

Molecular Identification of Flies

Padet Siriyasatien

Department of Parasitology

Faculty of Medicine , Chulalongkorn University



Problems of Morphological Identification

Similarities among species, especially in early larval stages

A taxonomic key covering all immature stages of common insect species in all geographical regions is yet unavailable



Rearing maggots to adult stage and traditional identification based on the adult morphological characteristics can be performed but, however, are time-consuming procedures

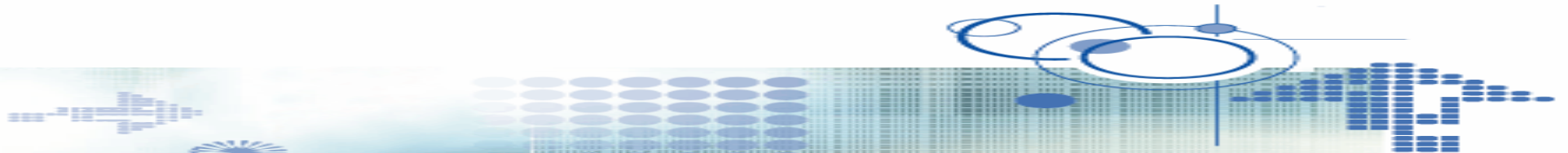
Specimens may be killed or damaged before arrival to the laboratory room



Molecular tools can overcome many difficulties associated with morphological problems

Ribosomal RNA transcription unit

Mitochondrial genome (mtDNA)

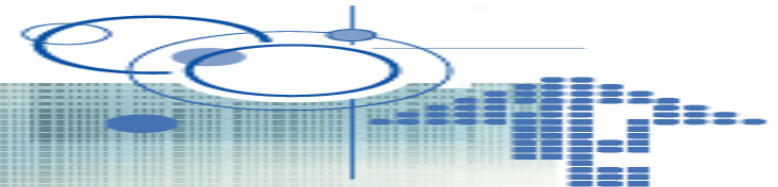


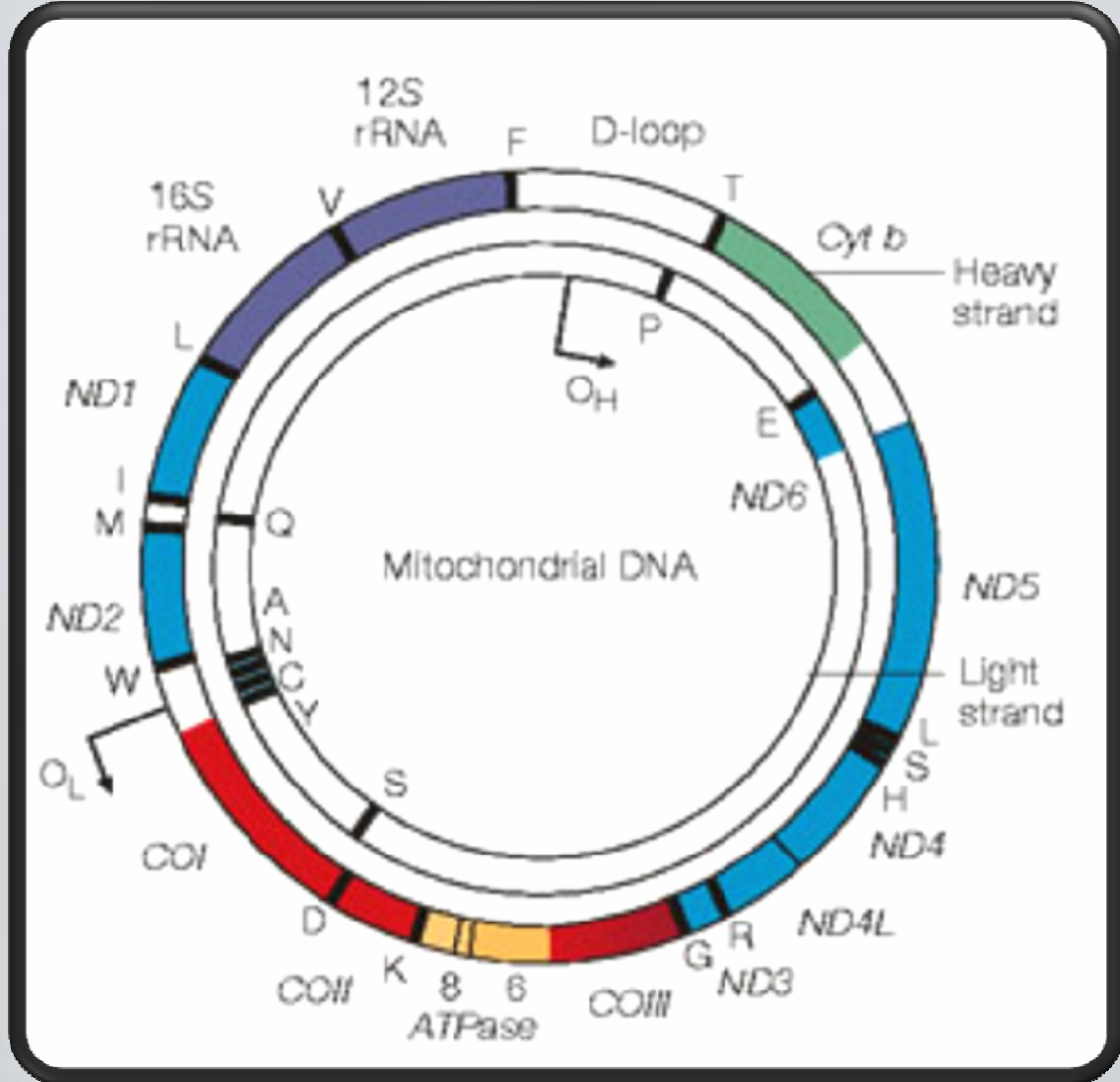
Ribosomal RNA transcription unit



- Short sequence (500-800 bp)
- Multi copies (cassette)
- High variation (spacer)

28S rDNA, ITS1, ITS2



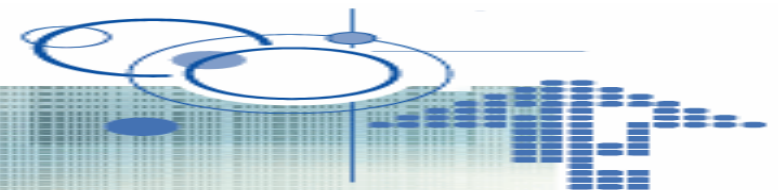


Greater abundance in tissues, when compared with nuclear DNA, makes it easier for extraction even from small amount of sample

High degree of genetic variations

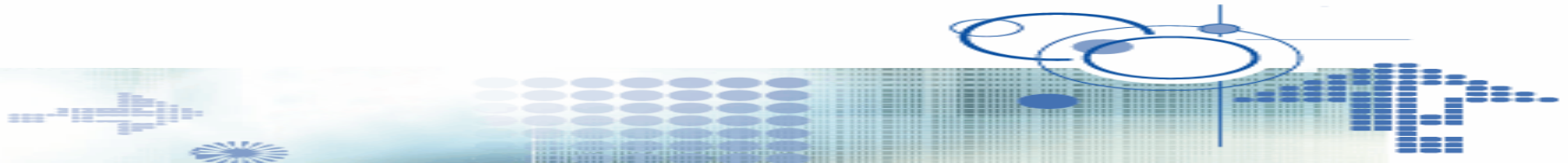
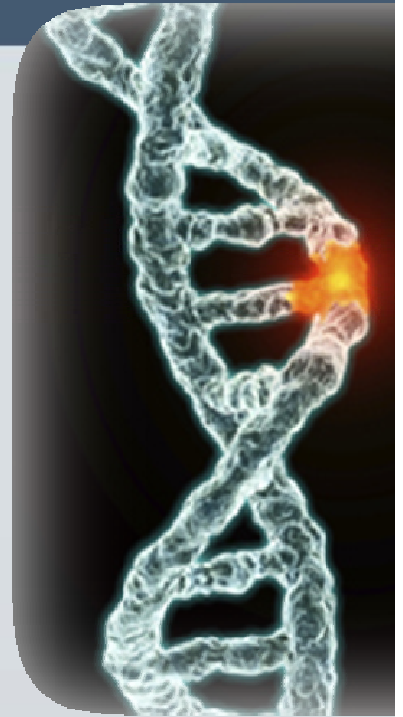
Strictly maternal inheritance and no genetic recombination, mtDNA haplotype is a good candidate for evolutionary and population genetics study

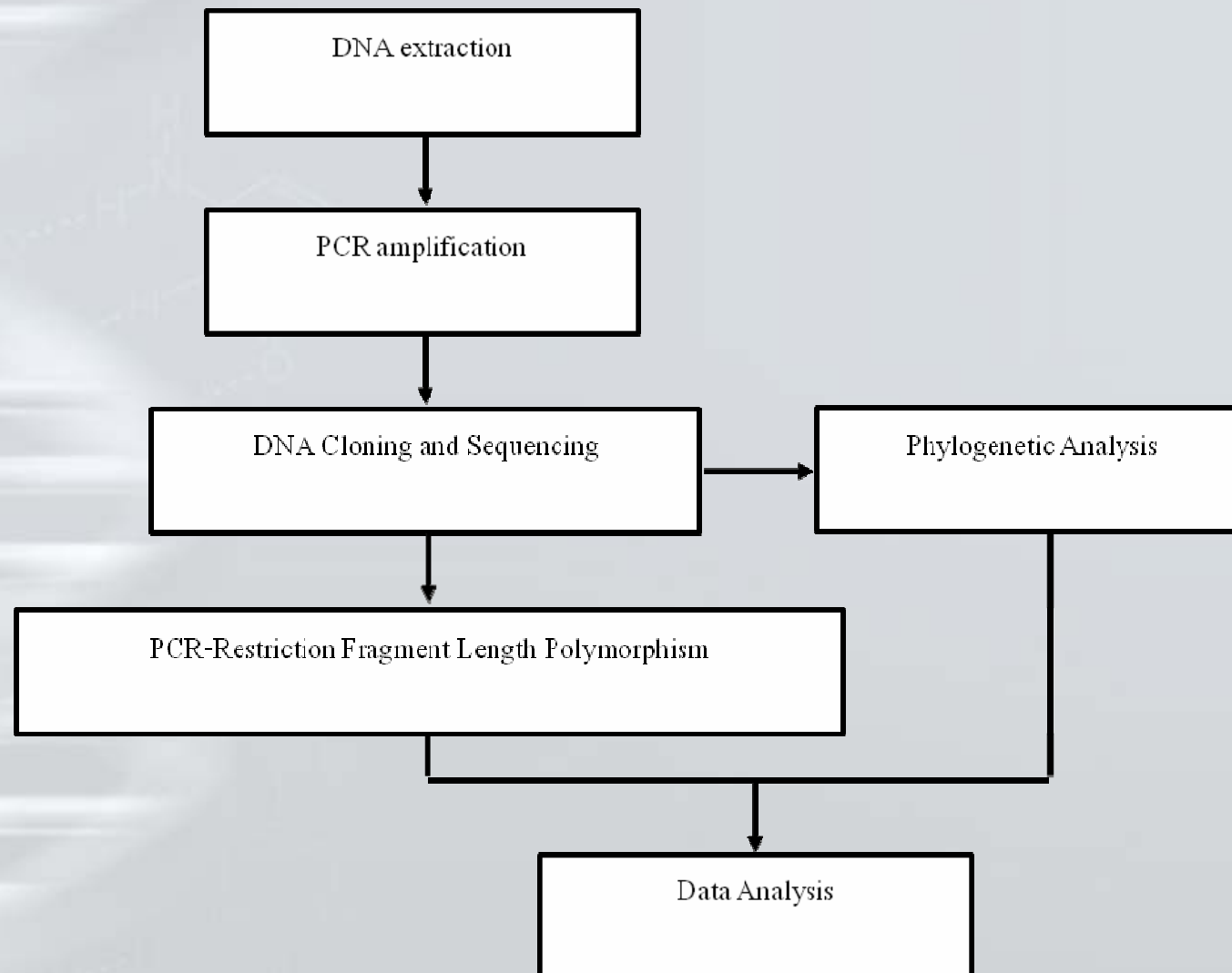
**COI, COII, ND5, 12S rDNA,
16S rDNA**



❖ DNA sequencing

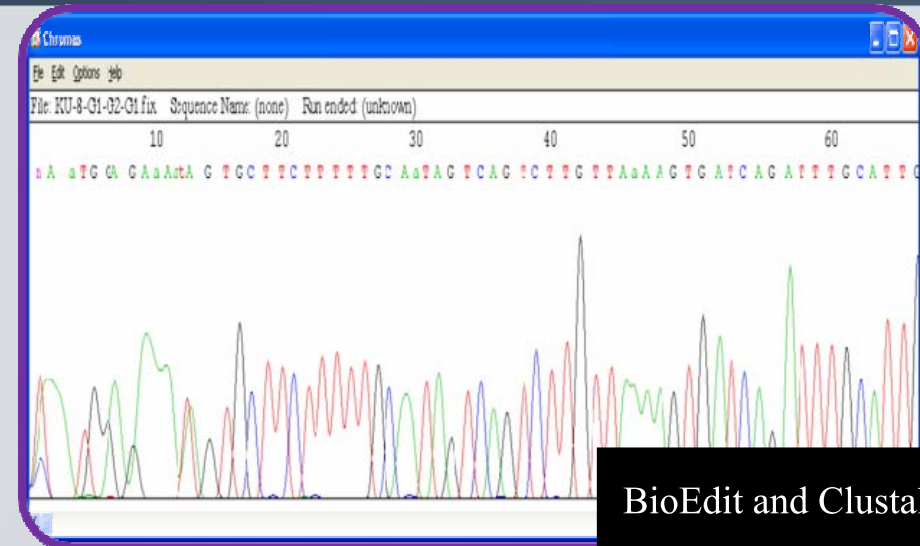
❖ Restriction Fragment Length Polymorphism (RFLP)







Sequencing



BioEdit and Clustal X



ref[[NP_114944.1](#)] **G** NADH dehydrogenase subunit 5 [Macaca sylvanus]
Length=608

GENE ID: 803075 NDS | NADH dehydrogenase subunit 5 [Macaca sylvanus]
(10 or fewer PubMed links)

Score = 796 bits (2056), Expect = 0.0, Method: Composite
Identities = 438/564 (77%), Positives = 478/564 (84%), Gap = 0

Query 24 VNFWKNSYPHYVKSIVASTFIISLFTIMFMCLOQEVIIISWENGA
+NPWKK+ YP+YVK+ V FI SL IT+M L+QE II +NEW TOT L+LSFKLD
Sbjct 24 INPWKZLYPNVYKTAIYAFITSLSTLYMFLMGETIINSWWMQTQLSLTSLFKLD 89

Query 84 YFSMNFIPVAFVWISMEFSLWYMSDENIQFTKYLLIFLITMLILVTANLQFLFIG 143
YFSMNF P+AL TWSIMEFSLWYMSDENIQFTKYLLIFLITMLILVTANLQ FIG
Sbjct 84 YFSMNFPIALLTWSIMEFSLWYMSDENIQFTKYLLIFLITMLILVTANLQFFIG 143

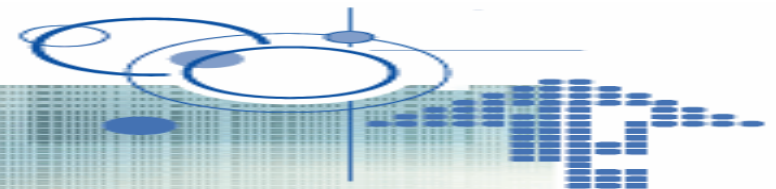
Query 144 WEGVGIMSFLISWYARADANTAAIQAVLYNRIGDIGFILALANFILHSUNDFQOMAL 203
WEG+GIMSFLISWYAR DANTAAIQAVLYNRIGDIG IL +WF+LH NSND QOM
Sbjct 144 WEGVGIMSFLISWYARADANTAAIQAVLYNRIGDIGILITMTWFLHYNSNDFQOMLA 203

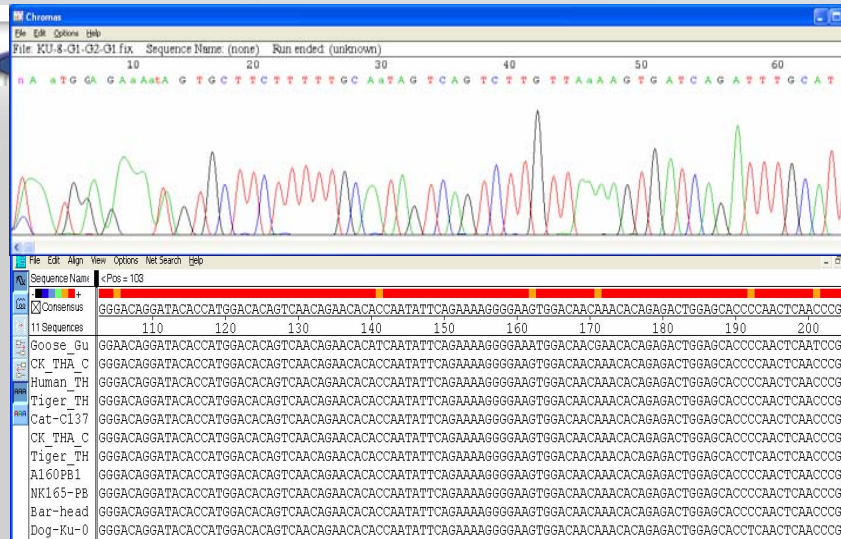


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Alignment:

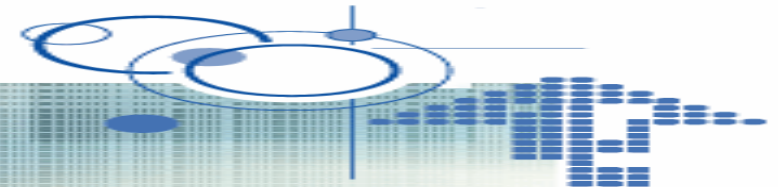
CLUSTALW

Phylogenetic tree :

Neighbor-joining (NJ) method

Nucleotide substitution: Kimura-2-parameter model

Bootstrap test 1,000 resampling test



PCR-RFLP

NEBcutter V2.0

sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and com sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence. The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBases.

[NEBcutter](http://tools.neb.com/NEBcutter2/index.php)

Local sequence file:

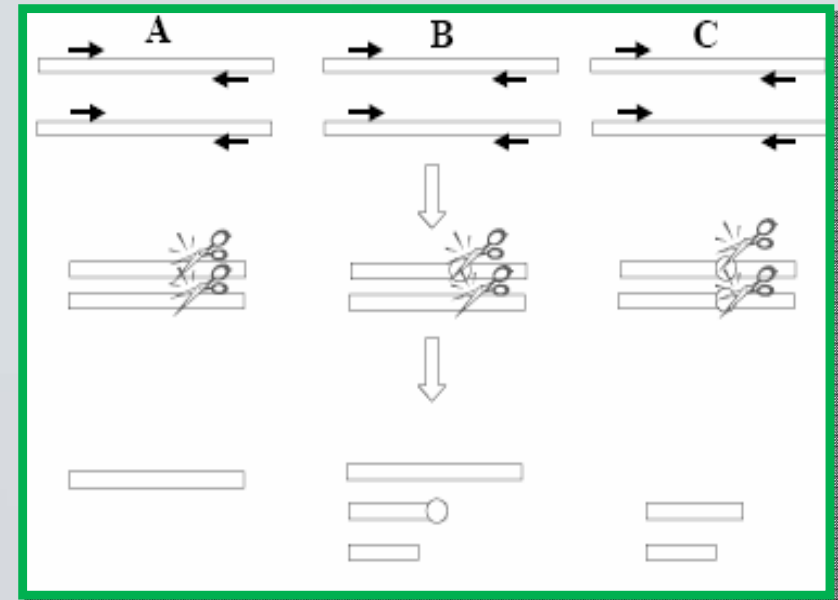
GenBank number:

or paste in your DNA sequence: (plain or FASTA format)

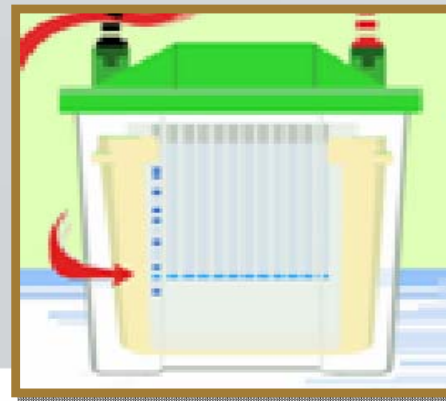
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TCAGAGTATTTATATAATTACAGAAGTATATAATAATTCTTTTTTATTGAGGAAGGCTAGCATA
AAATAATTTTATGAAGTGAAGTTGCTCTTTAATAAAGGATTTTTTTTTTTTATTGTTATAAATG
ATAAAGAAATGATTTTGTTCAGAGGTTAATGCATTTAAGAAATTTTAAAAATATATGT
```

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☐ All commercially available specificities
☐ All specificities

<http://tools.neb.com/NEBcutter2/index.php>



Restriction enzyme digestions



Gel electrophoresis



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Mitochondrial DNA-based identification of some forensically important blowflies in Thailand

Kanok Preativatanyou^a, Nantana Sirisup^b, Sunchai Payungporn^{c,d}, Yong Poovorawan^d,
Usavadee Thavara^e, Apiwat Tawatsin^e, Sivapong Sungpradit^f, Padet Siriyasatien^{a,*}

^aDepartment of Parasitology, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Bangkok 10330, Thailand

^bDepartment of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

^cDepartment of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

^dCenter of Excellence in Clinical Virology Unit, Chulalongkorn University, Bangkok 10330, Thailand

^eDepartment of Medical Science, National Institute of Health, Nonthaburi 11000, Thailand

^fFaculty of Veterinary Science, Mahidol University, Nakhonpathom 73170, Thailand

Table 1

Isolate code, sex, location and accession number of each blowfly used in this study.

Blowfly species	Sex	Location	Accession no.
<i>Chrysomya megacephala</i>			
A01	♂	Tak	FJ153258
A02	♀	Chiang-Mai	FJ153259
A03	♂	Chiang-Mai	FJ153260
A04	♂	Chiang-Mai	FJ153261
A05	♂	Chiang-Mai	FJ153262
A06	♀	Buri-Ram	FJ153263
A07	♀	Buri-Ram	FJ153264
A08	♀	Phitsanulok	FJ153265
A09	♂	Phitsanulok	FJ153266
A10	♂	Chumphon	FJ153267
A11	♂	Chumphon	FJ153268
A12	♀	Bangkok	FJ153269
<i>Chrysomya rufifacies</i>			
B01	♀	Bangkok	FJ153270
B02	♂	Bangkok	FJ153271
B03	♀	Phitsanulok	FJ153272
B04	♀	Chiang-Mai	FJ153273
B05	♂	Chiang-Mai	FJ153274
<i>Lucilia cuprina</i>			
C01	♀	Chiang-Mai	FJ153275
C02	♀	Chiang-Mai	FJ153276
C03	♂	Chiang-Mai	FJ153277
<i>Musca domestica</i> (Outgroup)			
D01	♂	Bangkok	FJ153278

Table 2

Comparative RFLP patterns among three blowfly species.

Blowfly species	Taq ^α I digestion	VspI digestion
<i>Chrysomya megacephala</i>	30, 111, 156, 264, 307 and 456 bp	4, 12, 160, 495 and 653 bp
<i>Chrysomya rufifacies</i>	30, 81, 186, 264, 307 and 456 bp	12, 653 and 659 bp
<i>Lucilia cuprina</i>	30, 81, 86, 177, 188 and 762 bp	210, 450 and 664 bp

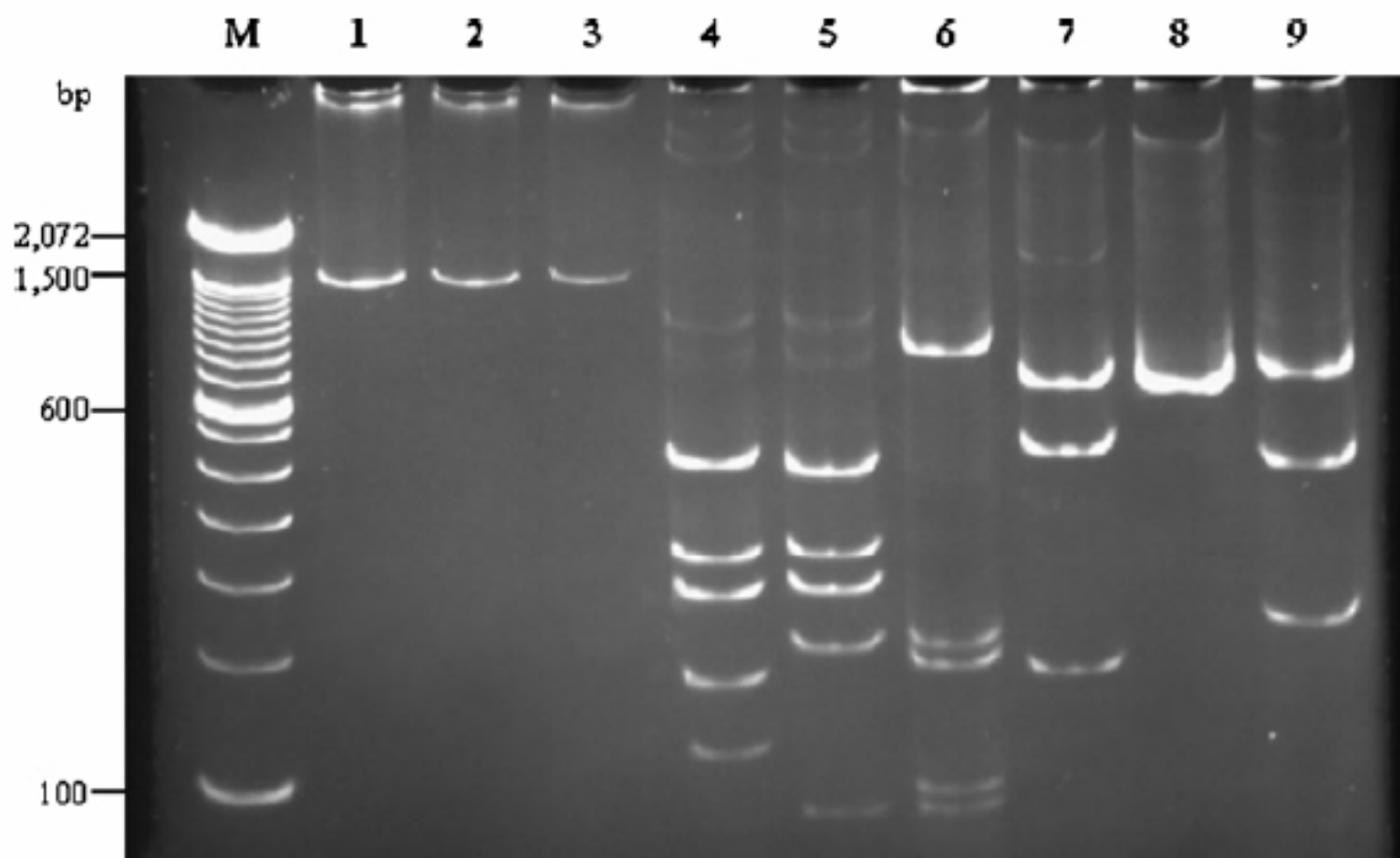


Fig. 2. The 6% native polyacrylamide gel shows different PCR-RFLP patterns of COI-COII amplicons digested with two restriction endonucleases. From left to right as follows: undigested PCR products from *C. megacephala*, *C. rufifacies* and *L. cuprina*, respectively (lanes 1–3); *C. megacephala*/*Taq*^αI (lane 4); *C. rufifacies*/*Taq*^αI (lane 5); *L. cuprina*/*Taq*^αI (lane 6); *C. megacephala*/*Vsp*I (lane 7); *C. rufifacies*/*Vsp*I (lane 8); *L. cuprina*/*Vsp*I (lane 9). Lane M is 100 bp DNA standard marker.

Table 3

Identity matrix reveals the percentage of partial COI–COII sequence similarity between each isolate.

Code	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12	B01	B02	B03	B04	B05	C01	C02	C03
A01																				
A02	99.8																			
A03	99.8	100																		
A04	99.8	100	100																	
A05	99.8	100	100	100																
A06	99.8	100	100	100	100															
A07	99.8	100	100	100	100	100														
A08	99.8	100	100	100	100	100	100													
A09	99.7	99.9	99.9	99.9	99.9	99.9	99.9	99.9												
A10	99.8	100	100	100	100	100	100	100	99.9											
A11	99.7	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.8	99.9										
A12	99.7	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.8	99.9	99.8									
B01	93.1	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.2	93.3	93.2	93.2								
B02	93.1	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.2	93.3	93.2	93.2	99.8							
B03	93	93.1	93.1	93.1	93.1	93.1	93.1	93.1	93	93.1	93	93	99.6	99.6						
B04	93.1	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.2	93.3	93.2	93.2	99.8	100	99.6					
B05	93	93.1	93.1	93.1	93.1	93.1	93.1	93.1	93	93.1	93	93	99.6	99.8	99.6	99.8				
C01	60.1	60	60	60	60	60	60	60	60	60	60.1	60	60	60.1	60.2	60.1	60.1			
C02	60.2	60.1	60.1	60.1	60.1	60.1	60.1	60.1	60	60.1	60.2	60	60.1	60.2	60.3	60.2	60.2	99.9		
C03	60.1	60	60	60	60	60	60	60	60	60	60.1	60	60	60.1	60.2	60.1	60.1	99.7	99.8	
D01	57.4	57.4	57.4	57.4	57.4	57.4	57.4	57.4	57.4	57.4	57.4	57.4	58.4	58.4	58.3	58.4	58.3	53.3	53.2	53.4

A01–A12, *Chrysomya megacephala*; B01–B05, *Chrysomya rufifacies*; C01–C03, *Lucilia cuprina*; D01, *Musca domestica*.

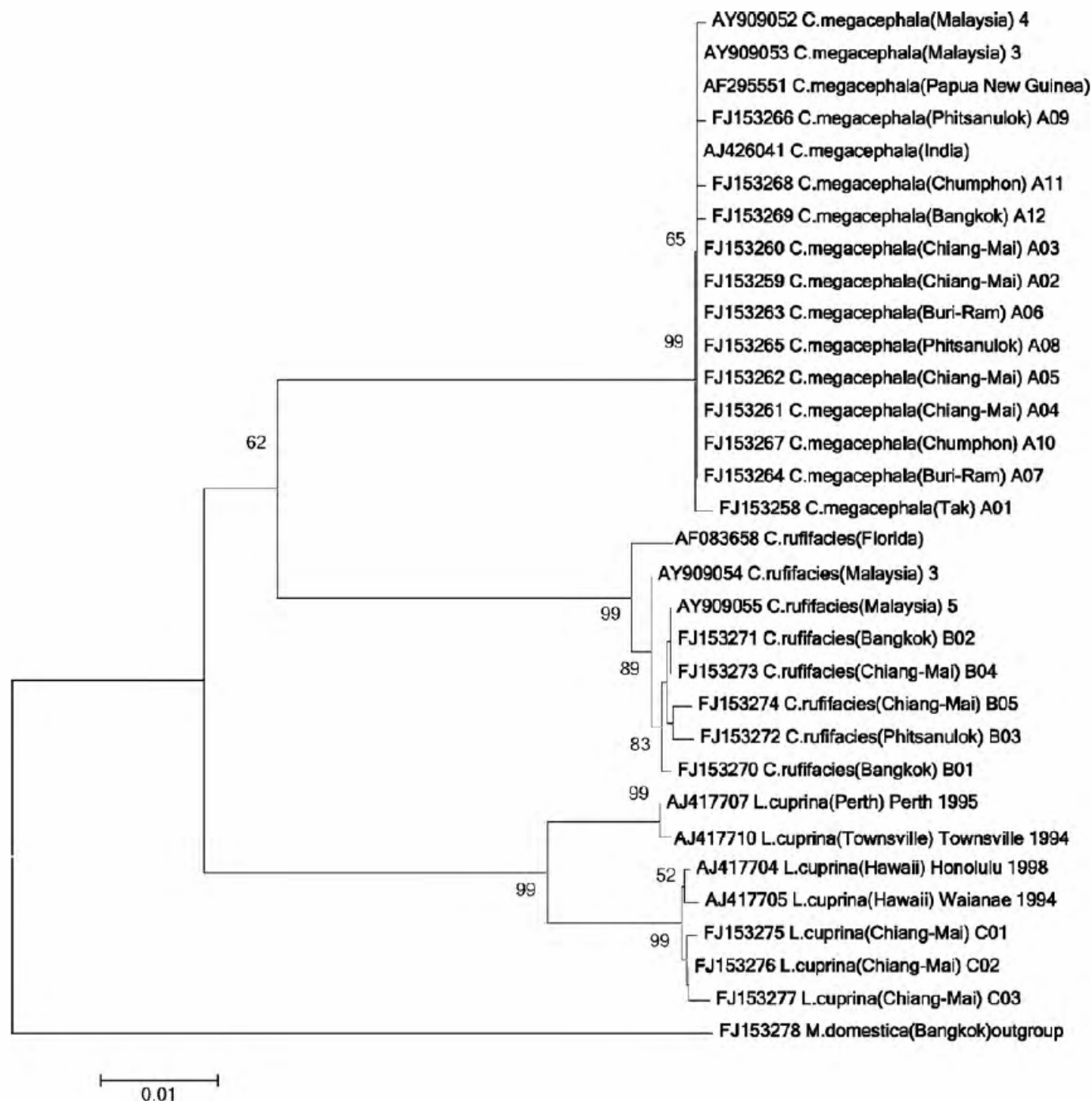
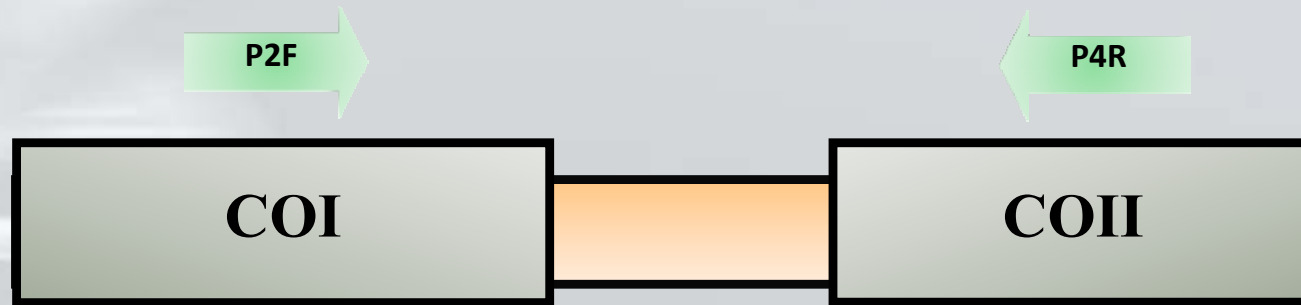
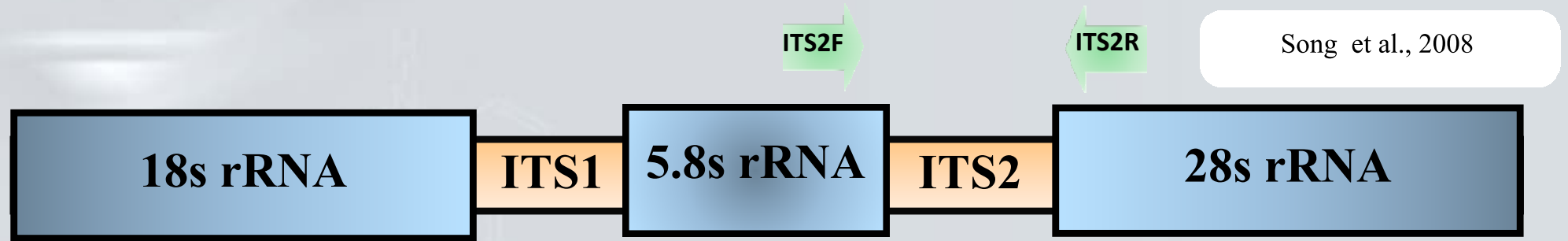


Fig. 3. The neighbor-joining tree using Kimura's 2-parameter model illustrating phylogenetic relationships among three blowfly species and one housefly outgroup, based on the COI-COII nucleotide sequences data.

***ITS* and *CO* sequences of *Dermatobia hominis*
recovered from Thai travelers returning from
Brazil**



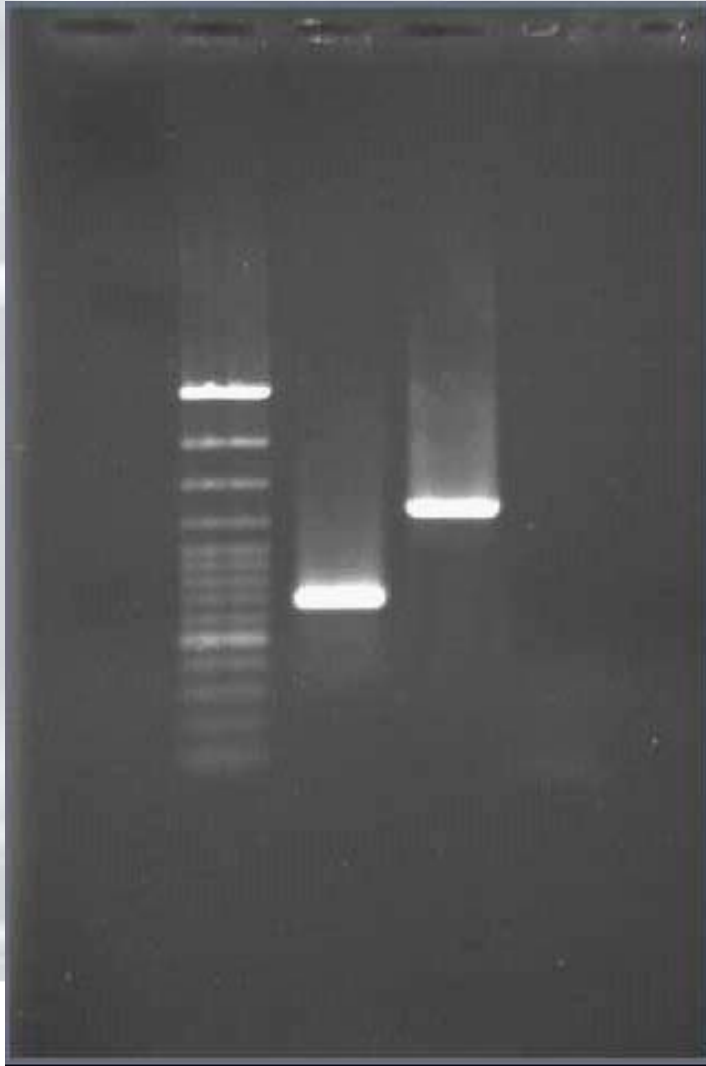
PCR amplification



Preativatanyou K et al., 2010



PCR amplicon verification




Lane 1: 100 bp ladder marker

Lane 2: 645 bp ITS2 from *Dermatobia hominis*

Lane 3: 1,324 bp COI-COII from *D. hominis*

Then, the amplicons were purified and direct-sequenced in both directions using the same forward and reverse primers used in the PCR.

ITS2 sequences

```
>  gb|EF560183.1| Dermatobia hominis 5.8S ribosomal RNA gene, partial sequence;  
internal transcribed spacer 2a, 2S ribosomal RNA gene, and  
internal transcribed spacer 2, complete sequence; and 28S ribosomal  
RNA gene, partial sequence  
Length=873  
  
Score = 1160 bits (628), Expect = 0.0  
Identities = 639/644 (99%), Gaps = 1/644 (0%)  
Strand=Plus/Plus  
  
Query 1 TGCTTGGA CTACATATGGTTGAGGGTTGTAAGATTATGTTTAACCAGGTTGCTTAAGGGA 60  
|||||  
Sbjct 142 TGCTTGGA CTACATATGGTTGAGGGTTGTAAGACTATGCTTAACCAGGTTGCTTAAGGGA 201  
  
Query 61 GAAAACAGAAAAGAGatattattattatatgattataatataatattatcattttcttgt 120  
|||||  
Sbjct 202 GAAAACAGAAAAGAGATATTATTATTATATGATTATAATATAATATTATCATTTCCTGT 261  
  
Query 121 tttatttttCTTCTAAGCACATATTGTATTATTGAATACCCTATAATTTTCATACATTTTA 180  
|||||  
Sbjct 262 TTTATTTTCTTCTAAGCACATATTGTATTATTGAATACCCTATAATTTTCATACATTTTA 321
```

Nucleotide sequence was analyzed using the BLAST search (<http://www.ncbi.nlm.gov/BLAST>) and showed the similarity with the identified database (Brazil) at > 99%

This sequence was assigned as HQ215834.1 in the NCBI database.

Dermatobia hominis isolate CL01 2S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: HQ215834.1

[FASTA](#) [Graphics](#)

[Go to:](#) ☐

LOCUS	HQ215834	645 bp	DNA	linear	INV 11-OCT-2010
DEFINITION	Dermatobia hominis isolate CL01 2S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.				
ACCESSION	HQ215834				
VERSION	HQ215834.1 GI:308196513				
KEYWORDS	.				
SOURCE	Dermatobia hominis (human botfly)				
ORGANISM	Dermatobia hominis Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Oestroidea; Oestridae; Dermatobia.				
REFERENCE	1 (bases 1 to 645)				
AUTHORS	Thanapatcharoen,A., Preativatanyou,K. and Siriyasatien,P.				
TITLE	Cutaneous myiasis caused by Dermatobia hominis in Thai travelers: First report in Thailand				
JOURNAL	Unpublished				
REFERENCE	2 (bases 1 to 645)				
AUTHORS	Thanapatcharoen,A., Preativatanyou,K. and Siriyasatien,P.				
TITLE	Direct Submission				
JOURNAL	Submitted (02-SEP-2010) Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Rama 4 Road, Pathumwan, Bangkok 10330, Thailand				
FEATURES	Location/Qualifiers				
source	1..645 /organism="Dermatobia hominis" /mol_type="genomic DNA" /isolate="CL01" /db xref="taxon: 115427 "				

COI-COI sequences

```
>gb|AY463155.1| D Dermatobia hominis mitochondrion, complete genome
Length=16360

Score = 2183 bits (1182), Expect = 0.0
Identities = 1194/1200 (99%), Gaps = 0/1200 (0%)
Strand=Plus/Plus

Query 1      TTGTTTTAGCAAATTCATCAATTGATATTATCTTACATGATACATATTATGTAGTAGCCC 60
            |||
Sbjct 2482    TTGTTTTAGCAAATTCATCAATTGATATTATCTTACATGATACATATTATGTAGTAGCCC 2541

Query 61     ATTTTCATTATGTTCTTTCAATAGGAGCAGTATTTGCTATTATAGGAGGATTGTACATT 120
            |||
Sbjct 2542    ATTTTCATTATGTTCTTTCAATAGGAGCAGTATTTGCTATTATAGGAGGATTGTACATT 2601

Query 121    GATATCCACTATTTACTGGACTAACAATAAATAACAATATTAAAAAGTCAATTTACTA 180
            |||
Sbjct 2602    GATATCCACTATTTACTGGACTAACAATAAATAACAATATTAAAAAGTCAATTTACTA 2661
```

The 99% similarity was presented, compared to the *D. hominis* mitochondrial genome database (Brazil).

This sequence was assigned as HQ334260.1 in the NCBI database.

Dermatobia hominis cytochrome oxidase subunit I (COI) and cytochrome oxidase subunit II (COII) genes, partial cds; mitochondrial

GenBank: HQ334260.1

[FASTA](#) [Graphics](#)

[Go to:](#) ☒

LOCUS	HQ334260	1200 bp	DNA	linear	INV 08-NOV-2010
DEFINITION	Dermatobia hominis cytochrome oxidase subunit I (COI) and cytochrome oxidase subunit II (COII) genes, partial cds; mitochondrial.				
ACCESSION	HQ334260				
VERSION	HQ334260.1 GI:311034085				
KEYWORDS	.				
SOURCE	mitochondrion Dermatobia hominis (human botfly)				
ORGANISM	Dermatobia hominis Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Oestroidea; Oestridae; Dermatobia.				
REFERENCE	1 (bases 1 to 1200)				
AUTHORS	Thanapatcharoen,A., Preativatanyou,K. and Siriyasatien,P.				
TITLE	Cutaneous myiasis caused by Dermatobia hominis in Thai travelers: First report in Thailand				
JOURNAL	Unpublished				
REFERENCE	2 (bases 1 to 1200)				
AUTHORS	Thanapatcharoen,A., Preativatanyou,K. and Siriyasatien,P.				
TITLE	Direct Submission				
JOURNAL	Submitted (01-OCT-2010) Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Pathumwan, Bangkok 10330, Thailand				

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