

Additional file 1:

Figure S1. Alignment of rDNA sequences (ITS2 to D3 domain of 28S) of different molecular forms of *An. subpictus* and *An. sundaicus* complex. MW078484: *An. subpictus* Form A (haplotype 1), MW078485: *An. subpictus* Form A (haplotype 2), MW078486: *An. subpictus* Form B, MW078487: *An. subpictus* Form C, MW078488: *An. epiroticus* (variant I) from Myanmar, MW078489: *An. sundaicus* (TK) from Car Nicobar, MW078490: *An. sundaicus* D (variant III). MT068436, MT068425 and MT068434 are representative sequences of *An. subpictus* from Thailand and Indonesia [1]. Dots in alignment represent similarity in nucleotide base with the first row of sequence and dash represents missing nucleotide. The demarcation of ITS2-boundaries was done using online ITS2 database tool available at <http://its2.bioapps.biozentrum.uni-wuerzburg.de/> (Selig et al., 2008) [2]

	5.8S	><	ITS2	
MW078484	CGCATATGGCGCATCGGACGTTTCAACCCGACCGATGCACACATCCTTGAGTGCCTACTAGGTACTGAGAGATTCCTATA			[80]
MW078485				
MW078486	TC..TT.....			
MW078487	TC..TT.....			
MW078488	TC..TT.....			
MW078489	TC..TT.....			
MW078490	TC..TT.....			
MT068436	TC..TT.....			
MT068425	TC..TT.....			
MT068434	Y.....TC..TT.....			
	ITS2			
MW078484	ACTTGACTACAGACGGGCGCCACAAACGGGCTGACGGGCCATCCGTCGTCGGCGTGCGACTGTGCGAGCATGGCGTGCTC			[160]
MW078485				
MW078486	.T.A.....T..T.....TT.....T.....			
MW078487	CT.A.....T.....TT.....T.....			
MW078488	.T.A.....T.....T.....T.....			
MW078489	.T.A.....T.....T.....T.....			
MW078490	.T.A.....T.....T.....T.....			
MT068436	CT.A.....T.....TT.....T.....			
MT068425	.T.A.....T.....TT.....T.....			
MT068434	.T.A.....T.....TT.....T.....			
	ITS2			
MW078484	GGGTCTCGGCGTGGACCTTGGGCGCTGAAAGTGACACT--GTTTG-GCGGCACCTGCGCGTGTGCTCTC---AGTGTT			[240]
MW078485	--.....			
MW078486	T..CT...A.....TT.....CTA....C			
MW078487	T..CT...A.....TT.....CTA....C			
MW078488	T..CT...A.....TT.....CTA....C			
MW078489	T..CT...A.....TT.....CTA....C			
MW078490	T..CT...A.....TT.....CTA....C			
MT068436	T..CT...A.....TT.....CTA....C			
MT068425	T..CT...A.....TT.....CTA....C			
MT068434	T..CT...A.....TT.....CTA....C			
	ITS2			
MW078484	GACGTATGGTGAGGGTAGTGTCAAATCGCACGGTTCGACAACA--AGCGTACCGTCGAGTTGGTGCAATCGGATGCCTA			[320]
MW078485	--.....			
MW078486	GC.....G.....CA.....T.....			
MW078487	GC.....G.....CA.....T.....			
MW078488	GC.....G.....CA.....T.....			
MW078489	GC.....G.....CA.....T.....			
MW078490	GC.....G.....CA.....T.....			
MT068436	GC.....G.....CA.....T.....			
MT068425	GC.....G.....CA.....T.....			
MT068434	GC.....G.....CA.....T.....			
	ITS2			
MW078484	CTACCATGGGCGGAGCCGGCGTGCATTCAACACTCGACGT-----CCTGTATCAACCGGATGCCAACTTGGTTGGTGGT			[400]
MW078485	-----			
MW078486	T.....GTGCGT.....			
MW078487	T.....A.....GCGTGT.....R.....			
MW078488	T.....GCGTGT.....			
MW078489	T.....GCGTGT.....			
MW078490	T.....GCGTGT.....			
MT068436	T.....A.....GCGTGT.....			
MT068425	T.....GTGTGT.....			
MT068434	T.....GTGTGT.....			

ITS2

MW078484 GCCGGCGCAGACAGGACACTGAATCGATCTTGGTGGTACAACCCACATGTGGGTTAGTAGGTAGGTGGTCGATGTGTGCA [480]
 MW078485
 MW078486
 MW078487
 MW078488
 MW078489
 MW078490
 MT068436
 MT068425
 MT068434

ITS2

MW078484 GGTGACAACCGGATGCCAGCGATGGCGGTGCCGGCGCACACCAGCACACTCGCCCCTAGGTCGCTTGTGCGTGTAACGC [560]
 MW078485
 MW078486 -----T.C.--TCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....
 MW078487 -----T.C.--TCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....
 MW078488 -----T.C..TGTCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....
 MW078489 -----T.C..TGTCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....K.
 MW078490 -----T.CC.TGTCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....
 MT068436 -----T.C..TGTCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....
 MT068425 -----T.C.--TCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....
 MT068434 -----T.C.--TCA.T..T...T.....G..AG.---.G...G.G..---.....AGT.....

ITS2

MW078484 GTGTGATCCATACACATACCTGTTTGTGAGCTGTGCGTTGAACACAAGAGGATGAGAGTTGCCAAACA---CACAGACCACA [640]
 MW078485
 MW078486 ...C..C.....G.....C.....A.....-----C...TTG.....G..GGAGG.A.T.....
 MW078487 ...C..C.....G.....C.....A.....-----C...TTG.....G..GGAGG.G.T.....
 MW078488 ...C..C.....G.....C.....-----C...CTG.....G..GGAG..A.T.....
 MW078489 ...C..C.....G.....C.....-----C...CTG.....G..GGAG..A.T.....
 MW078490 ...C..C.....G.....C.....-----C...CTG.....G..GGAG..A.T.....
 MT068436 ...C..C.....G.....C.....A.....-----C...TTG.....G..GGAGG.G.T.....
 MT068425 ...C..C.....G.....C.....A.....-----C...TTG.....G..GGAG..A.T.....
 MT068434 ...C..C.....G.....C.....A.....-----C...TTG.....G..GGAG..A.T.....

ITS2-->< 28S

MW078484 ----CTCCAGTAGGCCTCAAGTGATGTGTGACTACCCCCAGAATTAAAGCATATTAATAAGGGGAGGAAGAGAAACCAAC [720]
 MW078485 ----
 MW078486 ACTA.....
 MW078487 AACT.....
 MW078488 --TA.....
 MW078489 --TA.....
 MW078490 --TA.....
 MT068436 AACT.....
 MT068425 CCTA.....
 MT068434 CCTA.....

28S

MW078484 CGGGATTCCCTGAGTAGCTGCGAGCGAAACGGGAGAAGCCCAGCACGTAGGGACGACGTGGAGACGCGCCTGTCCGGTTC [800]
 MW078485
 MW078486T.....G.....A.....
 MW078487T.....G.....
 MW078488T.....G.....
 MW078489T.....G.....
 MW078490T.....G.....

28S

MW078484 CGTGTATTGGACCGGTCCGTTATCTATCATGTACAGTGTGCATCAAGTCTAACTTGAAGGTGGCTCATTATCCCATAGAG [880]
 MW078485
 MW078486C.....CA.....
 MW078487C.....CA.....
 MW078488C.....CA.....
 MW078489C.....CA.....
 MW078490C.....CA.....

28S

MW078484 GGTGATAGGCCCGTTGCATGCGCGCGCCTGTGCTGGTAGACGGTCGGCTCCAGGGAGTCGTGTTGCTTGATAGTGCAGCA [960]
 MW078485
 MW078486A.....A.....
 MW078487A.....A.....
 MW078488A.....A.....
 MW078489A.....A.....
 MW078490A.....A.....

28S

MW078484 CTAAGTGGGAGGTAAACTCCTTCTAAGGCTAAATATGACCATGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGTTGA [1040]
MW078485
MW078486A.....CG.....
MW078487A.....TG.....
MW078488A.....TG.....
MW078489A.....TG.....
MW078490A.....TG.....

28S

MW078484 AAAGCACTCTGAATAGAGAGTCAAATAGTACGTGAAACTGCCTAGGGTACACAAACCCGTTGAACTCAATGATCCGGGCG [1120]
MW078485
MW078486
MW078487
MW078488
MW078489
MW078490

28S

MW078484 GCGATATTCAGCGGCGGTGCGAAGGCCACCGTGCCTTATCGTTCCGAGCAAACGGACATCGCGATCCATTACAATAGC [1200]
MW078485
MW078486T.....G.....
MW078487T.....G.....
MW078488T.....G.....
MW078489T.....G.....
MW078490T.....G.....

28S

MW078484 GAGTCGAGCGTTCTGCGCTGCGACCGTCCGGCAT-TACTGGCCCTGGCTCGTGGTGGACGGCTCCCTAGTAGGGGCGGC [1280]
MW078485-.....
MW078486 .G...G...C.T.....G.TA.....
MW078487 .GT...G...C.T.....GCGA.....
MW078488 .G...G...C.T.....GCGA.....
MW078489 .G...G...C.T.....GCGA.....
MW078490 .G...G...C.T.....GCGA.....

28S

MW078484 TTGACGGCCGCACCGAGCGGGGGTCTCCGCGCCTTCTTCTGAAGGGCGACTGGGTCCGACCGAGCGTGGTGTGCCGCTG [1360]
MW078485
MW078486 ..TG.....A..C.....
MW078487 ..TG.....A..C.....M.....
MW078488 ..TG.....A..C.....
MW078489 ..TG.....A..C.....
MW078490 ..TG.....A..C.....

28S

MW078484 GAACCGTGATGGATCCGAGCGTGGGGCCGACC--AAGGCTGCCCTC---GTGGCCAGCCAAGCGTGCCCCCAGATCGGC [1440]
MW078485--.....
MW078486A...GTTAG...A...T..TGCGAA..TTGT..GTT...T.....
MW078487A...GTTAG...A...T..TGCGAA..TTGT..GTT...T.....
MW078488A...GTTAG...A...T..TGCGAA..TTGT..GTT...T.....
MW078489A...GTTAG...A...T..TGCGAA..TTGT..GTT...T.....
MW078490A...GTTAG...A...T..TGCGAA..TTGT..GTT...T.....

28S

MW078484 GATGAGCAGCATGCATTGAGGCACCTCCGGGACCCGTCTTGAAACACGGACCAAGAAGTCTATCTTGCACGCGAGCCAAT [1520]
MW078485
MW078486T.....A.....
MW078487T.....A.....
MW078488T.....A.....
MW078489T.....A.....
MW078490T.....A.....

28S

MW078484 GGGACCGGTGCCACCTTCGGGTGGTGCCGCTCAATCGAACACCCATAGGCGAAGACAACCTCGAAGACGCATGTCACGGGA [1600]
MW078485
MW078486A.A....GGT.T....T.....T...TTC.....
MW078487A.A....GGT.T....T.....T...TTC.....
MW078488A.A....GGT.T....T.....T...TTC.....
MW078489A.A....GGT.T....T.....T...TTC.....
MW078490A.A....GGT.T....T.....T...TTC.....

28S

MW078484 TTACGGGTTTCGGCATTTGGCGCAAGCCTACGTCCGATCCCTCCATCCCAGGGTGCCCCGATACGGGTGGGAGCGGGCCTCC [1680]
MW078485
MW078486-.....T.....
MW078487-.....T.....
MW078488-.....T.....
MW078489-.....T.....
MW078490-.....T.....

28S

MW078484 GGGCGTCGTTCTACCCAGCGGGCATACCCCGAGTGTGCAGGATGCGACCCGAAAGATGGTGAACCTATGCCTGATCAGGTT [1760]
 MW078485
 MW078486
 MW078487
 MW078488
 MW078489
 MW078490

28S

MW078484 GAAGTCAGGGGAAACCCTGATGGAGGACCGAAGCAATTCTGACGTGCAAATCGAT [1815]
 MW078485
 MW078486
 MW078487
 MW078488
 MW078489
 MW078490

Reference

1. Wilai P, Ali RSM, Saingamsook J, Saeung A, Junkum A, Walton C, Harbach RE, Somboon P. Integrated systematics of *Anopheles subpictus* (Diptera: Culicidae) in the Oriental Region, with emphasis on forms in Thailand and Sulawesi, Indonesia. Acta Tropica. 2020;208:1-8.
2. Selig C, Wolf M, Müller T, Dandekar T, Schultz J. The ITS2 Database II: homology modelling RNA structure for molecular systematics. Nucleic Acids Res. 2008;36 (Database issue):D377-D380