**Figure 3**

GenBank search of the cloned *Ae. aegypti* mosquito larval APN reveals highest identity to the *An. gambiae* unannontated whole genome sequence.

(A) The top three results of the blast search.

(B) Sequence alignment between the *Ae. aegypti* mosquito larval APN (query) and that of *An. gambiae* (Sbjct) that showed the highest sequence identity (64%).

A

| Sequences producing High-scoring Segment Pairs: | Score | P(N) |
|---|---|---|
| gb\|AAAB01008817.1\| Anopheles gambiae str. PEST CRA_x9P1GA... | 864 | 2.4e-32 |
| gb\|AAAB01008987.1\| Anopheles gambiae str. PEST CRA_x54KRF... | 703 | 4.6e-25 |
| gb\|AAAB01008968.1\| Anopheles gambiae str. PEST CRA_x9P1GA... | 522 | 1.0e-16 |

B

>gb|AAAB01008817.1| Anopheles gambiae str. PEST CRA_x9P1GAV59A7, whole genome shotgun sequence
Score = 864 (135.7 bits), Expect = 2.4e-32, P = 2.4e-32
Identities = 360/561 (64%), Positives = 360/561 (64%), Strand = Plus / Plus

```
Query:      6 CTGCAAGTATAGAGAATCACTTGCTACTGTTCGATCCTGCCGTAACCACTTA-CGCACCC 64
              |||| ||| || |||  |  | || ||||||| || | ||| || || || ||  | |
Sbjct: 737670 CTGCTTGTACAGTGAAG-AGGTCCTCCTGTTCGAGCCGGGCGTTACGACGTATCGAGCGC 737728

Query:     65 GCACGTGGATTGATACCATTATCGCTCACGAATACATTCACCAGTGGTTCGGAAATATTA 124
               |||   ||    || || ||||| ||||| ||| | ||||||||||||||| ||  |||
Sbjct: 737729 AAACGACCATCACCACAATCATCGCGCACGAGTACGTGCACCAGTGGTTCGGCAACGTTA 737788

Query:    125 TTACCAACGAGTGGTGGAGCTACCTATGGCTCAACGAGGGATTCGCTACTCTTTACGAAT 184
              | |||||||||||||||||||| || ||||| |||||||| ||||| || || ||||| |
Sbjct: 737789 TCACCAACGAGTGGTGGAGCTATCTCTGGCTTAACGAGGGCTTCGCGACGCTGTACGAGT 737848

Query:    185 ACTACGGCGCCCATCTGGCTTCACCTGAAATGGAGTACTTCGAGCTGTTCACCCTGGATG 244
               |   || ||| || |||||| || ||     |||| |   || |||||||  | |  | |
Sbjct: 737849 TCCTGGGGGCCGATATGGCTTATCCGGAGCGCCAGTATCGGGACCTGTTCAACGTTCAGG 737908

Query:    245 TTACCCAGTGGGCACTAGATG-CTGATTCTCGCG-AGGGAACCCGACCCATGAGTTTCAG 302
              |   ||| | |  ||  ||  | ||  | |||| |  | || || || |||| ||||||
Sbjct: 737909 TGGTGCAGCGTGTTCTC-ATCACAGACGC-CGCGGAAAGCACGCGCCCGATGACTTTCAG 737966

Query:    303 TAATGGTGCAACATTTTATGCCATT-TGGGATCTGTTTGATAGTATAGCGTATTCGAAAG 361
                || ||||| ||| |||||||  |  |  ||||| || |   | ||||| || || |
Sbjct: 737967 CCGCGGCGCAACGTTTAATGCCATCCTCAGC-CTGTTCGACAACGTGGCGTACTCCAAGG 738025

Query:    362 CTGGTGCCGTTTTGAACATGTTCCGTGGTG-TACTTGGAGATGAAGTATGGCGTCTTATG 420
              |||  | ||  |  | ||||||| | || | ||    |||| |  ||||| |  |||
Sbjct: 738026 GTGGCTCGGTACTTCAGATGTTCCG-GCTGCTGCTGCCGGATGCCGCCTGGCGGCAGATG 738084

Query:    421 A-GCAGCGTTTACTTGTCAGA-CAATGAACTTGAAGCTGTTAATCCAGATGATTTGATCG 478
               || ||   ||| || |||  ||| ||  | |   | || ||  | ||| | ||  | |
Sbjct: 738085 TTGC-GCACGTACGTG-CAGGGCAACGAGTTCGGCACGGTCAACACGGATAACTTCGTGG 738142

Query:    479 CCGCAATGGAAGAAGCCTCCAAGGACATGGATATTT-TGCCAGATGGTATTTCAATGCGT 537
              ||||| ||   ||||| | | | |   | | |||  |||| || || |   | ||| |
Sbjct: 738143 CCGCACTGACGGAAGCGTTCGACGGTGTCG-TATCGCTGCCCGAGGGGAC--CGATGTG- 738198

Query:    538 GA-C--TTCGTCGAAAGCTGG 555
              || |  || ||  | ||||||
Sbjct: 738199 GAGCGGTTTGTTCATAGCTGG 738219
```

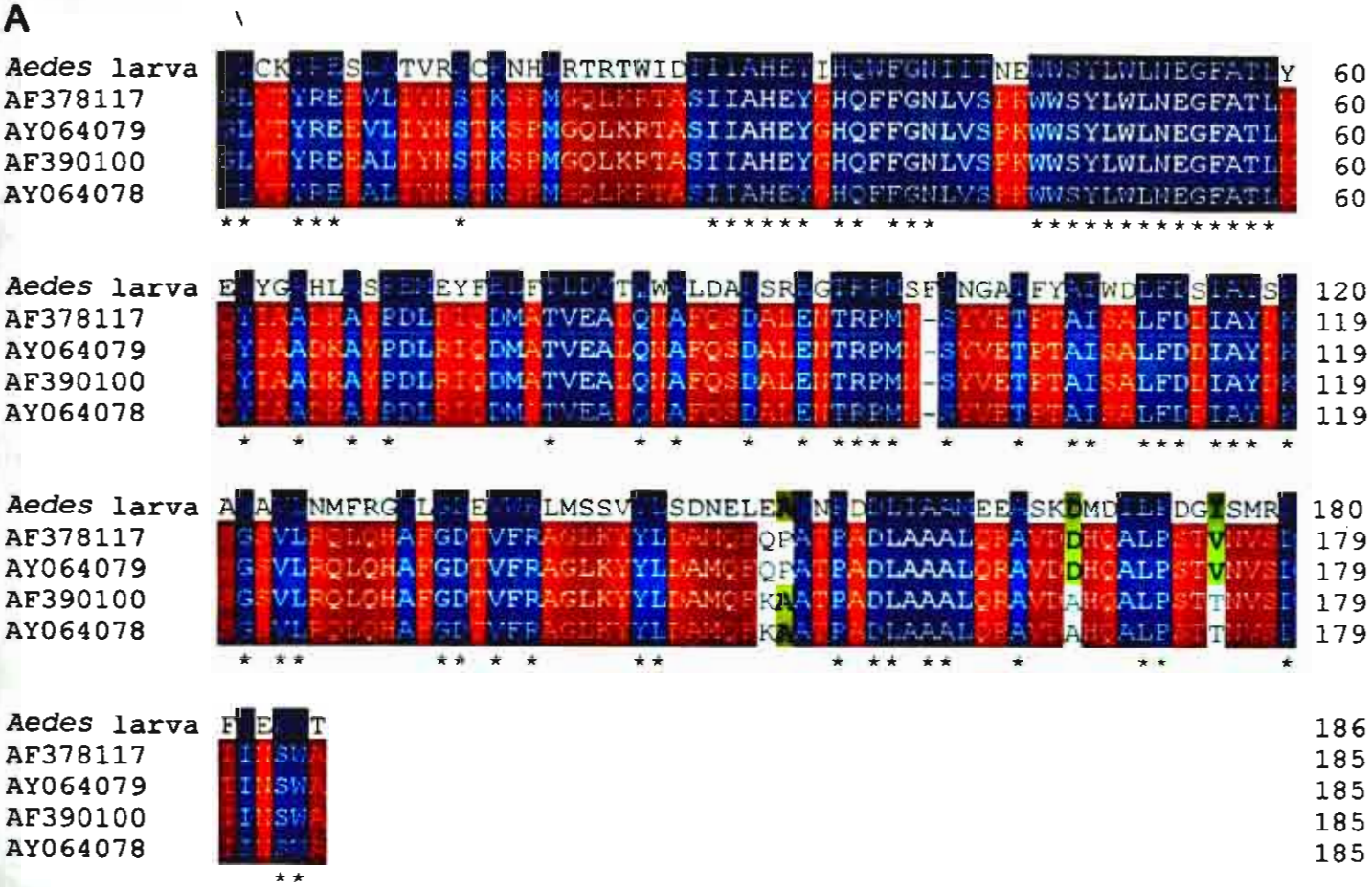
**Figure 4**

Comparison of *Ae. aegypti* larval APN to adult *Ae. aegypti* and other insect species reveals a low to moderate degree of identity.

(A) The deduced amino acid sequence of the *Ae. aegypti* larval APN fragment is aligned to those of adult *Ae. aegypti* APNs (AF378117; AF390100; AY064078-9). Shading of the alignment represents degree of conservation; blue, red and yellow shading denote 100%, 80% and 60% homology, respectively. Star denotes identical residues.

(B) The deduced amino acid sequence of the *Ae. aegypti* larval APN fragment is aligned to those of *H. punctigera* APN2 (Emmerling *et al.*, 2001), *M. sexta* APN3 (accession number AF498990), *H. armigera* APN4 (AF535165), *S. litura* APN (Agrawal *et al.*, 2002) and *H. virescens* 110 kD APN (AF378666). Shading of the alignment represents degree of conservation; blue, red and yellow shading denote 100%, 83% and 67% homology, respectively. Star denotes identical residues.

A


Aedes larva
AF378117
AY064079
AF390100
AY064078
60
120
180
186

**B**

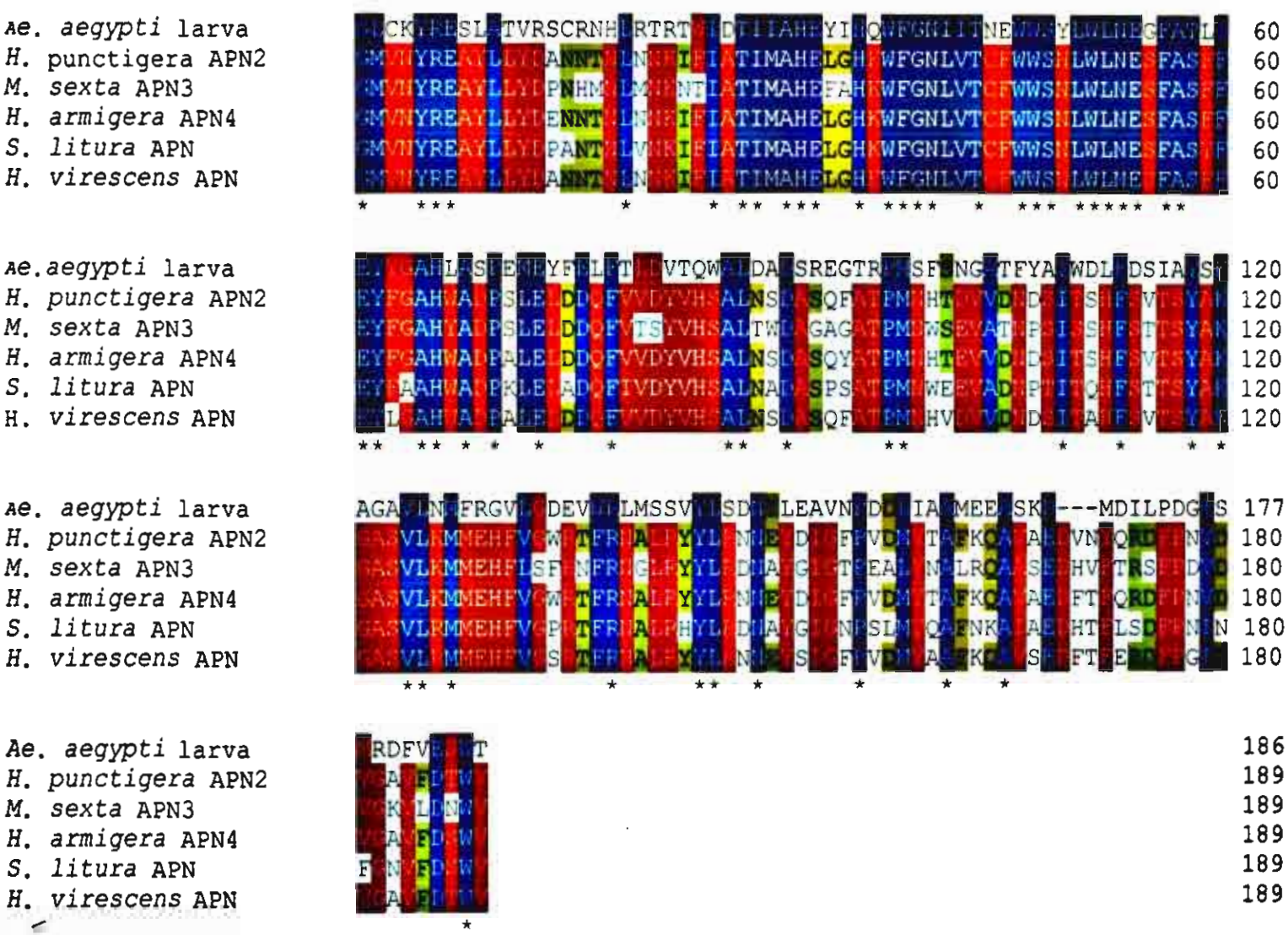

Ae. aegypti larva
H. punctigera APN2
M. sexta APN3
H. armigera APN4
S. litura APN
H. virescens APN
60
120
177
186
189

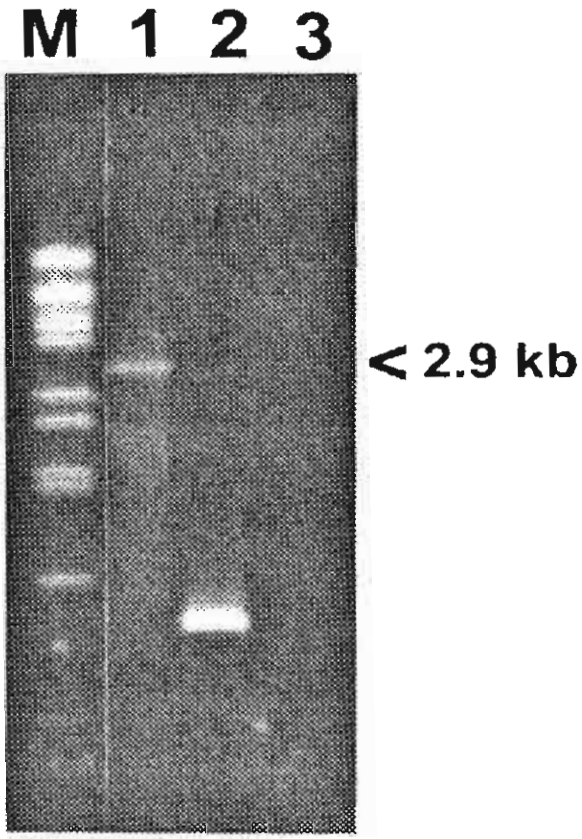
**Figure 5**

RT-PCR demonstrates expression of another APN isoform in the *Ae. aegypti* larval midgut.

(A) 1% gel electrophoresis of RT-PCR product. Lane 1, *Ae. aegypti* larval midgut APN amplicon (~2.9 kb, arrowhead). Lane 2, *Ae. aegypti* asparagine synthetase (positive control). The water control is lane 3. M = $\lambda$/*BstE* II digested DNA standard.

(B) Partial nucleotide sequence and the deduced amino acid sequence of the 2.9 kb APN clone.

A



B

atgtggaaaactgcgatcttgattctgagtgtaacttcggccgtttggtcgggagttcccttagagcga
M W K T A I L I L S V T S A V W S G V P L E R

cagaatcttccatcaactccccgacaggaggtgccagtttccggttatcggctaccaaacaacactatt
Q N L P S T P R Q E V P V S G Y R L P N N T I

ccactccggtacaacgtagaactgacaacgcacgttcacgaccaccaaagtccgaatcagtttgatttc
P L R Y N V E L T T H V H D H Q S P N Q F D F

aatggaaaagtcacaatttcactgcgtgtcctggaagaaaatgtacagaacattacgcttcattatcgt
N G K V T I S L R V L E E N V Q N I T L H Y R

caaattacggtaacccatgtgaagcttaccgatgccaccaatacagtgctcgtggacgatgattccagc
Q I T V T H V K L T D A T N T V L V D D D S S

ttcaccacggatgtcacctacgagttcctggtcattctagcgccttcaattttgaggatcggcgattat
F T T D V T Y E F L V I L A P S I L R I G D Y

tcactggaacttgaataccacggagagctccgtaccgataacgggggtttctaccgttcgtcctacacc
S L E L E Y H G E L R T D N G G F Y R S S Y T

gatgccagcggaaatactcggtggattgcaacgacacagttcgagtcgacggatgcccgacatgcgttc
D A S G N T R W I A T T Q F E S T D A R H A F

ccgtgctacgacgaacctggcacccgaactcctatcggattgaaattaacgcatggaaacgcataccat
P C Y D E P G T R T P I G L K L T H G N A Y H

gccatctccaatatgcccatcaaaagttccttgccctggaatgcgacctataccgttaccgaatttgag
A I S N M P I K S S L P W N A T Y T V T E F E

tataccttggcaatg
Y T L A M

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## Project Outputs

1. Publication

Pootanakit, K., Angsuthanasombat, C. and Panyim, S. (2003) Identification of two isoforms of aminopeptidase N in *Aedes aegypti* larval midgut. *Journal of Biochemistry and Molecular Biology*. Manuscript accepted.

2. Presentation

Pootanakit, K., Angsuthanasombat, C. and Panyim, S. (2001) Molecular cloning of aminopeptidase N – a potential *Bacillus thuringiensis* toxin receptor from *Aedes aegypti* mosquito larval midgut. The Second Annual Conference of The Thailand Research Fund Post-Doctoral Fellows (*Abstr.* OA1-11) held at Karnchanaburi, Thailand, from October 12-14.

# ภาคผนวก

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Subject: Acceptance of JBMB-07-03E

March 18, 2003

Dr. Kusol Pootanakit
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RE: JBMB-07-03E - K. Pootanakit, C. Angsuthanasombat, and S. Panyim - "Identification of two isoforms of aminopeptidase N in Aedes aegypti larval midgut"

Dear Dr. Pootanakit:

I have received comments concerning the above referenced manuscript from two reviewers and these are attached to this e-mail as separate files. Both reviewers indicate that the manuscript is suitable for publication in the Journal of Biochemistry and Molecular Biology. The acceptance date will be March 18, 2003.

Reviewer 2 suggests one minor correction. Please make that correction and send me the following items:

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