

# Supplementary Materials: Comparative Studies on the Stenogamous and Eurygamous Behavior of Eight *Anopheles* Species of the Hyrcanus Group (Diptera: Culicidae) in Thailand

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**Table S1.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (10 cubic cm cage, DRS = 3.6).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	28	30	52	66
<i>An. argyropus</i>	89	0	0	0
<i>An. crawfordi</i>	0	0	0	0
<i>An. nigerrimus</i>	61	24	2 <sup>a</sup>	2 <sup>a</sup>
<i>An. nitidus</i>	18	0	0	0
<i>An. paraliae</i>	28	4	10 <sup>b</sup>	14 <sup>b</sup>
<i>An. pursati</i>	114	26	12 <sup>c</sup>	12 <sup>c</sup>
<i>An. sinensis</i>	12	2	18 <sup>d</sup>	2 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S2.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (20 cubic cm cage, DRS = 3.6).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	29	23	35	97
<i>An. argyropus</i>	97	2	0	0
<i>An. crawfordi</i>	0	0	0	0
<i>An. nigerrimus</i>	38	56	11 <sup>a</sup>	29 <sup>a</sup>
<i>An. nitidus</i>	8	0	0	0
<i>An. paraliae</i>	77	10	9 <sup>b</sup>	3 <sup>b</sup>
<i>An. pursati</i>	93	26	21 <sup>c</sup>	20 <sup>c</sup>
<i>An. sinensis</i>	10	0	4 <sup>d</sup>	32 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S3.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (30 cubic cm cage, DRS = 3.6).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	9	37	28	21
<i>An. argyropus</i>	125	6	0	0
<i>An. crawfordi</i>	7	1	0	0
<i>An. nigerrimus</i>	63	60	4 <sup>a</sup>	9 <sup>a</sup>
<i>An. nitidus</i>	8	1	0	0
<i>An. paraliae</i>	59	13	5 <sup>b</sup>	6 <sup>b</sup>
<i>An. pursati</i>	89	67	8 <sup>c</sup>	11 <sup>c</sup>
<i>An. sinensis</i>	25	2	18 <sup>d</sup>	15 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S4.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (10 cubic cm cage, DRS = 7.2).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	15	5	25	95
<i>An. argyropus</i>	65	10	0	0
<i>An. crawfordi</i>	0	0	0	0
<i>An. nigerrimus</i>	44	14	14 <sup>a</sup>	9 <sup>a</sup>
<i>An. nitidus</i>	0	0	0	0
<i>An. paraliae</i>	52	10	0	4 <sup>b</sup>
<i>An. pursati</i>	100	10	0	12 <sup>c</sup>
<i>An. sinensis</i>	10	10	10 <sup>b</sup>	65 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S5.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (20 cubic cm cage, DRS = 7.2).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	12	4	22	156
<i>An. argyropus</i>	104	0	0	0
<i>An. crawfordi</i>	0	0	0	0
<i>An. nigerrimus</i>	100	4	4 <sup>a</sup>	50 <sup>a</sup>
<i>An. nitidus</i>	6	1	1 <sup>b</sup>	0
<i>An. paraliae</i>	40	6	6 <sup>c</sup>	36 <sup>b</sup>
<i>An. pursati</i>	92	22	8 <sup>d</sup>	38 <sup>c</sup>
<i>An. sinensis</i>	62	6	6 <sup>e</sup>	18 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d, e vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S6.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (30 cubic cm cage, DRS = 7.2).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	12	11	20	114
<i>An. argyropus</i>	138	1	0	0
<i>An. crawfordi</i>	1	0	0	0
<i>An. nigerrimus</i>	66	22	8 <sup>a</sup>	20 <sup>a</sup>
<i>An. nitidus</i>	2	0	1 <sup>b</sup>	0
<i>An. paraliae</i>	36	5	11 <sup>c</sup>	18 <sup>b</sup>
<i>An. pursati</i>	68	5	0	8 <sup>c</sup>
<i>An. sinensis</i>	19	6	13 <sup>d</sup>	36 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S7.** Frequency of inseminated grade based on sperm density in spermathecae of female mosquitoes of the eight species (40 cubic cm cage, DRS = 7.2).

Mosquito Species *	Grading of Sperm Density in Female Spermathecae **			
	1+	2+	3+	4+
<i>An. peditaeniatus</i>	18	38	42	80
<i>An. argyropus</i>	82	0	0	0
<i>An. crawfordi</i>	1	4	0	0
<i>An. nigerrimus</i>	46	24	7 <sup>a</sup>	7 <sup>a</sup>
<i>An. nitidus</i>	17	1	0	0
<i>An. paraliae</i>	60	0	5 <sup>b</sup>	5 <sup>b</sup>
<i>An. pursati</i>	85	4	0	4 <sup>c</sup>
<i>An. sinensis</i>	44	0	2 <sup>c</sup>	2 <sup>d</sup>

\* Dissected 200 spermathecae/species; \*\* Chi-square test: a, b, c, d vs. *An. peditaeniatus* ( $p < 0.05$ ).

**Table S8.** Comparisons of the results of statistical analyses of male genital measurements for the eight species of the Hyrcanus Group.

Character	Mosquito Species	p-Value
Length of aedeagus	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.000 *
	<i>An. peditaeniatus</i> vs. <i>An. crawfordi</i>	0.001 *
	<i>An. peditaeniatus</i> vs. <i>An. nigerrimus</i>	0.884
	<i>An. peditaeniatus</i> vs. <i>An. nitidus</i>	0.000 *
	<i>An. peditaeniatus</i> vs. <i>An. paraliae</i>	0.477
	<i>An. peditaeniatus</i> vs. <i>An. pursati</i>	1.000
Width of aedeagus	<i>An. peditaeniatus</i> vs. <i>An. sinensis</i>	0.000 *
	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.252
	<i>An. peditaeniatus</i> vs. <i>An. crawfordi</i>	0.025 *
	<i>An. peditaeniatus</i> vs. <i>An. nigerrimus</i>	0.294
	<i>An. peditaeniatus</i> vs. <i>An. nitidus</i>	1.000
	<i>An. peditaeniatus</i> vs. <i>An. paraliae</i>	1.000
Length between base of aedeagus and origin of gonocoxite (right)	<i>An. peditaeniatus</i> vs. <i>An. pursati</i>	0.194
	<i>An. peditaeniatus</i> vs. <i>An. sinensis</i>	0.017 *
	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.620
	<i>An. peditaeniatus</i> vs. <i>An. crawfordi</i>	0.889
	<i>An. peditaeniatus</i> vs. <i>An. nigerrimus</i>	0.493
	<i>An. peditaeniatus</i> vs. <i>An. nitidus</i>	0.958
Length between base of aedeagus and origin of gonocoxite (left)	<i>An. peditaeniatus</i> vs. <i>An. paraliae</i>	0.955
	<i>An. peditaeniatus</i> vs. <i>An. pursati</i>	0.984
	<i>An. peditaeniatus</i> vs. <i>An. sinensis</i>	0.009 *
	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.546
	<i>An. peditaeniatus</i> vs. <i>An. crawfordi</i>	0.987
	<i>An. peditaeniatus</i> vs. <i>An. nigerrimus</i>	0.419
Width of gonocoxite at origin of parabasal seta (right)	<i>An. peditaeniatus</i> vs. <i>An. nitidus</i>	0.962
	<i>An. peditaeniatus</i> vs. <i>An. paraliae</i>	0.733
	<i>An. peditaeniatus</i> vs. <i>An. pursati</i>	0.930
	<i>An. peditaeniatus</i> vs. <i>An. sinensis</i>	0.013 *
	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.809
	<i>An. peditaeniatus</i> vs. <i>An. crawfordi</i>	0.000 *
Width of gonocoxite at origin of parabasal seta (left)	<i>An. peditaeniatus</i> vs. <i>An. nigerrimus</i>	0.000 *
	<i>An. peditaeniatus</i> vs. <i>An. nitidus</i>	0.014 *
	<i>An. peditaeniatus</i> vs. <i>An. paraliae</i>	0.015 *
	<i>An. peditaeniatus</i> vs. <i>An. pursati</i>	0.876
	<i>An. peditaeniatus</i> vs. <i>An. sinensis</i>	0.000 *
	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.921
	<i>An. peditaeniatus</i> vs. <i>An. crawfordi</i>	0.017 *
	<i>An. peditaeniatus</i> vs. <i>An. nigerrimus</i>	0.000 *
	<i>An. peditaeniatus</i> vs. <i>An. nitidus</i>	0.001 *
	<i>An. peditaeniatus</i> vs. <i>An. paraliae</i>	0.016
	<i>An. peditaeniatus</i> vs. <i>An. pursati</i>	0.668
	<i>An. peditaeniatus</i> vs. <i>An. sinensis</i>	0.000 *

**Table S8. Cont.**

Character	Mosquito Species	p-Value
Length of gonocoxite (right)	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	1.000
	<i>An. crawfordi</i>	1.000
	<i>An. nigerimus</i>	0.000 *
	<i>An. nitidus</i>	0.000 *
	<i>An. paraliae</i>	0.000 *
	<i>An. pursati</i>	1.000
	<i>An. sinensis</i>	0.000 *
Length of gonocoxite (left)	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	1.000
	<i>An. crawfordi</i>	1.000
	<i>An. nigerimus</i>	0.000 *
	<i>An. nitidus</i>	0.000 *
	<i>An. paraliae</i>	0.000 *
	<i>An. pursati</i>	1.000
	<i>An. sinensis</i>	0.000 *
Length of gonostylus (right)	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.002 *
	<i>An. crawfordi</i>	0.000 *
	<i>An. nigerimus</i>	0.000 *
	<i>An. nitidus</i>	0.000 *
	<i>An. paraliae</i>	0.000 *
	<i>An. pursati</i>	0.010 *
	<i>An. sinensis</i>	0.000 *
Length of gonostylus (left)	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.002 *
	<i>An. crawfordi</i>	0.000 *
	<i>An. nigerimus</i>	0.000 *
	<i>An. nitidus</i>	0.000 *
	<i>An. paraliae</i>	0.000 *
	<i>An. pursati</i>	0.010 *
	<i>An. sinensis</i>	0.000 *
Ratio length and width of gonocoxite	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.646
	<i>An. crawfordi</i>	0.013 *
	<i>An. nigerimus</i>	1.000
	<i>An. nitidus</i>	0.997
	<i>An. paraliae</i>	0.646
	<i>An. pursati</i>	0.739
	<i>An. sinensis</i>	0.018 *
Ratio length and width of gonocoxite + gonostylus	<i>An. peditaeniatus</i> vs. <i>An. argyropus</i>	0.443
	<i>An. crawfordi</i>	0.013 *
	<i>An. nigerimus</i>	0.000 *
	<i>An. nitidus</i>	0.000 *
	<i>An. paraliae</i>	0.000 *
	<i>An. pursati</i>	0.512
	<i>An. sinensis</i>	0.000 *

\* Significantly different ( $p < 0.05$ , Tukey's HSD test).



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