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Genetic and morphological differences of *Haematobia irritans* and *H. exigua*, and molecular phylogeny of Japanese Stomoxyini flies (Diptera, Muscidae)

Mitsuhiro Iwasa[1] and Naotaka Ishiguro[2]

[1] *Laboratory of Entomology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, 080–8555 Japan*
[2] *Laboratory of Food and Environmental Hygiene Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Yanagido 1–1, Gifu, 501–1193 Japan*

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Abstract: Molecular analysis based on mitochondrial DNA (mtDNA) sequence was performed to elucidate the genetic difference between *Haematobia irritans* (Linnaeus) and *H. exigua* (de Meijere), and the phylogenetic relationship among Japanese Stomoxyini species (*Stomoxys calcitrans* (Linnaeus), *S. indicus* Picard, *S. uruma* Shinonaga et Kano, and *Haematobosca sanguinolenta* (Austen) including *Haematobia*). Two populations obtained from Obihiro and Morioka which were morphologically identified as *H. irritans* were genetically identical. Two populations obtained from Taiwan and Vietnam which were morphologically identified as *H. exigua* were almost genetically identical, but there was a small divergence (0.3–0.4%) between them. Nucleotide sequence divergences between *H. irritans* from Obihiro-Morioka and *H. exigua* from Taiwan-Vietnam were 1.8–1.9%, and those between *Haematobia* and other separate species of *Stomoxys* and *Haematobosca* were 6.7–10.9%. In cluster analysis based on mtDNA, *Haematobia* species were close to *Haematobosca sanguinolenta*. *S. calcitrans* and *S. uruma* were closely related to each other, and *S. indicus* was distantly related to these two species. We discussed the status of *H. irritans* and *H. exigua* based on mtDNA, and the discrepancies of molecular phylogeny versus the previous hypotheses based on morphological criteria in Japanese Stomoxyini.

Key words: *Haematobia irritans*, *Haematobia exigua*, mtDNA sequence, Japanese Stomoxyini, phylogeny

Introduction

Stomoxyini biting flies are important ectoparasites of domestic animals, comprising about 50 species in the world. In Stomoxyini, the horn fly *Haematobia irritans* (Linnaeus) and the buffalo fly *H. exigua* (de Meijere) are closely related to each other. *H. irritans* is mainly distributed in the Holarctic Region, though it was introduced to South America by humans and livestock; *H. exigua* is found throughout the Oriental and Australasian Regions. Morphological differences between these two species are very minor—*H. exigua* is distinguished from *H. irritans* by the presence of 4–6 long hairs with curled tips on the second segments of the male's hind tarsus (Malloch, 1932; Mackerras, 1933; Hennig, 1964; Van Emden, 1965; Kano et al., 1972). The two flies have often been regarded as different species (Bezzii, 1911; Malloch, 1932; Mackerras, 1933; Hennig, 1964; Van Emden, 1965; Snyder, 1965; Pont, 1986), or as subspecies (Schat, 1903; Zumpt, 1973; Pont, 1977). Genetic

[1] To whom correspondence shoud be addressed at: e-mail: iwasa@obihiro.ac.jp

surveys of *Haematobia* species are limited to a few papers that examined the molecular characterization and variability of *H. irritans* populations (Castiglioni and Bicudo, 2005; Brito et al., 2007). No such reports have been published on *H. exigua*.

Molecular phylogenetic studies of Diptera have been done by Clary and Wolstenholme (1985), Vossbrinck and Friedman (1989), DeSalle and Grimaldi (1991), and Friedrich and Tautz (1997). In particular, analysis of the COI and COII sequences of mitochondrial DNA (mtDNA) is useful for taxonomic classification and phylogenetic relationships of cyclorrhaphous flies (Baker et al., 2001; Wells and Sperling, 2001; Wells et al., 2002; Savage et al., 2004). Regarding the molecular phylogeny of Stomoxyini flies, however, there are only a few reports on the genus *Stomoxys* within Calypterate Diptera (Vossbrinck and Friedmann, 1989) and within Muscidae (Schuehli et al., 2007).

Therefore, comparative and phylogenetic analyses based on molecular data are necessary to resolve the species status of these species and to establish a phylogeny in Stomoxyini flies.

The purpose of this study is to determine the genetic and morphological differences of *H. irritans* and *H. exigua* and the molecular phylogeny of the six Japanese species of Stomoxyini based on analysis of mtDNA.

Materials and Methods

Insects used

In Japan, *H. irritans* is mainly distributed in the northern district (Hokkaido and Tohoku), and is absent in the Okinawa Islands. Taiwan is one of the northernmost margins in the Oriental distribution of *H. exigua.* We chose Vietnam for a comparison of *H. exigua* from a more southern and continental location. Therefore, the specimens of *H. irritans* and *H. exigua* were collected from four localities: [Japan] Obihiro (42°55′ N, 143°12′ E), Morioka (39°42′ N, 140°06′ E (Fig. 1); [Taiwan] Henchun (22°0′ N, 120°44′ E); [Vietnam] Hue (16°23′ N, 107°42′ E (Fig. 1).

Before total DNA extraction, the specimens were initially identified to species based on morphological criteria. *H. irritans* and *H. exigua* were identified by characteristics of long hairs with curled tips on the second segments of the male's hind tarsus, after Kano et al. (1972). For comparison of morphological traits in populations of *H. irritans* and *H. exigua* from 4 localities, we examined 16–38 individuals with distinction of sex in each population; we measured the body length with an ocular micrometer and counted numbers of frontal setulae on the head under a stereoscopic microscope. Details (species location, sampling date, no. of samples for analysis, and accession numbers) of the specimens of *Haematobia*, *Stomoxys*, *Haematobosca* used in this study are listed in Table 1. In addition, we examined three *Musca* species for determination of their genetic relationships with the genera of Stomoxyini (Table 1).

DNA extraction

For mtDNA extraction, the specimens were killed with cyanide and preserved in 99% ethanol. Small thoracic muscles of adults were removed from specimens in preserved 99% ethanol; the remainder of each specimen was deposited at Obihiro University of Agriculture and Veterinary Medicine. Total DNA was extracted from the muscle samples using DNeasy Tissue Kit (Qiagen Science, MD) according manufacturer's instruction.

PCR and direct sequencing of mtDNA

Nucleotide sequences from COI to COII region in mtDNA were amplified by PCR using five primer sets (TY-J-1460: TACAATTTATCGCCTAAACTTCAGCC and C1-N-2191: CCCGGTAAAATTAAAATATAAACTTC; Jer2: TTACCAGTATTAGCTGGAGG and C1-N-2800: CATTTCAAGT/CTGTGTAAGCATC; C1-J-2495: CAGCTACTTTATGAGCTTTAGG and Hae6: AAAGGAGAAGAACTATCTTG; Hae5: CCACC-

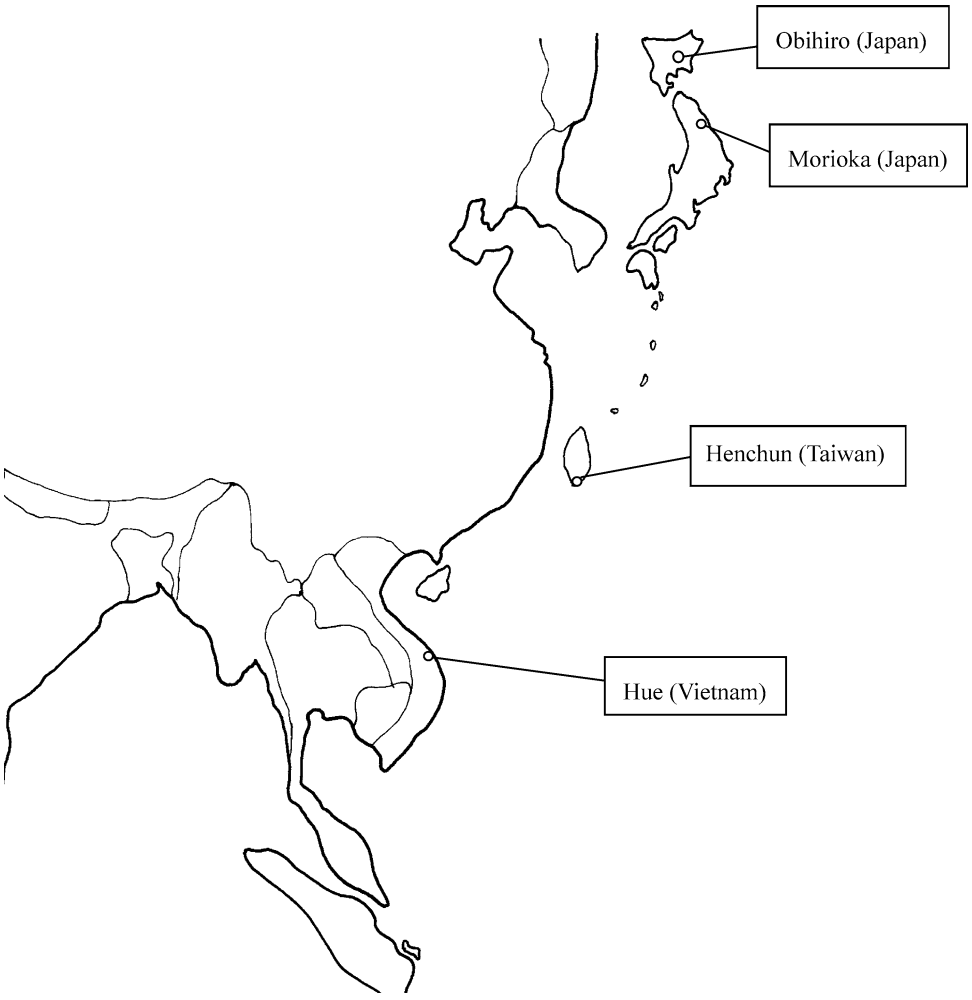

Fig. 1. Map showing sampling localities of *Haematobia irritans* and *H. exigua* in Japan, Taiwan, and Vietnam.

Table 1. Specimen details: Species, location, sampling date and gene bank accession number.

Species	Collection location	Country	Sampling date	No. of samples for DNA analysis	Accession number
Stomoxyini					
Haematobia irritans (Linnaeus)	Obihiro, Hokkaido	Japan	28-Aug-03	2	AB479522, AB479523
Haematobia irritans (Linnaeus)	Morioka, Honshu	Japan	1-Oct-03	2	—
Haematobia exigua (de Meijere)	Henchun	Taiwan	21-Dec-03	2	AB479524, AB479525
Haematobia exigua (de Meijere)	Hue, Thua Thien Hue	Vietnam	19-Nov-04	2	AB479526, AB479527
Stomoxys calcitrans (Linnaeus)	Obihiro, Hokkaido	Japan	4-Oct-03	2	AB479520, AB479521
Stomoxys indicus Picard	Ishigaki Is., Okinawa	Japan	28-Mar-04	2	AB479534, AB479535
Stomoxys uruma Shinonaga et Kano	Yonaguni Is., Okinawa	Japan	29-Mar-04	2	AB479518, AB479519
Haematobosca sanguinolenta (Austen)	Sapa, Lao Cai	Vietnam	26-Oct-02	2	AB479536, AB479537
Musca species					
Musca domestica Linnaeus	Obihiro, Hokkaido	Japan	13-Jun-04	2	AB479528, AB479529
Musca bezzii Patton et Cragg	Obihiro, Hokkaido	Japan	13-Jun-04	2	AB479532, AB479533
Musca crassirostris Stein	Ninh Binh near Hanoi	Vietnam	29-Oct-02	2	AB479530, AB479531

AGCAGAACATAGTTA and C2-N-3389: TCATAAGTTCA [R] TATCATTG; C2-J-3138: AGAGCCTCTCCTTTAATAGAACA and TK-N-3775: GAGACCATTACTTGCTTTCAGTCATCT) (Wells and Sperling, 2001; Jer2, Hae5 and Hae6 were originally synthesized). The PCR products were purified by using a QIAquick Kit (Qiagen) and used for a sequencing template. Nucleotide sequences were determined with an ABI PRISM 301 (Applied Biosystem, Foster, CA) with BigDye Terminater Cycle sequencing Kits (Applied Biosystems). The 2,272-bp nucleotide sequences of the partial region from COI to COII were constructed by connecting five DNA fragments amplified by the five primer sets.

Data analysis

Multiple-sequencing alignment of the PCR products was performed using GENETYX-MAC software (Software Development Co., Ltd., Tokyo, Japan). Neighbor-joining analysis was made using the PHYLIP program package, version 3.572 (Felsenstein, 1995). A distance matrix for the neighbor-joining tree was constructed with Kimura's two-parameter distance (1980). We used 1,000 bootstrap replications (Felsenstein, 1985) to determine the confidence intervals of the phylogeny.

Results

Table 2 shows the body length and numbers of frontal setulae of the populations of *H. irritans* and *H. exigua* collected from the four localities. We found significant differences in male body length among four populations (Obihiro > Morioka > Taiwan > Vietnam). Pictures of male adults of *H. irritans* and *H. exigua* collected from these localities can be seen in Fig. 2. There were also significant differences in average body length of females and in the number of frontal setulae on male heads between *H. exigua* populations of Taiwan and Vietnam (Table 2).

Percent uncorrected mtDNA sequence divergence of the six Japanese Stomoxyini is shown in Table 3. Sequences of the specimens from Obihiro and Morioka identified as *H. irritans* were the same, and their divergence was zero. Sequence divergence between two populations from Taiwan and Vietnam identified as *H. exigua* were 0.3–0.4%. In the four populations of *Haematobia* (Obihiro, Morioka, Taiwan, Vietnam), the most variable (0.4%) sequence divergence was that of *H. exigua* from Vietnam. Sequence divergences between *H. irritans* (Obihiro and Morioka) and *H. exigua* (Taiwan and Vietnam) were 1.8–1.9% and those between *Haematobia* species and other species (*Stomoxys* and *Haematobosca*) were 6.7–10.6%. Divergences between *Haematobosca* and *Haematobia* (6.7–7.0%) were smaller than those between *Haematobosca* and *Stomoxys* (8.5–10.8%). Among the three *Stomoxys* species, sequence divergences between *uruma* and *indicus* were larger (10.8–10.9%) than those between *calcitrans* and

Table 2. Body length and numbers of frontal setulae of *Haematobia irritans* and *H. exigua* collected from four localities.

Localities	Species	Average body length (mm)±SD		Average no. of frontal setulae±SD	
		Male	Female	Male	Female
Obihiro (Japan)	*H. irritans*	4.90±0.44a (22)	5.07±0.49a (21)	11.55±0.21a (22)	10.77±0.23a (22)
Morioka (Japan)	*H. irritans*	4.61±0.48b (20)	5.10±0.81a (16)	11.06±0.17a (18)	10.75±0.14a (16)
Henchun (Taiwan)	*H. exigua*	3.92±0.33c (29)	4.20±0.31b (35)	8.33±0.16b (30)	7.81±0.99b (38)
Hue (Vietnam)	*H. exigua*	3.35±0.42d (28)	3.69±0.47c (36)	7.61±0.09c (28)	7.48±0.98b (33)

Values in parentheses are numbers of specimens examined.
Values followed by the different letters within the columns are significantly different (Fisher's PLSD; $p < 0.05$).

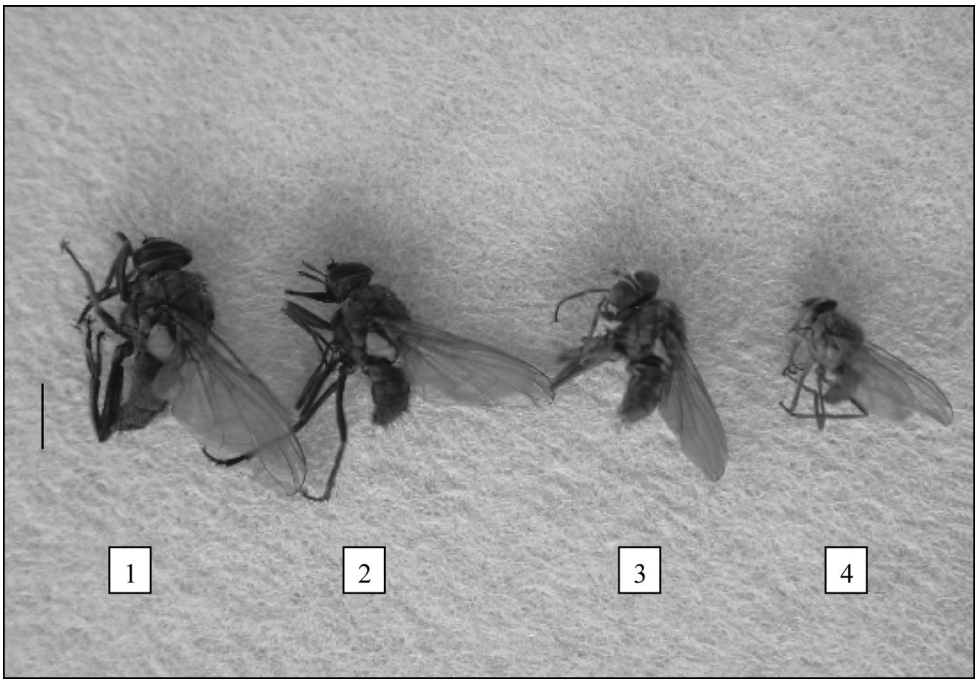

Fig. 2. Male adults of *Haematobia irritans* and *H. exigua* collected from four localities. 1: *H. irritans*, Obihiro (Japan); 2: *H. irritans*, Morioka (Japan); 3: *H. exigua*, Henchun (Taiwan); 4: *H. exigua,* Hue (Vietnam). Scale: 2 mm.

uruma, and between *calcitrans* and *indicus*. Those between *Musca* and Stomoxyini species (*Haematobia, Stomoxys* and *Haematobosca*) were 8.8–13.1%.

Based on mtDNA from COI to COII sequences, we constructed the neighbor-joining dendrogram of Japanese Stomoxyini species and three *Musca* species (Fig. 3). *H. irritans* (Obihiro) and *H. exigua* (Taiwan, Vietnam) cluster together, and *Haematobosca* clusters with the *Haematobia* species. There was some genetic distance between *H. irritans* and *H. exigua*, it was less than half of that between *Haematobosca* and *Haematobia*. Whereas the three *Stomoxys* species constitute another cluster, *S. calcitrans* and *S. uruma* are more closely related to each other. The genetic distances among the three *Stomoxys* species were also longer than that between *H. irritans* and *H. exigua*. *Musca* species form a sister group to a clade consisting of *H. irritans, H. exigua* and *Haematobosca sanguinolenta*. *M. crassirostris* is more closely related to *M. domestica* than to *M. bezzii*.

Discussion

Kano et al. (1972) reported that there were significant differences in body length and numbers of frontal setulae between the populations of *H. irritans* and *H. exigua* collected from various localities. Our data show that these morphological traits of *H. irritans* and *H. exigua* vary with latitudinal gradient within each species (Table 1, Fig. 1). Both species may have evolved and diverged at morphological and genetic levels, being strongly influenced by climate. Castiglioni and Bicudo (2005) suggested that genetic variability allows us to infer the origin of Brazilian populations of *H. irritans* which have been introduced to and dispersed throughout Brazil by cattle trade. High genetic homogenity between the specimens of *H. irritans* from Obihiro and

Table 3. Percent uncorrected sequence divergence among Stomoxyini and *Musca* species based on mtDNA analysis.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*H. irritans* (Obihiro 1)																						
2	*H. irritans* (Obihiro 2)	0																					
3	*H. irritans* (Morioka 1)	0	0																				
4	*H. irritans* (Morioka 2)	0	0	0																			
5	*H. exigua* (Taiwan 1)	1.8	1.8	1.8	1.8																		
6	*H. exigua* (Taiwan 2)	1.8	1.8	1.8	1.8	0.2																	
7	*H. exigua* (Vietnam 1)	1.8	1.8	1.8	1.8	0.3	0.3																
8	*H. exigua* (Vietnam 2)	1.9	1.9	1.9	1.9	0.4	0.3	0.4															
9	*S. calcitrans* 1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.3														
10	*S. calcitrans* 2	9.2	9.2	9.2	9.2	9.0	9.1	9.1	9.3	0.04													
11	*S. indicus* 1	10.4	10.4	10.4	10.4	10.4	10.4	10.5	10.5	10.4	10.3												
12	*S. indicus* 2	10.4	10.4	10.4	10.4	10.5	10.5	10.6	10.6	10.4	10.4	0.1											
13	*S. uruma* 1	9.1	9.1	9.1	9.1	8.8	8.8	8.9	8.9	7.5	7.5	10.8	10.9										
14	*S. uruma* 2	9.1	9.1	9.1	9.1	8.8	8.8	8.9	8.9	7.5	7.5	10.8	10.9	0									
15	*Hb. sanguinolenta* 1	7.0	7.0	7.0	7.0	6.7	6.7	6.8	6.8	9.1	9.0	10.7	10.8	8.5	8.5								
16	*Hb. sanguinolenta* 2	6.9	6.9	6.9	6.9	6.7	6.7	6.9	6.8	8.9	8.9	10.5	10.6	8.5	8.5	0.3							
17	*M. domestica* 1	9.4	9.4	9.4	9.4	8.9	8.9	8.8	8.9	10.9	10.8	11.5	11.5	10.2	10.2	9.0	9.0						
18	*M. domestica* 2	9.4	9.4	9.4	9.4	8.8	8.9	8.8	8.9	10.9	10.8	11.5	11.5	10.2	10.2	9.0	9.0	0					
19	*M. bezzii* 1	10.2	10.2	10.2	10.2	10.2	10.2	10.1	10.8	10.0	10.0	13.0	13.1	10.3	10.3	10.3	10.2	8.0	8.0				
20	*M. bezzii* 2	10.2	10.2	10.2	10.2	10.1	10.1	10.1	10.1	10.1	10.1	13.0	13.1	10.8	10.8	10.2	10.2	8.0	8.0	0			
21	*M. crassirostris* 1	9.5	9.5	9.5	9.5	9.0	9.0	9.0	9.1	10.5	10.4	12.2	12.2	9.9	9.9	9.7	9.7	7.2	7.2	7.9	7.9		
22	*M. crassirostris* 2	9.6	9.6	9.6	9.6	9.1	9.0	9.0	9.1	10.5	10.5	12.2	12.2	9.9	9.9	9.7	9.7	7.2	7.2	7.9	7.9	0.04	

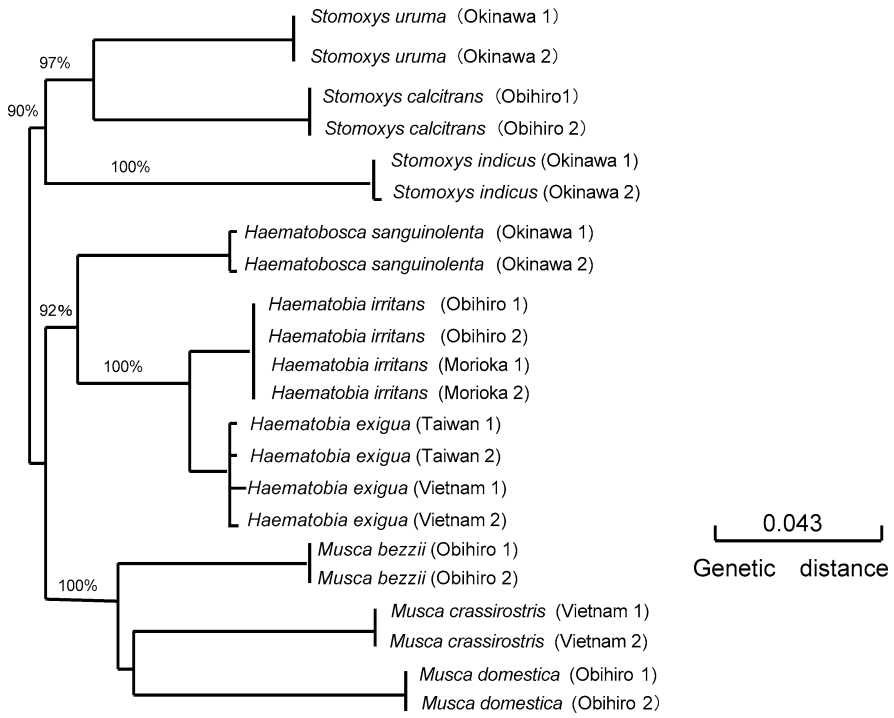

Fig. 3. Phylogenetic tree based on 2,272 bp from COI to COII gene in mtDNA using the neighbor-joining method. Percentages are bootstrap values.

Morioka may be ascribed to the same geographic origin, probably from North America (Tohoku Agricultural Research Center, personal information). In *H. exigua*, differences in body length in both sexes and numbers of frontal setulae in males between Taiwan and Vietnam may reflect some genetic variabilities (0.3–0.4%) between them. The most variable sequence in the specimens from Vietnam (0.4%) may suggest rich intraspecies gene diversity in tropical regions.

Sequence divergences between *H. irritans* and *H. exigua* (1.8–1.9%) in the present study are larger than the range of the estimated values for intraspecies differentiation based on mtDNA in various insect species which have been reported by DeSalle et al. (1986), Martin and Simon (1990), and Vargas and Espin (1995). mtDNA analysis of haplotypes of the calliphorid flies *Chrysomya albiceps* and *C. rufifacies* (which are closely related to each other) showed 0.04–0.8% intraspecies differences and 2.9–3.1% interspecies differences (Wells and Sperling, 1999). Genetic divergences between *H. irritans* and *H. exigua* in the present study may indicate an intermediate status between intraspecies and interspecies. Sizes of sequence divergences (6.7–10.9%) between *Haematobia* and other separate species of *Stomoxys* and *Haematobosca* are similar to those between the genera *Chrysomya, Phormia, Lucilia, Calliphora*, and *Dyscritomyia* of Calliphoridae (Wells et al., 2002) and larger than those between *Thricops* species of Muscidae (Savage et al., 2004). In phylogenetic tree, results of sequence divergence are reflected in the genetic distance between *H. irritans* and *H. exigua*; this distance is considerably small as compared with those among the separate species mentioned above. However, this genetic distance does not necessarily follow that they are subspecies.

There is some evidence to support the idea that these are separate species. In

insects, the evolution of cytochrome oxidase sequence of mtDNA is estimated to be about 2.3% per million years (Brower, 1994). If we accept this rate, sequence divergences (1.8–1.9%) between *H. irritans* and *H. exigua* in the present study may show that they have diverged about 0.4 million years ago. This is enough time for divergence from a common ansestor as shown by Brower (1994), Maekawa et al. (1998), and Wells et al. (2002).

We can also consider geographic factors which may have led to selection of separate species. *H. irritans* exists exclusively as parasites on cattle in the Holarctic Region, specimens from the Hawaiian islands and South America may have been the results of artificial dispersal by humans and livestock (Zumpt, 1973; Castiglioni and Bicudo, 2005). On the other hand, *H. exigua* prefers water buffalo in the Oriental Region (Handschin, 1933); actually, our field observations in Vietnam show that adult *H. exigua* are much more heavily infested on water buffalo than cattle. Furthermore, adult *H. exigua* strongly react to water and abound in greatest numbers in swampy areas, and the larvae favor buffalo dung (Krijigsman and Windred, 1933). Consequently, *H. irritans* and *H. exigua* have separated geographically, adapting to different climates and hosts.

Urech et al. (2005) showed that there are differences in cuticular hydrocarbon composition of between *H. irritans* and *H. exigua.* These data supported their recognition as separate species rather than as subspecies. To more fully determine the status of *H. irritans* and *H. exigua*, further genetic and crossbreeding studies are needed, involving the specimens in the margin of the Palaearctic and Oriental regions where the two species coexist.

According to Zumpt (1973), the genus *Haematobosca* is more primitive than the genera *Stomoxys* and *Haematobia,* when scored on the number of plesiomorphic features, and the former is distantly related to the latter. The close molecular phylogenetic relationship of *Haematobosca sanguinolenta* and *Haematobia* in the present study may conflict with hypotheses based on morphological traits. To resolve this problem, it will be necessary to reassess the morphological characteristics which are better suited to phylogenetic analysis.

According to Shinonaga (2003), *Stomoxys calcitrans* is distinctly different from the other *Stomoxys* species in having dichoptic eyes in males and dark spots on the abdominal tergites. While *S. indicus* and *S. uruma* are closely related to each other with only minor differences, the former are distinguished from the latter by the color of tibiae and tarsi, shape of genitalia, and body size (Shinonaga, 2003). In the present molecular tree, however, the close relationship between *S. calcitrans* and *S. uruma* is not linked to morphological characteristics. Gene phylogenesis may not necessarily reflect the phylogeny based on morphological traits (Vogler and DeSalle, 1993; Doyle, 1997; Maddison, 1997). A possible explanation for this contradiction is that morphological characteristics are diverging very slowly between *S. indicus* and *S. uruma*. Such a phenomenon can occur in two species that have been under similar conditions of natural selection during their evolutionary history after geographical separation (Muraji, 2001). In fact, habitat, life cycles, mating, and blood-sucking behaviors of these two species appear to be very similar. Thus, evolutionary factors may not have resulted in two morphologically distinct species. Another possible explanation is that this conflict could be a result of the different modes of inheritance of mtDNA and of genes controlling morphology (Volger and DeSalle, 1993). Though samples sizes for DNA analysis may be small in the present study, but the results show an outline of genetic differences in mtDNA among *Haematobia* species and other genera of Stomoxyini and raise several points at issue. Further study of morphology and molecular analyses using

other genes may clarify the phylogeny of Stomoxyini flies.

Vossbrinck and Friedman (1989) grouped together *S. calcitrans* and *Musca domestica* based on molecular analysis. In the present study, clustering of *Musca* species with *Haematobosca* and *Haematobia* suggests that *Musca* is closely related to *Haematobosca* than *Stomoxys*. Among three *Musca* species, mouthparts of *M. crassirostris* are sclerotized, adapted for blood-sucking, such as those of Stomoxyini. Our analysis by mtDNA also confirmed that the sclerotized blood-sucking mouthparts of *Musca* evolved independently to those of Stomoxyini species.

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References

Baker, R. H., Wilkinson, G. S. and DeSalle, R. 2001. Phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diopsidae). *Syst. Biol*., 50: 87–105.

Bezzii, M. 1911. Etudes systématiques sur les muscides hématophages du genere *Lyperosia*. *Arch. Parasitol*., 15: 110–143.

Brito, L. G., Regitano, L. C. A., Huacca, M. E. F., Carrilho, E. and Borja, G. E. M. 2007. Genotype characterization of *Haematobia irritans* from different Brazilian geographic regions based on randomly amplified polymorphic DNA (RAPD) analysis. *Pesq. Vet. Bras*., 27: 1–5.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Nat. Acad. Sci. USA,* 91: 6491–6495.

Castiglioni, L. and Bicudo, H. E. M. D. 2005. Molecular characterization and relatedness of *Haematobia irritans* (horn fly) populations, by RAPD-PCR. *Genetica,* 124: 11–21.

Clary, D. O. and Wolstenholme, D. R. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol*., 22: 252–271.

DeSalle, R. and Grimaldi, D. A. 1991. Morphological and molecular systematics of the Drosophilidae. *Annu. Rev. Ecol. Syst*., 22: 447–475.

DeSalle, R. Giddings, L. V. and Templeton, A. R. 1986. Mitochondrial DNA variability in natural populations of Hawaiian *Drosophila*. I. Methods and levels of variability in *D. silverstris* and *D. heteroneura* populations. *Heredity,* 56: 75–85.

Doyle, J. J. 1997. Trees within trees: Genes and species, molecules and morphology. *Syst. Biol*., 46: 537–553.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 39: 783–791.

Felsenstein, J. 1995. PHYLIP Version 3.572 Executables for PowerMac. University of Washington, Seattle.

Friedrich, M. and Tautz, D. 1997. Evolution and phylogeny of the Diptera: A molecular phyogenetic analysis using 28S rDNA sequence. *Syst. Biol*., 46: 674–698.

Handschin, E. 1933. *Lyperosia exigua* in Java und Nordoaustralien. *Revue suisse Zool.*, 40: 449–528.

Hennig, W. 1964. 63b Muscidae. *In*:Die Fliegen der palaearktischen Region (ed. E. Lindner). pp. 1048–1058, Schweizerbart, Stuttgart.

Kano, R., Shinonaga, S. and Hasegawa, T. 1972. On the specific name of *Haematobia* (Diptera, Muscidae) from Japan. *Jpn. J. Sanit. Zool*., 23: 49–56 (in Japanese).

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequence. *J. Mol. Evol.,* 16: 111–120.

Krijgsman, B. J. and Windred, G. L. 1933. Investiga-

tions on the buffalo fly *Lyperosia exigua* de Meij. *Coun. Sci. Ind. Res. Aust. Pamp*., No. 43, 40 pp.

Mackerras, I. M. 1933. Taxonomy of *Lyperosia exigua* De Meijere (Diptera, Muscidae). *Ann. Mag. Nat. Hist*., 11 (Ser. 10): 58–64.

Maddison, W. P. 1997. Gene trees in species trees. *Syst. Biol*., 46: 523–536.

Maekawa, K., Miura, T., Kidate, O. and Matsumoto, T. 1998. Genetic variation and molecular phylogeny based on the mitochondrilal genes of the Damp Wood Termite *Hodotermopsis japonica* (Isoptera: Termopsidae). *Entomol. Sci*., 1: 561–571.

Malloch, J. R. 1932. Exotic Muscaridae (Diptera)-XXXVI. *Ann. Mag. Nat. Hist*. 9 (Ser. 10): 501–514.

Martin, A. and Simon, C. 1990. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. *Evolution,* 44: 1066–1080.

Muraji, M. 2001. Molecular, morphological, and behavioral analyses of Japanese *Gerris* (*Macrogerris*) water striders (Heteroptera; Gerridae): Evidence for a new species. *Entomol. Sci*., 4: 321–334

Pont, A. C. 1977. Family Muscidae. *In*: A Catalog of the Diptera of the Oriental Region 3 (ed. M. D. Delfinado and D. E. Hardy), pp. 451–525, Univ. Press of Hawaii, Honolulu.

Pont, A. C. 1986. Family Muscidae. *In*: Catalogue of Palaearctic Diptera 9 (ed. A. Soós and L. Papp), pp. 57–215, Académiai Kaidó, Budapest.

Savage, J., Wheeler, T. A. and Weigmann, B. M. 2004. Phyogenetic analysis of the genus *Thricops* Rondani (Diptera: Muscidae) based on molecular and morphological characters. *Syst. Entomol*., 29: 395–414.

Schat, P. 1903. Verdere mededeelingen over《surra》. *Meded. Proefstn Oost- Java*, 44: 1–19.

Schuehli, G. S. E., Carvalho, C. J. B. and Wiegmann, B. M. 2007. Molecular phylogenetics of the Muscidae (Diptera: Calyptratae): new ideas in a congruence context. *Inverteb. Syst*., 21: 263–278.

Shinonaga, S. 2003. A monograph of the Muscidae of Japan. 347 pp, Tokai Univ. Press, Tokyo.

Snyder, F. M. 1965. Insects of Micronesia, Diptera: Muscidae. pp. 311–313, B. P. Bishop Museum, Honolulu.

Urech, R., Brown, G. W., Moore, C. J. and Green, P. E. 2005. Cuticular hydrocarbons of buffalo fly, *Haematobia exigua*, and chemotaxonomic differentiation from horn fly, *H. irritans*. *J. Chem. Ecol*., 31: 2451–2461.

Van Emden, F. I. 1965. The fauna of India and the adjacent countries, Diptera 7, Muscidae Part 1. 647 pp, Government of India, Delhi.

Vargas, M. E. I. and Espin, A. N. M. A. 1995. Genetic variability in mitochondrial DNA of the screwworm, *Cochliomyia hominivorax* (Diptera: Calliphoridae), from Brazil. *Biochem. Genet*., 33: 237–256.

Vogler, A. P. and DeSalle, R. 1993. Phylogeographic patterns in coastal North American Tiger Beetles (*Cicindela dorsalis* Say) inferred from mitochondrial DNA sequences. *Evolution,* 47: 1192–1202.

Vossbrinck, C. R. and Friedman, S. 1989. A 28s ribosomal RNA phylogeny of certain cyclorrhaphous Diptera based upon a hypervariable region. *Syst. Entomol*., 14: 417–431.

Wells, J. D., Goff, M. L., Tomberlin, J. K. and Kurahashi, H. 2002. Molecular systematics of the endemic Hawaiian blowfly genus *Dyscritomyia* Grimshaw (Diptera: Calliphoridae). *Med. Entomol. Zool*., 53: 231–238.

Wells, J. D. and Sperling, F. A. H. 1999. Molecular phylogeny of *Chrysomya albiceps* and *C. rufifacies* (Diptera: Calliphoridae). *J. Med. Entomol.*, 36: 222–226.

Wells, J. D. and Sperling, F. A. H. 2001. DNA-based identification of forensically important Chrysominae (Diptera: Calliphoridae). *Forensic Sci. Int*., 120: 109–114.

Zumpt, F. 1973. The Stomoxyine biting flies of the world. Diptera, Muscidae. 175 pp, Gustav Fischer Verlag, Stuttgart.