Edwards (1932) considered *Cx. pseudomimeticus* Sergent from Algeria to be a synonym of this species. Sirivanakarn (1976) provided a morphological key to distinguish the adults of *Cx. mimeticus* from the adults of other species of the Mimeticus Complex, principally based on wing

spots, mesonotal scales, and pale scaling on the legs and abdomen. According to Sirivanakarn, *Cx. mimeticus* and *Cx. fasyi* are morphologically indistinguishable in the adult, larval, and pupal stages, but are distinguished by features of the male genitalia. The adults of both species closely resemble those of *Cx. jacksoni*, but can be distinguished by having predominantly pale mesonotal scales and the midtibia without an anterior pale stripe. However, according to other investigators (Barraud 1934, Edwards 1935, Gutsevich et al. 1974, Tanaka et al. 1979, Harbach 1988), the pattern of wing spots and mesonotal scaling are variable and the presence of the pale stripe on the anterior surface of the midtibia of *Cx. mimeticus* was previously known. Such differences may be attributed to intraspecific variation, and may also be influenced by environmental conditions, in particular hyperpigmentation under cool conditions, as reported for other mosquito species, e.g., *Anopheles minimus* Theobald (Harrison 1980), *An. yaeyamaensis* Somboon & Harbach (Somboon et al. 2010), and species of the *An. lindesayi* complex (Somboon et al. 2022c). Gutsevich et al. (1974) stated that adults of *Cx. mimeticus* closely resemble those of *Cx. jacksoni*, but the larvae exhibit distinct differences. The male genitalia of both species are distinct; however, Sirivanakarn (1976) noted that the male genitalia of *Cx. mimeticus* figured by Lien (1968) from Taiwan did not appear to agree with the typical form of this species, but he did not provide details to distinguish them. Tanaka et al. (1979) suggested that *Cx. mimeticus* and *Cx. jacksoni* can be separated by the apical pale band on abdominal tergum VII, which is broader in *Cx. jacksoni*. Rattanarithikul et al. (2005) used this character to distinguish the two species in Thailand, but they also noted that adults are difficult to distinguish. We also had difficulty separating the adults of *Cx. mimeticus* and *Cx. jacksoni*, as well as those of a species near *Cx. tsengi* in Bhutan, using morphological features, which are quite variable and overlap.

Larvae of *Cx. mimeticus* from its type locality in Italy are not known because the species was described from the adult female(s). In general, larvae of *Cx. mimeticus* have a moderate to long siphon with setae on the posterior midline (seta 1-S) longer than the diameter of the siphon at the point of attachment, and comb scales with a strong median apical spine. However, those setae figured and described for the larva of *Cx. mimeticus* by Gutsevich et al. (1974) and Tanaka et al. (1979) appear to be shorter than those described and illustrated by Sirivanakarn (1976) and Rattanarithikul et al. (2005), and also the larvae of *Cx. mimeticus* found in Bhutan (personal observation), in which the setae are about 2–3 times longer than the diameter of the siphon. In addition, larvae of *Cx. mimeticus* described and figured by Barraud (1934), La Casse and Yamaguti (1950), and Tanaka et al. (1979) share similarities in setal branching and the shape and number of the dorsomental teeth (15–19, 15 or 16, and 15–17, respectively). We, hereafter, refer to specimens with 15 or more dorsomental teeth as *Cx. mimeticus* form A, as opposed to the form with fewer dorsomental teeth (13) described and figured by Sirivanakarn (1976), which is hereafter referred to as *Cx. mimeticus* form B. In Bhutan, *Cx. mimeticus* is a common species widely distributed at high elevations where larval forms A and B are occasionally found in sympatry. Bhutanese form A has seta 6-C double, seta 13-T usually 2-branched (2,3), and seta 2-S is strong and curved, similar to these setae described for *Cx. mimeticus* by Tanaka et al. (1979). In form B, seta 6-C is usually 3-branched, seta 13-T is usually 4-branched (3–7), and seta 2-S is strong but rather straight or slightly curved, similar to these setae described by Sirivanakarn (1976). In published studies, the thoracic integument of *Cx. mimeticus* larvae is either not mentioned or is said to be smooth, whereas in larvae of forms A and B in Bhutan the thoracic integument is lightly or moderately aculeate (spiculation of Sirivanakarn 1976).

Of 28 *COI* sequences attributed to *Cx. mimeticus* in GenBank (Table 1), 22 from European (Portugal, Spain, and Turkey) and Asian countries (China, Japan, and Pakistan), including the three sequences of larval form A from Bhutan (GenBank: OP208064–OP208066), are very similar or identical (98.60–100%; K2P 0.00–0.87%). The sequences formed a clade with BS of 87%, which was unresolved in comparison to the clades consisting of sequences of species of the Mimeticus Complex (Fig. 1). The sequence of *Cx. mimeticus* form B from Bhutan (GenBank: OP208067) and one sequence from China (GenBank: JQ728151) are more divergent from those of *Cx. mimeticus* (K2P 2.04–3.23%), but the sequence from China is almost identical to the sequence of larval form B from Bhutan (99.85%). Both sequences are quite similar to sequences of the species near *Cx. tsengi* (99.24–99.38%; K2P 0.00–0.58%), differing by two fixed positions (Supp Fig. S1 [online only]), and they were recovered in the same clade (BS 94%). Larvae of the species near *Cx. tsengi* differ from larvae of *Cx. mimeticus* form B in having 1–4 strong posterior spines on the distal half of the siphon. The morphological distinctions suggest that *Cx. mimeticus* form B is probably a novel species, but the small divergence of *COI* provides no support for this. The presence of aculeae on the thoracic integument of larvae of Bhutanese form A is probably a variable character of *Cx. mimeticus*, based on the molecular and phylogenetic evidence.

Two *COI* sequences from Korea (GenBank: KT358433 and KT358434) and two unverified sequences from China (GenBank: JQ728150 and JQ728152) identified as *Cx. mimeticus* are similar to *Cx jacksoni* from Bhutan (GenBank: MW476157) (>99%), and those sequences were recovered in a clade consisting of sequences for specimens of *Cx. jacksoni* from Bhutan and India (BS 79%). This is probably due to morphological misidentification of the adults used for DNA sequencing.

Culex mimulus Edwards, 1915

Culex mimulus was described from numerous adult specimens (number not specified) collected at unspecified localities in Sarawak, Malaysia (Edwards 1915). Three nominal species have been synonymized with *Cx. mimulus*, i.e., *Cx. confusus* Baisas (Balabac Island, the Philippines) (synonymy by Delfinado 1966), *Cx. mossmani* Taylor (Queensland, Australia) (synonymy by Edwards 1924), and *Cx. neomimulus* Lien (Taiwan) (synonymy by Sirivanakarn 1976). Although differences in the wing spots of *Cx. neomimulus* were considered to be normal variation of *Cx. mimulus*, larvae of the former differ from larvae of the latter in having 1) a greater number of dorsomental teeth (15 vs 13), 2) comb scales similar to those of *Cx. murrelli* in lacking a distinct median apical spine, but are quite different than the comb scales of *Cx. mimulus*, which have a small median apical spine, and 3) seta 2-X is 3-branched as opposed to usually being 2-branched (1,2) in *Cx. mimulus*. Further study of topotypic specimens of *Cx. neomimulus* and comparison of DNA sequences with those of *Cx. mimulus* are needed to clarify the taxonomic status of this nominal species.

Culex mimulus has been recorded in many countries of southern and southeastern Asia and northern Australia. According to Sirivanakarn (1976), adults of *Cx. mimulus* are similar to those of *Cx. murrelli* in the development of wing spots, although in the former the first median costal spot often involves the costa, subcosta, and veins R_1 and R_s, occasionally also extending onto M and CuA, whereas in *Cx. murrelli* the first median costal spot only involves the costa, subcosta, and vein R_1. Rattanarithikul et al. (2005), however, did not use wing spots as a character to distinguish the two species. The male genitalia of the two species are quite similar, with minor differences exhibited by the subapical claw of the gonostylus
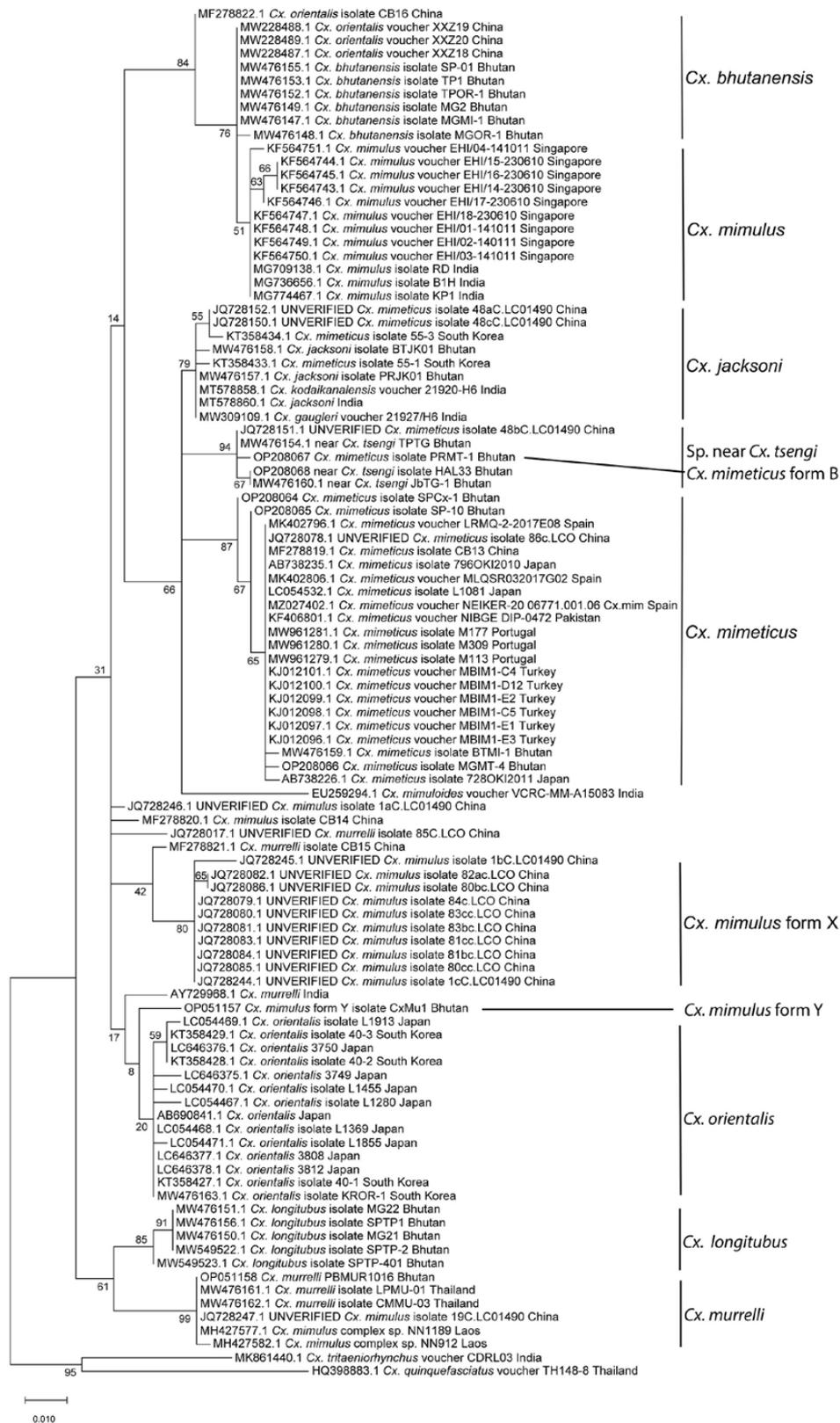
Fig. 1. Maximum Likelihood tree of 100 *COI* sequences (348 bp) from specimens of the Mimeticus Complex deposited in GenBank, with *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* as the outgroup taxa. Bootstrap values are shown at each node. The best-fit model of nucleotide substitution was GTR+I.

(Sirivanakarn 1976). In contrast, the larvae of the two species are clearly distinct in having differently developed comb scales and siphons of different length and shape.

Twenty-eight *COI* sequences, including unverified sequences, identified as *Cx. mimulus* from several countries are deposited in GenBank (Table 1). One sequence (544 bp) of *Cx. mimulus* from

Table 2. Kimura two-parameter genetic distance (K2P, %) of species of the Mimeticus Subgroup, based on 348 pairwise positions of *COI* sequences

Culex	bhutanensis	mimulus	orientalis	longitubus	murrelli	mimulus form X	mimulus form Y OP051157	mimulus JQ728246	mimulus MF278820	murrelli JQ728017	murrelli MF278821	murrelli AY729968	mimeticus	jacksoni	sp. near Cx. tsengi	mimeticus form B
bhutanensis	0.00–1.16															
mimulus	0.29–1.74	0.00–0.87														
orientalis	2.04–3.53	2.93–4.45	0.00–1.16													
longitubus	2.98–4.75	4.17–5.68	1.18–2.94	0.00–0.29												
murrelli	4.14–5.37	4.78–6.31	2.06–3.55	1.18–2.93	0.00–0.29											
mimulus form X	2.64–4.46	3.53–5.08	1.16–1.75	3.00–4.78	2.66–4.15	0.00–1.16										
mimulus form Y OP051157	2.64–3.53	3.53–4.45	1.16–1.75	2.69–3.55	3.85–4.16	2.05–2.95	–									
mimulus JQ728246	1.75–2.93	2.63–3.54	1.46–2.05	2.08–2.93	2.96–3.54	2.05–2.94	1.75	–								
mimulus MF278820	2.04–3.23	2.93–3.84	2.05–2.65	2.38–3.24	3.27–3.85	1.75–2.65	2.05	0.58	–							
murrelli JQ728017	2.93–3.54	3.23–4.15	2.05–2.66	2.38–3.24	3.26–3.85	2.95–3.86	2.65	1.45	1.75	–						
murrelli MF278821	2.94–4.14	4.14–4.76	1.45–2.05	2.38–3.24	2.66–3.24	1.16–2.05	2.66	1.45	1.16	1.75	–					
murrelli AY729968	2.93–4.14	3.83–4.75	2.93–4.77	2.99–3.85	3.88–4.46	2.35–3.25	2.05	1.45	1.75	2.34	2.35	–				
mimeticus	3.54–5.37	4.44–6.31	2.34–3.84	3.60–5.37	4.17–5.68	3.24–5.09	3.85–4.77	2.63–3.53	2.93–3.54	3.54–4.45	3.24–4.15	3.54–4.14	0.00–0.87			
jacksoni	2.33–3.83	3.23–4.75	2.93–4.14	2.98–4.44	3.55–4.75	2.63–3.85	3.24–3.84	2.04–2.63	2.34–2.93	2.93–3.53	2.63–2.93	2.93–3.53	1.46–2.94	0.00–0.87		
sp. near Cx. tsengi	3.83–4.44	4.13–5.37	3.23–4.44	3.59–4.74	4.16–5.05	3.53–4.76	4.14–4.45	2.93–3.23	3.23–3.53	3.83–4.14	3.53–3.83	3.83–4.14	2.04–3.23	1.45–2.34	0.00–0.58	
mimeticus form B	3.83–4.44	4.13–5.36	3.53–4.45	3.90–5.05	4.47–5.36	3.53–4.75	4.14–4.44	2.93–3.23	3.23–3.53	3.83–4.13	3.53–3.83	3.83–4.13	2.04–3.23	1.45–2.33	0.00–0.58	0.29–0.58
mimuloides	4.45–5.05	5.05–5.99		4.84–5.68	5.40–5.99	3.84–4.45	4.45	4.45	4.76	5.06	4.45	4.75	3.55–5.47	2.64–3.24	3.24–3.55	3.24–3.54

Sarawak, Malaysia (GenBank: MN540352), the type locality of this species (Edward 1915), is assumed to represent this species. Pairwise comparisons of the sequence from Malaysia and sequences from India (GenBank: MG709138, MG736656, and MG774467) and Singapore (GenBank: KF564743–KF564751) showed similarities of greater than 98.39%. This suggests that the specimens identified as *Cx. mimulus* from the three countries are conspecific. However, because the *COI* sequences of *Cx. mimulus* from Malaysia, India, and Singapore exhibited an overlap of about 50, 65, and 70%, respectively, with those of *Cx. mimulus* elsewhere and other species in the Mimeticus Subgroup (Supp Figs. S1 and S2 [online only]), comparison of sequence similarity and estimation of K2P genetic distance was limited to 348 bp. The phylogenetic analysis (based on 348 bp) revealed that the sequences of *Cx. mimulus* from Singapore and India (the sequence from Malaysia excluded) were closely related to the sequences of *Cx. bhutanensis*, and formed a clade in an unresolved relationship to sequences of *Cx. bhutanensis* (BS 51%; K2P 0.29–1.74%). Larvae of *Cx. bhutanensis* differ from larvae of *Cx. mimulus* in many ways (see *Cx. bhutanensis* below). Comparison of substantially longer pairwise sequences is needed to support a definite conclusion about the phylogenetic relationship of the two taxa.

Of 14 *COI* sequences from China identified as *Cx. mimulus* in GenBank, one sequence (539 bp, GenBank: KU530127) was largely different from sequences of other species of the Mimeticus Subgroup, and its identity is obscure, probably due to misidentification or contamination. The sequence is closest to that of *Cx. torrentium* Martini (95.38%), which is a species of the Trifilatus Subgroup of the Pipiens Group. One unverified sequence identified as *Cx. mimulus* (GenBank: JQ728247) is identical to the sequence of *Cx. murrelli* from Thailand (GenBank: MW476161). The other sequences identified as *Cx. mimulus* from China (539–663 bp; GenBank: MF278820) and 11 unverified sequences (GenBank: JQ728079–JQ728086 and JQ728244–JQ728246) were largely divergent from the clade of *Cx. mimulus* sequences from India and Singapore (K2P > 2.60%) (Fig. 1; Table 2). Ten of the former sequences (GenBank: JQ728079–JQ728086, JQ728244, and JQ728245) were identical or very similar (>98–100%; K2P 0.00–1.16%) and formed a clade with a highly supportive bootstrap value (80%), suggesting that these sequences possibly represent an unknown species. We hereafter provisionally refer to them as *Cx. mimulus* form X. The morphology of specimens of this form is unknown. The other two *Cx. mimulus* sequences (GenBank: JQ728246 and MF278820) and two sequences identified as *Cx. murrelli* from China (GenBank: MF278821 and JQ728017) were slightly divergent from the sequences of *Cx. mimulus* form X (K2P 2.05–2.94, 1.75–2.65, 1.16–2.05, and 2.95–3.86%, respectively; Table 2).

The only sequence of *Cx. mimulus* from Bhutan (658 bp, GenBank: OP051157), which overlaps about a 50, 65, and 90% with the sequences from Sarawak, Singapore, and India, respectively, exhibits low similarity (95.31–96.44%; K2P 3.53–4.45%) with those sequences. The sequence is also largely different from the sequences of *Cx. mimulus* form X from China (96.20–96.83%; K2P 2.05–2.95%), but less divergent from the sequences of *Cx. orientalis* from Japan and Korea (97.85–98.33%; K2P 1.16–1.75%). Although the Bhutanese *Cx. mimulus* has some affinity with *Cx. orientalis*, the larval specimen, which was used for DNA sequencing, is largely morphologically different from larvae of *Cx. orientalis* (see *Cx. orientalis* below), which have not been found in Bhutan and India. The larva of the Bhutanese specimen mostly agrees with the morphological description of *Cx. mimulus* provided by Sirivanakarn (1976), except the pecten spines have stronger lateral barbs and those near the base of the siphon have weaker denticles (delicate denticles in

Cx. mimulus), seta 2-X is 2- or 3-branched (1,2 in *Cx. mimulus*), and the thoracic integument has a light or moderate covering of aculeae (not mentioned in Sirivanakarn's description, but the integument is smooth in synonym *Cx. neomimulus*). Based on the molecular distinction, coupled with morphological differences, it is quite possible that the Bhutanese *Cx. mimulus* is an unrecognized species. We hereafter provisionally refer to it as *Cx. mimulus* form Y. The morphology of the adults is unknown.

Culex orientalis Edwards, 1921

Culex orientalis was described by Edwards (1921) from an unspecified number of males and females from Tokyo, Kobe, and Yokohama, Japan. [Sirivanakarn (1976) indicated that he examined the holotype male from Tokyo, but according to Townsend (1990), the type series consists of 15 syntypes: '4 male, 5 female, Tokyo; 2 male, 4 female, Yokohama']. The female of this species is easily separated from those of other species in the Mimulus Complex in having the basal costal pale spot of the wing extending onto vein R. However, this spot is inconspicuous or practically absent in males (LaCasse and Yamaguti 1950), which may cause confusion with males of *Cx. mimulus* and *Cx. murrelli*. *Culex orientalis* is probably restricted to the Palaearctic Region. It is common in Japan and the Korean Peninsula (including Jeju Island) (Tanaka et al. 1979), and is also recorded from Russia (Gutsevich et al. 1974) and northern China (Lu et al. 1997). The records of this species from Sri Lanka by Carter (1950), Taiwan by Lien (1962), and the Philippines by Baisas (1938) are very doubtful as the species is unlikely to occur in those countries. Larvae of *Cx. orientalis* are distinguishable from larvae of other species of the Mimeticus Subgroup, but closely resemble larvae of *Cx. bhutanensis*, particularly in the shape and length of the siphon and siphonal setae (see *Cx. bhutanensis* below). The proximal siphonal setae of *Cx. orientalis* are slightly longer than the diameter of the siphon at the point of attachment and the comb scales have a moderately developed median apical spine and rather stiff lateral spicules, whereas the comb scales of most species of the Mimeticus Complex, except *Cx. mimuloides*, have a strong median apical spine. The comb scales of *Cx. mimulus* and *Cx. murrelli* do not have a long median apical spine. We examined a number of larval specimens of *Cx. orientalis* from Japan and South Korea and found that the thoracic integument is not smooth but has a light or moderate covering of aculeae. This character was not mentioned by Sirivanakarn (1976) and Tanaka et al. (1979).

The *COI* sequences of *Cx. orientalis* from Japan and South Korea are identical or very similar (99.23–100%; K2P 0.00–1.16%), and formed a clade in an unresolved relationship to the sequence of *Cx. mimulus* form Y from Bhutan (K2P 1.46–2.95%) and a sequence listed as *Cx. murrelli* (GenBank: AY729968) from India (K2P 1.45–2.05%). The interspecific K2P genetic distances between the sequences of *Cx. orientalis* and *Cx. longitubus* and the sequences of *Cx. orientalis* and *Cx. mimulus* form X are low, i.e., 1.18–2.94 and 1.46–2.95, respectively. Four sequences in GenBank from China identified as *Cx. orientalis*, three (GenBank: MW228487–MW228489) from southeastern Tibet (Linzhi or Nyingchi, Xizang, China) and one (GenBank: MF278822) from an unspecified locality, are distinct from a sequence of *Cx. orientalis* from Japan (GenBank: LC054471) (97.23–98.56%), but they are similar to a sequence of *Cx. bhutanensis* from Bhutan (GenBank: MW476149) (98.94–99.09%; K2P 0.00–0.87%), differing at only one fixed position (Supp Fig. S2 [online only]), and were recovered in a clade with that species (BS 84%). The sequences from China were submitted to GenBank before *Cx. bhutanensis* was recognized as a new species. For that reason, the Chinese specimens were probably misidentified,

particularly if larvae were used for DNA analysis because larvae of *Cx. orientalis* and *Cx. bhutanensis* are quite similar.

Culex mimuloides Barraud, 1924

Culex mimuloides was described as a variety of *Cx. mimeticus* based on adults of both sexes from the Nilgiri Hills in southern India (Barraud 1924). The species is also known from Yunnan, China. The wing venation of the adults is very distinctive in having the furcation of vein M_{1+2} proximal to the furcation of vein R_{2+3}. This is in contrast to the other species of the Mimeticus Complex in which the two furcations are approximately at the same level. Larvae of *Cx. mimuloides* have seta 4-P 2-branched, but this seta is single in all other species of the Mimeticus Subgroup. The comb scales are similar to those of *Cx. murrelli* in having an evenly rounded apical fringe of fine spicules. The posterior setae (seta 1-S) of the siphon are as long as or shorter than the diameter of the siphon at the point of attachment, as in *Cx. murrelli*. Two *COI* sequences (669 bp, GenBank: EU259294, and 531 bp, GenBank: MT578859) from Indian specimens identified as *Cx. mimuloides* are deposited in GenBank (Table 1). Only 318 base pairs overlap, but they are identical. Only sequence MT578859 was included in the phylogenetic analysis, and it was placed in an unresolved relationship with the clades of other species of the Mimeticus Complex (Fig. 1).

Culex diengensis Brug, 1931

Culex diengensis was described from adults (both sexes) and is only known from localities on the island of Java, Indonesia (Brug 1931). The larval and pupal stages remain unknown. According to Sirivanakarn (1976), veins R_{4+5} and M_{3+4} of the wings of this species are largely pale-scaled from near their bases to near their tips, which is slightly different from other species of the Mimeticus Complex in which vein R_{4+5} is entirely dark-scaled or with a pale streak at midlength and vein M_{3+4} has a short median pale streak. However, we found that some females of *Cx. mimeticus*, *Cx. jacksoni*, *Cx. longitubus*, and the species near *Cx. tsengi* from Bhutan exhibit variation in the pale scaling of these veins. In contrast, the male genitalia of *Cx. diengensis* are the most distinctive among the species of the Mimeticus Complex, being characterized by several peculiar modifications. The aedeagus and proctiger of this species are apparently more similar to those parts of the male genitalia in species of the Mimulus Complex than they are in other species of the Mimeticus Complex.

Culex jacksoni Edwards, 1934 (in Barraud 1934)

Culex jacksoni was described from a male and a female, with associated larval exuviae, from Hong Kong (Edwards, in Barraud 1934), and is recorded from localities in Bhutan, mainland China, India, the Korean Peninsula, Nepal, Russia, Sri Lanka, Taiwan, and Thailand. According to Sirivanakarn (1976), adults of *Cx. jacksoni* closely resemble the adults of *Cx. mimeticus*, but differ in having predominantly brownish mesonotal scales, the midtibia with an anterior pale stripe, and the proboscis of the male has a distinct ventral cluster of several long setae. However, Edwards (1935) and Tanaka et al. (1979) revealed that these characters are variable and overlap. Tanaka et al. (1979), however, found that the females differ from the females of *Cx. mimeticus* by usually having a broad pale apical band on abdominal tergum VII (see also *Cx. mimeticus* above). The male genitalia of *Cx. jacksoni* are quite different from the male genitalia of *Cx. mimeticus* and the other species of the Mimeticus Subgroup in lacking the basal sternal process of the proctiger. While the adults are quite similar to the adults of other species of the Mimeticus

Complex, the larva of *Cx. jacksoni* is unique in having 2–9 (or more) prominent, strong spines borne posteriorly on the distal half of the siphon. Sirivanakarn (1976) revealed that seta 6-I,II is usually 4- or 5-branched in larvae of *Cx. jacksoni*, but in larvae from Bhutan we found that this seta is usually 3-branched, occasionally 4-branched, as in other species of the Mimeticus Subgroup, agreeing with Tanaka et al. (1979). We therefore consider that differences in branching may be due to intraspecific variation in *Cx. jacksoni*. Sirivanakarn (1976) considered *Cx. fuscifurcatus* Edwards (in Barraud 1934) and *Cx. kangi* Lien (Lien 1968) to be synonyms of *Cx. jacksoni* based on similarities of the male genitalia. However, the larval siphon of *Cx. kangi* is apparently more slender than the siphon of *Cx. jacksoni*, and the former has more dorsomental teeth (15 vs 13). Since we have not seen much variation in the shape of the siphon in specimens of *Cx. jacksoni* from Bhutan, we are reluctant to accept the observation of Sirivanakarn (1976). Further study of *Cx. kangi* is needed, particularly a comparison of DNA sequences of topotypic specimens with specimens of *Cx. jacksoni*, to better classify and assess the taxonomic status of that nominal species.

The *COI* sequences of two larvae of *Cx. jacksoni* from Bhutan (GenBank: MW476157 and MW476158) are similar (99.85%). The sequence MW476157 is similar to the *COI* sequences of *Cx. jacksoni* (99.67%), *Cx. gaugleri* (99.81%), and *Cx. kodaikanalensis* (99.82%) from India, which were all recovered in a single clade (BS 79%) (Fig. 1), indicating that these three nominal species are conspecific (also see *Cx. gaugleri* and *Cx. kodaikanalensis* below). As stated previously, two *COI* sequences from South Korea (GenBank: KT358433 and KT358434) and two unverified sequences from China (GenBank: JQ728150 and JQ728152) identified as *Cx. mimeticus* are in fact sequences of *Cx. jacksoni*.

Culex fasyi Baisas, 1938

Culex fasyi was described from five males and three females collected at Baguio, a mountain town on Luzon Island, the Philippines, but according to the author (Baisas 1938) the females were only 'provisionally regarded as the same species.' Very little is known about the species, which is probably the only representative of the Mimeticus Complex in the Philippines. According to Sirivanakarn (1976), the adults, larva, and pupa of *Cx. fasyi* are extremely similar to those life stages of *Cx. mimeticus*, but the former can be distinguished by having a shorter basal sternal process on the proctiger of the male genitalia. The male genitalia of *Cx. fasyi* and *Cx. tsengi* are indistinguishable. Due to the absence of DNA sequences for *Cx. fasyi* in GenBank, it is impossible to determine its phylogenetic relationship with other species of the Mimeticus Subgroup.

Culex solitarius Bonne-Wepster, 1938

Bonne-Wepster (1938) described *Cx. solitarius* from 'a series of males and females' (number not specified) collected at Anggi Lakes in present-day West Papua Province, Indonesia. *Culex solitarius* is only known from West Papua Province, which encompasses the two western peninsulas of New Guinea Island, and very little is known about the species. Sirivanakarn (1976) noted that *Cx. solitarius* is similar to *Cx. fasyi* in the adult, larval, and pupal stages, including the male genitalia, and suggested that the two species were conspecific. However, due to the limited number of specimens available for examination, Sirivanakarn was reluctant to synonymize *Cx. solitarius* with *Cx. fasyi*. Further morphological study and molecular data are needed to confirm the specific status of *Cx. solitarius*.

Culex propinquus Colless, 1955

Culex propinquus was described from three females reared from pupae collected from a well in Pulau Blakang Mati, Singapore (Colless 1955). This species is only known from Singapore, and the male and the larval and pupal stages are unknown. The adult female is 'Very similar to the Malayan form of *C. mimulus*, differing in the presence of white mottling in the dark areas of legs, wings [veins CuA and 1A] and proboscis' (Colless 1955). As noted by Sirivanakarn (1976), and in the present study, the adults of other species of the Mimeticus Subgroup do not appear to exhibit an overlap in these characters with *Cx. propinquus*.

Culex tsengi Lien, 1968

Culex tsengi was described from males and females with associated larval and pupal exuviae (Lien 1968). The species is known only from its type locality: Chihtuan, Loshui, Tatung, Ilan Hsien, Taiwan. Contrary to the online Systematic Catalog of Culicidae (http://www.mosquitocatalog.org/taxon_descr.aspx?ID=17553), *Cx. tsengi* is not found in Thailand (Rattanarithikul et al. 2005, Wilkerson et al. 2021). Sirivanakarn (1976) observed that adults of this species can be separated from the adults of *Cx. mimeticus* and *Cx. jacksoni* by having the median costal pale spot of the wing largely involving the subcosta and the third or apical costal pale spot coving only the apical portion of vein R_1. However, as stated by Lien (1968), 'It is almost impossible to separate this species from *Cx. mimeticus*… by pale markings on wings. Although the costa at middle of wing is entirely dark in the type specimens, it is pale in the specimens from other localities on Taiwan.' We follow Lien's description and consider that the identification of *Cx. tsengi* should not be based solely on adult morphology. In contrast, the larva of *Cx. tsengi* is unique in having 1–3 large simple pecten spines located between the second and seventh elements of siphonal seta 1-S. Although the larva of *Cx. jacksoni* also has more distally located pecten spines, it differs from the larva of *Cx. tsengi* in having a stouter and shorter siphon, shorter seta 1-S in relation to the diameter of the siphon, and more numerous and stronger distal siphonal spines.

A species near *Cx. tsengi* was recently found in many districts of Bhutan (Somboon et al. 2021a). It was previously misidentified as *Cx. tsengi* due to the similarity of the larval siphon, i.e., the presence of distal pecten spines (Somboon et al. 2020). The adults are morphologically similar to *Cx. jacksoni*, *Cx. mimeticus*, and *Cx. tsengi*. However, the larva differs from the larva of *Cx. tsengi* described and figured by Lien (1968) in having relatively longer and stronger distal spines on the posterior side of the siphon, fewer dorsomental teeth (11–13 vs 15), and the presence of aculeae on the thoracic integument. Also, seta 4-P is occasionally 2-branched. Pupae of the Bhutanese form differ in having seta 6-III–VI single or 2-branched as opposed to having 3 branches in the Taiwanese form. Unfortunately, there are no DNA sequences for *Cx. tsengi* from Taiwan in GenBank for comparison. The *COI* sequences of the species near *Cx. tsengi* comprise a clade closely related to sequences of *Cx. mimeticus* form B and a specimen of *Cx. mimeticus* from China as stated previously.

Culex murrelli Lien, 1968

Culex murrelli was described from a series of adults (both sexes) reared from larvae collected at Hsinhsien, Wulai, Taipei, Hsien, Taiwan (Lien 1968). Years earlier, Barraud (1934) reported that *Cx. mimulus* in India consisted of larvae with both short and long siphons. Similarly, Bram (1967) observed that larvae of *Cx. mimulus* in northern Thailand generally had a short and stout siphon, contrasting with specimens from southern Thailand,

Indonesia, and the Philippines, which have a long, narrow siphon. It is likely that the long-siphon form refers to *Cx. mimulus* and the short-siphon form corresponds with *Cx. murrelli*. Besides the length of the siphon, the comb scales of *Cx. mimulus* have a median apical spine that is slightly longer than the lateral spicules, whereas the comb scales of *Cx. murrelli* have apical spicules similar to the lateral spicules. Lien (1968) noted that in *Cx. murrelli* the first median costal pale spot of the wing extends only onto vein R_1, whereas in *Cx. mimulus* that spot also extends onto vein R_s. However, Sirivanakarn (1976) found that the wing patterns of the two species are variable and often overlap, in agreement with Rattanarithikul et al. (2005), who did not use wing spots to distinguish the two species in Thailand. We also found variation in the pale spots on the wings of *Cx. murrelli* from Thailand, not only at vein R_s but also at the furcations of veins R_{2+3} and M_{1+2}, and also the length of the pale spot on vein R_{4+5}, which is occasionally completely dark. We therefore conclude that these two species cannot be reliably distinguished based on pale wing spots. Their larvae, however, are clearly distinct.

Culex murrelli has been recorded throughout southern and southeastern Asia. Unfortunately, no DNA sequences of this species are available from its type locality (Taiwan) for comparison with specimens collected in other countries. The morphology of larvae of *Cx. murrelli* from Thailand and Bhutan, which we used for DNA sequencing, are similar and mostly resemble the larva of the species described by Sirivanakarn (1976) and Rattanarithikul et al. (2005). However, they differ from larvae of Taiwanese *Cx. murrelli* described and figured by Lien (1968) in having 13 dorsomental teeth (vs 15), with one lateral tooth on each side relatively larger (vs more or less equal in size to the lateral teeth), seta 6-C usually 2-branched (2,3) (vs 3- or 4-branched), and the thoracic integument with a light or moderate covering of aculeae (vs smooth).

Pairwise comparisons of *COI* sequences derived from larvae of *Cx. murrelli* from Thailand (GenBank: MW476161 and MW476162) and Bhutan (GenBank: OP051158) revealed a high degree of similarity (99.09–99.85%). Two *COI* sequences of the *Cx. mimulus* complex in GenBank from Laos (GenBank: MH427577 and MH427582) and one unverified *COI* sequence from China (GenBank: JQ728247) identified as *Cx. mimulus* are identical or very similar to sequences of *Cx. murrelli* from Thailand and Bhutan (99.23–100%). All of the sequences form a single clade (BS 99%; K2P 0.00–0.29%), in an unresolved relationship to the clade consisting of sequences of *Cx. longitubus* (Fig. 1). However, two sequences from China (GenBank: MF278821 and unverified JQ728017) identified as *Cx. murrelli* are distinct from a sequence of *Cx. murrelli* from Thailand (GenBank: MW476161) (97.44 and 97.13%, respectively; K2P > 2.6%). The two sequences from China are 97.92 and 96.67% similar, respectively, to a sequence within the clade of *Cx. mimulus* form X (GenBank: JQ728080) and are not included within that clade (Fig. 1). In addition, one sequence from India listed as *Cx. murrelli* (494 bp, GenBank: AY729968) is largely different from the MW476161 sequence of *Cx. murrelli* from Thailand (95.68%; K2P > 3.8%) and is more closely associated with but divergent from the sequence of *Cx. mimulus* form Y from Bhutan (97.41%; K2P 2.05%). Also, the K2P genetic distances between the Indian sequence and the sequences of *Cx. orientalis* from Japan and Korea that form a sister relationship with *Cx. mimulus* form Y from Bhutan is 1.45–2.05%. Due to insufficient information, it is not certain whether sequences AY729968, JQ728017, JQ728246, MF278820, and MF278821 are an unknown species or a variant of a currently recognized species of the Mimulus Complex.

Culex tianpingensis Chen, 1981

Culex tianpingensis, known only from China, was described from adults (both sexes) and larvae collected in the Zhuang Autonomous Region (Chen 1981). Adults have wings similar to other species of the Mimulus Complex, but differ slightly in having the first costal pale spot extended onto the subcosta and veins R_1, R_s, and M, and pale scales on the proximal portion of the costa before the first costal pale spot. The male genitalia of *Cx. tianpingensis*, however, are similar to those of *Cx. longitubus* and other species of the Mimeticus Complex, particularly in having a relatively narrow foliform seta *g* and the absence of minute spicules near the tip of the gonostylus. Larvae generally resemble larvae of *Cx. longitubus* in the shape of the siphon and the development of setae 1,2-S, but differ in many other characters, as described by Somboon et al. (2021b) (see *Cx. longitubus* below). The characters of the larval stage and male genitalia are somewhat similar to characters in species of the Mimeticus Complex, but based on wing spots *Cx. tianpingensis* bears a closer resemblance to species of the Mimulus Complex. Therefore, it is difficult to confidently place *Cx. tianpingensis* in either of the two complexes of the Mimeticus Subgroup recognized by Sirivanakarn (1976). The pupa of *Cx. tianpingensis* is known (Chen and Zhao 1987), but no DNA sequences are available for the species, indicating that further study is needed to resolve its relationship with the species of the Mimeticus Subgroup. For the present time, we prefer to treat *Cx. tianpingensis*, like *Cx. longitubus*, as a species of the Mimeticus Subgroup without assigning it to one of the two complexes.

Culex katezari Karlekar, Andrew & Deshpande, 2020

Culex katezari was described as a species of the Mimulus Complex from specimens collected in or around the village of Katezari in the Gadchiroli District of Maharashtra State, India (Karlekar et al. 2020). It was described as different from other species of the Mimeticus Subgroup based primarily on minor differences in the wing spots of five females reared from wild-caught larvae. The authors described the holotype female and a paratype male, but did not describe the larva, pupa, and male genitalia. They compared the wing spots of their purported new species in a table with the 'generalized' pattern of wing spots in the other species of the Mimeticus Subgroup with disregard for variation, which makes it difficult to accurately identify and distinguish the species with certainty (Delfinado 1966, Bram 1967, Lien 1968, Tanaka et al. 1979, Sirivanakarn 1976, Rattanarithikul et al. 2005). In addition to wing spots, the authors generated a 16S rRNA sequence for the species (the methodology and source were not provided), but the sequence is not available in GenBank for comparison with the 16S rRNA sequences that are available for other species of the subgroup. Ironically, the authors stated that confirmation of *Cx. katezari* was made by BLAST comparison of the 16S sequence 'with all the species' of the Mimeticus Subgroup despite the fact that 16S sequences are not available for 'all' species of the group.

Culex bhutanensis Somboon, Namgay & Harbach, 2021

Culex bhutanensis was described from Bhutan (Somboon et al. 2021a). The wing spots of this species are similar to the wing spots of *Cx. mimulus* and *Cx. murrelli*, except for the presence of a distinct line of pale scales along the posterior margin of the costa from near the base of the wing to the first costal pale spot, which is usually absent in *Cx. mimulus* and *Cx. murrelli*, as noted by Sirivanakarn (1976). The wings of *Cx. bhutanensis* females differ from the wings of *Cx. orientalis* in the absence of a basal costal pale spot, which

extends onto vein R. Larvae of *Cx. bhutanensis* mostly resemble larvae of *Cx. orientalis* in setal branching and the form of the siphon and siphonal setae, which may cause misidentification. However, seta 2-X of *Cx. bhutanensis* is usually 2-branched and the comb scales are thorn-shaped with a strong apical spine and fine lateral spicules, whereas in larvae of *Cx. orientalis* seta 2-X is usually 3- or 4-branched and the comb scales have a moderately developed apical spine. Larvae of *Cx. bhutanensis* differ from larvae of *Cx. murrelli* and *Cx. mimulus* in the shape of the siphon, the length of seta 1-S, and, in particular, the comb scales have a strong median apical spine. Pupae of *Cx. bhutanensis* can be separated from pupae of other members of the Mimeticus Subgroup occurring in Bhutan, and *Cx. orientalis* in Japan, by having seta 8-C with 3–5 branches (usually 4) whereas this seta in the other species is usually 2- or occasionally 3-branched.

Six *COI* sequences of *Cx. bhutanensis* (GenBank: MW476147–MW476149, MW476152, MW476153, and MW476155) (Table 1) show a high degree of similarity (99.24–100%). As stated previously, four *COI* sequences (GenBank: MW228487–MW228489 and MF278822) of specimens listed as *Cx. orientalis* from China were recovered in a clade along with sequences of *Cx. bhutanensis* (BS 84%; K2P 0.00–1.16%) (Fig. 1), indicating that *Cx. bhutanensis* is not confined to Bhutan but also occurs in southern Tibet, which borders Bhutan to the north and west.

Culex longitubus Somboon, Namgay & Harbach, 2021

Culex longitubus was described from Bhutan, and it is not known at present to occur elsewhere (Somboon et al. 2021b). It was previously misidentified as *Cx. tianpingensis* by Somboon et al. (2020) due to similarity of the larval siphon and siphonal setae 1,2-S, and was later regarded as a species near *Cx. tianpingensis* by Somboon et al. (2021a). Larvae of *Cx. longitubus* differ from larvae of *Cx. tianpingensis* as follows: 1) Seta 7-P is 2-branched vs 3- or 4-branched in *Cx. tianpingensis* and other species of the Mimeticus Subgroup (Lien 1968, Sirivanakarn 1976), 2) seta 13-T is usually longer than the distance from its insertion to the base of seta 14-P whereas in *Cx. tianpingensis* it does not extend beyond the base of seta 14-P, 3) the comb scales have a longer apical spine, 4) the siphon is longer (index 5.6–8.6 vs 5.5–6.6), 5) seta 1-S is longer (5.0–7.0 vs 4.5–5.0 times the diameter of the siphon at the point of attachment), 6) the distal pecten spines are simple or have weak basal denticles, 7) seta 1-X is usually 2-branched (2,3) but single (Chen 1981) or 2-branched (Lu et al. 1997) in *Cx. tianpingensis*, 8) the ventral brush (seta 4-X) of *Cx. longitubus* consists of 10 setae whereas it consists of 12 setae in *Cx. tianpingensis* and most other species of the Mimeticus Subgroup, and 9) the anal papillae are longer (3.5–4.0 vs 3.0 times the length of the saddle). The male genitalia of both species are similar to the male genitalia of other species of the Mimeticus Complex, particularly in having a relatively narrow foliform seta *g* and the absence of minute spicules near the tip of the gonostylus. The wing spots of *Cx. longitubus* are very distinct from the wing spots of *Cx. tianpingensis*, in which the first costal pale spot involves only the costa and vein R_s, similar to that of *Cx. jacksoni*, *Cx. mimeticus*, and the species near *Cx. tsengi*, except for the presence of a distinct line of pale scales on the posterior margins of the proximal areas of the costa and subcosta.

Although most morphological characters of the adults, male genitalia, and larva of *Cx. longitubus* indicate the species is a member of the Mimeticus Complex, the pupa is intermediate between the pupae of species of the Mimeticus and Mimulus Complexes in having seta 1-II usually 3-branched (2–5-branched), seta 1-III,IV usually 4-branched (2–8), seta 6-III,IV usually 2-branched (2–4-branched), and seta 4-VIII 2-branched. Five *COI* sequences of *Cx. longitubus* (GenBank: MW476150, MW476151, MW146156, MW549522, and MW549523) show a moderate degree of similarity (97.87–100%), but intraspecific genetic distances are small (K2P 0.00–0.29%). The sequences formed a clade in an unresolved relationship to the clade consisting of sequences of *Cx. murrelli* (Fig. 1). Consequently, it is not possible to confidently place *Cx. longitubus* in either the Mimeticus or Mimulus Complex. For the present time, we prefer to treat *Cx. longitubus* as a member of the Mimeticus Subgroup without placement in one of the two complexes recognized by Sirivanakarn (1976).

Culex gaugleri Suman, 2021 and Cx. kodaikanalensis Suman, Veer & Chandra, 2022

Culex gaugleri and *Cx. kodaikanalensis* were described as new species of the Mimeticus Complex based on specimens collected in the Kodaikanal Hills in Tamil Nadu State, India (Suman et al. 2021, 2022, respectively). Both nominal species were described from only two adult females, a holotype and a paratype, which were reared from wild-caught larvae; however, the authors did not describe the larval and pupal stages, and the males are unknown. The first costal wing spot of both species involves the costa and subcosta as in species of the Mimeticus Complex. However, according to the authors, *Cx. gaugleri* differs from other species of the Mimeticus Subgroup in 1) the absence of a pale spot at the furcation of vein M, 2) having the furcations of veins R_{2+3} and M at the same level, and 3) the absence of a pale stripe on the anterior surfaces of the fore-, mid- and hindtibiae (the authors stated that the tibia of all legs are entirely dark-scaled, but anterior pale stripes are clearly visible on the mid- and hindtibiae in the image of the female of *Cx. gaugleri* shown in Figure 1 of Suman et al. 2021). According to Suman et al. (2022), *Cx. kodaikanalensis* is distinguished by the absence of a pale stripe on the anterior surface of all tibiae, the presence of pale spots at veins R and M, and the length of the pale spot on vein R_{4+5} (extending from the basal 0.18 to the distal 0.9 of the vein). As mentioned above, many species of the Mimeticus Complex, in particular *Cx. fasyi*, *Cx. jacksoni*, *Cx. mimeticus*, and *Cx. tsengi*, exhibit considerable variation in the pale scaling of the wings (pale spots), legs, and abdomen; hence, these variable characteristics cannot be used for reliable identification of the species. More importantly, the authors deposited *COI* sequences for *Cx. gaugleri* (voucher 21927/H6) and *Cx. kodaikanalensis* (as *Cx.* sp. n.1 DS-2021, voucher 21920/H6) in GenBank (GenBank: MW309109 and MT578858, respectively). They also submitted a *COI* sequence for a specimen of *Cx. jacksoni* (GenBank: MT578860) that was collected on the same day and in the same area as the type specimens of *Cx. gaugleri* and *Cx. kodaikanalensis* (Table 1). Surprisingly, the pairwise *COI* sequences (454 bp) of *Cx. gaugleri* and *Cx. kodaikanalensis* are identical. Similarly, the pairwise *COI* sequence of the specimen of *Cx. jacksoni* is identical with that of *Cx. gaugleri* (534 bp) and is very nearly identical (one base difference) with the *COI* sequence of *Cx. kodaikanalensis* (550 bp) (99.81%). Also, the *COI* sequence (463 bp) of the Indian specimen of *Cx. jacksoni* is very similar to the *COI* sequences of *Cx. jacksoni* from Bhutan (GenBank: MW476157, 99.67% and MW476158, 99.51%), differing at only two and three bases, respectively. All such nucleotide substitutions have no effect on the encoding of amino acids. In the phylogenetic analysis of the *COI* sequences (Fig. 1), the three Indian species were recovered in the same clade as *Cx. jacksoni* from Bhutan, and the likely misidentified *Cx. mimeticus* from China and South Korea (BS 79%; K2P 0.00–0.87%). Based on the analyses of the

mitochondrial sequences, it appears, therefore, that *Cx. gaugleri* and *Cx. kodaikanalensis* are both conspecific with *Cx. jacksoni*. To further support this, the authors also generated 16S rRNA sequences for *Cx. gaugleri* (GenBank: MW298532), *Cx. kodaikanalensis* (GenBank: MW064134), and *Cx. jacksoni* (GenBank: MW064136), all of which are identical (data not shown in this study).

Bionomic and Medical Importance

With the exception of *Cx. mimulus*, *Cx. murrelli*, and *Cx. propinquus*, species of the Mimeticus Subgroup that occur in tropical and subtropical areas are usually found at high elevations, but where they occur in the Palaearctic Region they can be found in plains and mountainous areas. Larvae inhabit in a variety of ground pools and natural and artificial container habitats fully exposed to sunlight or in partial or heavy shade in forested areas (LaCasse and Yamaguti 1950, Gutsevich et al. 1974, Sirivanakarn 1976, Tanaka et al. 1979, Rattanarithikul et al. 2005). In Bhutan, *Cx. bhutanensis*, *Cx. longitubus*, *Cx. jacksoni*, *Cx. mimeticus* (forms A and B), and the species near *Cx. tsengi* are often found in sympatry at high elevations (1,500 to > 3,000 m), whereas *Cx. mimulus* and *Cx. murrelli* are more common at lower elevations (100 to 1,000 m). Larvae are found in stagnant pools, roadside pools, ponds, wells, ditches, stream pools, stream margins, marshes, rock pools, and rice fields. These sites usually contain green algae, grasses, and other aquatic vegetation. We often found that larvae of *Cx. longitubus* had fed on green algae. Lien (1968) reported that *Cx. murrelli* is widely distributed in foot-hill regions, whereas *Cx. tsengi* is widely distributed in mountainous areas throughout Taiwan; larvae of the latter species are found most frequently associated with green algal mats covering pools in riverbeds. In Thailand, larvae of *Cx. mimulus* and *Cx. murrelli* are commonly found in rural and forested areas in a wide variety of ground water habitats, including cement and plastic tanks, drums, animal hoof prints, stream pools, rock holes, sphagnum pools, swamps covered with vegetation, tree holes, and bamboo stumps (Bram 1967, Rattanarithikul et al. 2005, P. Somboon, unpublished).

At high elevations in Bhutan, larvae of species of the Mimeticus Subgroup were often associated with larvae of *Aedes pulchriventer* (Giles), *Ae. shortti* (Barraud), *Ae. vexans* (Meigen), *Anopheles aberrans* Harrison & Scanlon, species of the *An. baileyi* complex (*An. baileyi* Edwards, *An. bhutanensis* Somboon, Namgay & Harbach, and *An. monticola* Somboon, Namgay & Harbach), *An. bengalensis* Puri, species of the *An. culicifacies* complex, species of the *An. lindesayi* complex (*An. druki* Somboon, Namgay & Harbach, *An. himalayensis* Somboon, Namgay & Harbach, *An. lindesayi* Giles, and *An. thimphuensis* Somboon, Namgay & Harbach), *An. peditaeniatus* (Leicester), *An. pseudowillmori* (Theobald), *An. willmori* (James), *Cx. bitaeniorhynchus* Giles, *Cx. pseudovishnui* Colless, *Cx. sasai* Kano, Nitahara & Awaya, *Cx. theileri* Theobald, *Cx. tenuipalpis* (Edwards), and *Cx. vagans* Wiedemann. At lower elevations, *Cx. mimulus* and *Cx. murrelli* were collected in association with *Ae. elsiae* (Barraud), *Ae. macfarlanei* (Edwards), *An. pseudowillmori*, *Cx. bitaeniorhynchus*, *Cx. minor* (Leicester), and *Cx. pallidothorax* Theobald (Somboon et al. 2021a,b, 2022a,b, and unpublished observations).

In the former U.S.S.R., larvae of *Cx. mimeticus*, *Cx. jacksoni*, and *Cx. orientalis* were occasionally found with *Ae. vexans*, *An. claviger* (Meigen), *An. hyrcanus* (Pallas), *An. marteri* Senevet & Prunnelle, *An. superpictus* Grassi, *Cx. bitaeniorhynchus*, *Cx. modestus* Ficalbi, *Cx. tritaeniorhynchus* Giles, *Cx. vagans*, and *Culiseta longiareolata* (Macquart) (Gutsevich et al. 1974). In Japan, *Cx. mimeticus* larvae were frequently collected in association with larvae of *Cx. orientalis* and other species, including *Ae. dorsalis* (Meigen), *An. sinensis* Wiedemann, *Cx. bitaeniorhynchus*, *Cx. hayashii* Yamada, *Cx. infantulus* Edwards, *Cx. pseudovishnui*, and *Cx. tritaeniorhynchus* (LaCasse and Yamaguti 1950, Sirivanakarn 1976, Tanaka et al. 1979).

Little is known about the biting behavior and possible medical importance of species of the Mimeticus Subgroup. Adults of this group rarely enter houses; hence, humans are certainly not primary or even secondary hosts (Bram 1967). In Bhutan and Thailand, adults of the group were rarely found in cowsheds and females were rarely captured attempting to bite humans. Colless (1959) reported that females of *Cx. mimulus* in Singapore probably feed on birds and are not known to bite humans.

Discussion

Knowledge of species of the Mimeticus Subgroup remains incomplete. Several species of the subgroup exhibit considerable intra- and interspecific variation in the adult and immature stages that may cause confusion and lead to misidentification. The present study showed that some *COI* sequences of species of this subgroup deposited in GenBank were obtained from misidentified specimens. Misidentification was common for specimens (sequences) of *Cx. mimeticus*, *Cx. mimulus*, *Cx. murrelli*, and *Cx. orientalis*. The life stage of voucher specimens is not usually specified for nucleotide sequences submitted to GenBank, and, as a result, it is impossible to determine the reason for misidentification. Basically, the identification of species of the Mimeticus Subgroup based on adult morphology could more easily result in errors than identifications based on larvae, which are generally morphologically distinct, except for the similarities in larvae of *Cx. bhutanensis* and *Cx. orientalis*. Adults of species of the subgroup are usually difficult to collect in the wild; therefore, collections of larvae and pupae are preferable, no less because they can be readily reared individually to adults under field or laboratory conditions. It is important to retain associated exuviae and one or more killed larvae to complement the identification of adults. An abdominal segment (e.g., segment VI) of larvae or one or two legs of adults can be used for DNA extraction, and the rest of the body retained for morphological identification and to serve as voucher specimens. DNA sequences from misidentified specimens are not uncommon in GenBank and other databases, e.g., *Ae. vittatus* (Bigot) (Pagac et al. 2021). Outdated or obsolete names, forms, variants, or *sensu lato* listed for some sequences have not been corrected to currently valid names, e.g., *An. campestris*-like (Thongsahuan et al. 2011), which was more recently described as *An. wejchoochotei* by Taai and Harbach (2015). These issues are likely to cause confusion and difficulties for researchers who have no knowledge of recent formal taxonomic changes and fail to apply integrated approaches for the collection, rearing, morphological study, and molecular analysis of mosquitoes. The integration of these methods provides a testable means of delimiting species and unambiguously identifying specimens.

These problems are evident in the case of *Cx. gaugleri* and *Cx. kodaikanalensis*, both of which were merely based on minor differences in the pale scaling of the wings and legs of only two females, without considering the immature stages (Suman et al. 2021, 2022, respectively). Analyses of *COI* sequences in the present study clearly indicate that *Cx. gaugleri* and *Cx. kodaikanalensis* are conspecific with *Cx. jacksoni*. As noted above, the authors also generated identical 16S rRNA sequences for *Cx. gaugleri*, *Cx. kodaikanalensis*, and *Cx. jacksoni*. In view of the vague morphological evidence,

doubtful description, similar *COI* sequences, and identical 16S rRNA sequences, we hereby formally recognize *Cx. gaugleri* Suman, 2021 and *Cx. kodaikanalensis* Suman, Veer & Chandra, 2022 as junior subjective synonyms of *Cx. jacksoni* Edwards, 1934 (in Barraud 1934).

Problems regarding *Cx. katezari* include the description being based on minor differences in wing spots and no comparison of DNA sequences with other species in the Mimeticus Subgroup (Karlekar et al. 2020). Although sequences of rRNA are largely unavailable for species of the Mimeticus Subgroup, the authors generated a 16S rRNA sequence for this species, which has not been deposited in any online database; hence, it is impossible to determine whether *Cx. katezari* is a distinct species. Therefore, due to insufficient morphological and molecular evidence to support the specific status of *Cx. katezari*, it is henceforth formally regarded as a *nomen dubium* until sufficient information becomes available to support or refute its identification as a separate species.

The present study recognizes the possible existence of at least three additional species of the Mimeticus Subgroup, i.e., *Cx. mimeticus* form B from Bhutan, *Cx. mimulus* form X from China, and *Cx. mimulus* form Y from Bhutan. *Culex mimeticus* form B differs from *Cx. mimeticus* form A in larval morphology and *COI* sequence (K2P 2.04–3.23%). It differs from the species near *Cx. tsengi* primarily in the absence of large distal spines on the siphon, but the *COI* sequences of both are very similar. We have found variation in the size, shape, position, and number of the distal spines in larvae of the latter. Therefore, it is not certain at this stage whether the presence of the distal spines reflects intra- or interspecific variation. Examination of a large number of specimens and additional morphological and molecular studies are required to establish the status of *Cx. mimeticus* form B and the species near *Cx. tsengi*.

The *COI* sequences of *Cx. mimulus* form X from China are distinct from the sequences of *Cx. mimulus* from Malaysia, Singapore, and India, and *Cx. mimulus* form Y from Bhutan (K2P 3.53–5.08 and 2.05–2.95%, respectively). However, both *Cx. mimulus* forms X and Y have some affinity with *Cx. orientalis* (K2P 1.46–2.95 and 1.16–1.75%, respectively). In general, a sequence divergence greater than 2% at the *COI* locus corresponds to variation at the interspecific level in *Anopheles* mosquitoes (e.g., Wang et al. 2012, Wang et al. 2017). However, recently diverged species can show little divergence at mtDNA and rDNA loci, e.g., the *An. gambiae* complex (Paskewitz et al. 1993, Besansky et al. 1997). We also found <2% interspecific divergence of *COI* sequences in some species groups of mosquitoes, e.g., subgenus *Culiciomyia* Theobald of *Culex* and subgenus *Metalutzia* Tanaka of *Lutzia* Theobald (Phanitchakun et al. 2017, 2019, respectively). In the present study, intraspecific K2P genetic distance within species of the Mimeticus Subgroup, based on 348 positions, was quite low, usually <1%, with a maximum variation of 1.16% as found for sequences of *Cx. bhutanensis*, *Cx. mimulus* form X, and *Cx. orientalis* (Table 2). Minimum interspecific divergence was about 1.45% between *Cx. jacksoni* and *Cx. mimeticus* and the species near *Cx. tsengi*, and only 1.18% between *Cx. longitubus* and *Cx. murrelli*, and *Cx. longitubus* and *Cx. orientalis* (K2P 1.18–2.93 and 1.18–2.94%, respectively). The minimum genetic divergence between *Cx. orientalis* and *Cx. mimulus* form Y was 1.16% (K2P 1.16–1.75%), which is, on average, greater than the range of intraspecific variation seen in the other wide ranging taxa studied here. The morphology of larvae of *Cx. mimulus* form Y, which is largely different from larvae of *Cx. orientalis*, strongly supports interspecific difference, although the genetic distance between them is small. Since the larval morphology of *Cx. mimulus* form X from China is not known, it is not possible to make

a comparison with *Cx. mimulus* form Y from Bhutan. However, the two forms have a genetic divergence of 2.05–2.95%, which supports their interspecific difference. This finding may encourage the authors who submitted the sequences of form X to undertake further study. In addition, it is not certain whether a few specimens of *Cx. mimulus* and *Cx. murrelli*, which have *COI* sequences that differ slightly from those of form X and *Cx. orientalis* (Table 2), represent variants of form X or another species of the Mimeticus Subgroup. Further analysis and comparison of rRNA and other gene sequences, as well as morphological study, are needed to assess the specific status of these forms.

The present study shows that the Mimeticus Subgroup is more complex than previously thought. In view of the results, we emphasize the significance of integrated morphological and molecular analyses to resolve the status of species within this subgroup. In the past, when molecular tools were not available, several species were regarded as synonyms of species of the subgroup despite some differences in morphology. It is possible to test the status of synonymous nominal forms using molecular methods. To achieve this, DNA sequences from topotypic specimens are essential. The application of *COI* barcodes for all members of the subgroup would provide an important resource for elucidating the diversity of species. Finally, sequences should be deposited in an international database (e.g., GenBank or BOLD) with appropriate data so that other researchers can use the sequences with confidence. It would be helpful to indicate the life stage or stages used for sequencing and retain voucher specimens to enable users to assess the identification of specimens because, as we have shown, larvae are more distinct than adults, and vice versa, in some species groups.

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Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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