Asia-Pacific Journal of Science and Technology: Volume: 30. Issue: 02. Article ID.: APST-30-02-10. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by Research Department,
Khon Kaen University, Thailand

Morphology of sting, venom duct, venom sac and venom gland of wasps (Hymenoptera: Vespidae) found in northeastern Thailand

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Received 27 April 2024
Revised 17 September 2024
Accepted 25 September 2024

Abstract

Wasps (Hymenoptera: Vespidae) play a role in the ecosystem as predators. The wasp's sting is a vital weapon that has varying structures across species. This study aims to investigate morphological characteristics of sting, venom duct, venom sac and venom gland of wasps in three species: *Vespa affinis* Linnaeus, 1764, *V. tropica* Linnaeus, 1764, and *Provespa barthelemyi* Buysson, 1905. Samples were collected from 7 provinces in northeastern Thailand: Kalasin, Khon Kaen, Maha Sarakham, Yasothon, Sakon Nakhon, Sisaket, and Amnat Charoen. Three nests per species, 50 individuals per nest, were collected and preserved in 80% ethanol at -20°C. Thirty wasps per species were studied under a stereo microscope and a scanning electron microscope. Average width and length of the stings and its associated glands were analyzed. The results showed that *V. tropica* had the most curved stylet tip, with average width and length of 320.60 ± 3.15 and 3634.77 ± 80.89 µm. *V. affinis* had slightly curved stylet tip, with average width and length of 259.42 ± 3.93 and 3199.24 ± 33.51 µm. *P. barthelemyi* had very slightly curved, with average width and length of 233.18 ± 1.84 and 2272.74 ± 13.27 µm. One-way ANOVA, Cluster Analysis, and Scatter plotting were performed to classify the populations. The venom gland, venom duct, and venom sac among species had similar characteristics but differed significantly in size, muscle bundle arrangement, and internal width of the venom duct. In conclusion, these characteristics of sting and its associated glands can be used to identify wasp species.

Keywords: Morphology, Sting, Venom duct, Venom gland, Venom sac, Wasp

1. Introduction

Wasps in the order Hymenoptera, family Vespidae are primarily eusocial insects. Each nest consists of three castes: the queen, the workers, and the males. Wasps play an important role as predators in the food chain of the ecosystem and can also be used as biological control agent [1]. There are more than 5,000 species of wasps in family Vespidae found globally [2]. In Thailand, there have been few reports on species diversity of wasps. Seventeen species of Vespidae attributed to 4 subfamilies have been reported in northern Thailand, Eumeninae (4 species), Polistinae (6 species), Stenogastrinae (1 species) and Vespinae (6 species) [3]. Several morphological characteristics have been used for species identification such as body size, body color, wing size and body structures [1, 4, 5-7]. Interestingly, sting and venom apparatus are structures not yet employed for identifying wasp species [1].

The sting of a wasp is an important weapon, with structures varying between species. It is a sharp needle with venom canal, modified from ovipositor. It has a potent venom used in attacks that cause pain to enemies or prey. The primary roles of sting include killing or paralyzing prey as well as defense against enemies. Wasps can sting multiple times without dying due to the sting's barbs are not hooked by prey or enemy's tissue fibers [4, 8, 9].

Sting morphology of insects had been investigated in some taxa, including honeybees in the genus Apis [4, 10, 11] and social wasps [1, 8]. The reports showed differences of sting morphology in each species and enable diverse biological and mechanical functions [4]. Functional morphology of the sting of several species of bees and wasps have been demonstrated [4, 8, 12]. Because of their elegant structures and functionalities, insect stings are of interest

in the development of a painless and mechanically durable micro syringe-needle system for biomedical purposes [13].

In Thailand, 3 wasp species in the family Vespidae, *Vespa affinis* Linnaeus, 1764, *V. tropica* Linnaeus, 1764, and *Provespa barthelemyi* Buysson, 1905 are commonly found. *V. affinis* and *V. tropica* are similar in body colors, whereas *P. barthelemyi* differs from the others. Their nests could be seen in the forest area and nearby houses which can caused troubles to animals and humans. However, study on biology of wasps in Thailand is currently little explored. The purpose of this study was to investigate sting morphology of 3 wasp species in the family Vespidae, *V. affinis*, *V. tropica*, and *P. barthelemyi* using steromicroscope and scanning electron microscopy. We found differences within the same genus (*V. affinis* and *V. tropica*) and between 2 genera (*Vespa* and *Provespa*). This information can be used for identification of wasp species and may lead to better understanding of the initial mechanism of the wasp sting which can be applied in future studies.

2. Materials and methods

2.1 Sample collection and species identification

Adult wasps of the order Hymenoptera, family Vespidae were randomly collected from the northeastern Thailand during December 2020 – March 2021. Three nests per species with 50 individuals per nest were sampled. The insects were rendered unconscious by exposing them to low temperatures for 24 hours and then preserved in 80% ethyl alcohol at -20°C. Body length, right wing length and other morphological characteristics of 30 wasps per species were observed to use for species identification [14] which followed the keys of [15-17].

2.2 Morphology of sting

The sting of a wasp was carefully removed from the abdomen using forceps under a stereo microscope. During this process, the venom apparatus was carefully pulled out with the sting [8, 18]. Thirty wasps per species were dissected. The sting and venom apparatus were treated with 10% KOH for 8 h at room temperature [1, 8]. Then, they were washed in distilled water, transferred to 80% ethanol and kept separately in a 1.5 ml Eppendorf tube at -20°C. The sting samples were subsequently photographed under a stereo microscope (Zeiss Model Stemi 305 Lab Set) and a scanning electron microscope (SEM, Hitachi Model TM400Plus). From photographs we took 6 measurements from each specimen: 1) length of stylet, 2) width of stylet (about midway along the stylet), 3) length of lancets, 4) width of lancets (about midway along the straight part), 5) number of lancet barbs, 6) distance between each barb [1]. To reduce measurement error, each part of the sample was measured three times, and the averages calculated. All measurements were conducted using ImageJ software.

2.3 Morphology of venom gland, venom duct, and venom sac

The sting apparatus attached to the stings were carefully dissected and removed the soft tissues except for the muscles surrounding the venom sac. The sting apparatus were photographed under a stereo microscope (n=30) and a scanning electron microscope (n=10). The length and width of venom gland and venom duct of each wasp were measured using ImageJ software for further analysis [19, 20]. Unfortunately, the length of venom gland could not be determined because the structure was very fragile. Sting and venom apparatus morphology of each species were drawn to compare among different species [1].

2.4 Data analysis

Data obtained from the sting measurements were analyzed to calculate average (mean) and standard deviation. Analysis of Variance (ANOVA) was performed to analyze morphological differences of the stings between species, followed by a cluster analysis to classify the wasps into groups.

3. Results and Discussion

3.1 Sample collections and species identification

Wasp samples were collected from 7 provinces in the Northeastern Thailand (Kalasin, Khon Kaen, Maha Sarakham, Yasothon, Sakon Nakhon, Sisaket, and Amnat Charoen). Three species of wasps were identified: *V. affinis*, *V. tropica* and *P. barthelemyi* (Figure 1) based on external morphology [15-17]. ANOVA analysis showed significant differences in body size and wing size ($P<0.05$). The largest body size and wing size was *V. tropica* followed by *V. affinis* and *P. barthelemyi*, respectively (Table 1).

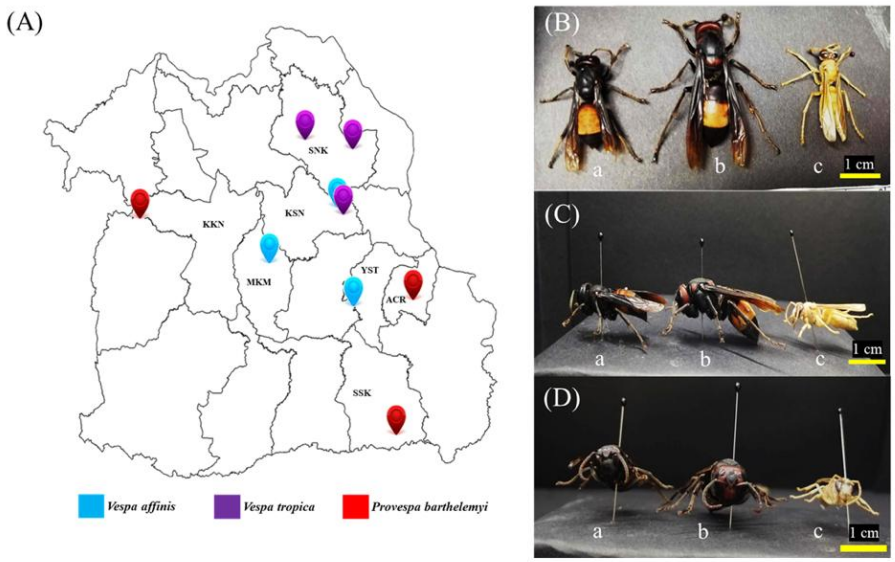

Figure 1 (A) Sampling locations in the Northeastern Thailand: Kalasin (KSN), Khon Kaen (KKN), Maha Sarakham (MKM), Yasothon (YST), Sakon Nakhon (SNK), Sisaket (SSK), and Amnat Charoen (ACR). External morphology of 3 species of wasps: (a) *Vespa affinis* (b) *Vespa tropica* (c) *Provespa barthelemyi*, (B) Dorsal view (C) Lateral view (D) Anterior view.

Table 1 Average body length and wing length of 3 wasp species: *Vespa affinis, Vespa tropica,* and *Provespa barthelemyi.*

Species (N = 30)	Body length (cm)			Wing length (cm)		
	Mean	±SD	Min-max	Mean	±SD	Min-max
V. affinis	2.20[a]	0.07	2.07 - 2.32	1.99[a]	0.09	1.81 - 2.17
V. tropica	2.61[b]	0.11	2.37 - 2.92	2.52[b]	0.10	2.26 - 2.67
P. barthelemyi	2.01[c]	0.08	1.85 - 2.21	1.64[c]	0.08	1.48 - 1.84

P < 0.05 *different letters in the same column indicate significant difference of means at the 95 % confidence level.

3.2 Morphology of sting

During morphological investigation of sting and venom apparatus in 3 wasp species under a stereo microscope and a scanning electron microscope (SEM), we found that there were slightly differences in wasp stings within the genus *Vespa*, whereas there were noticeable differences in the stings between *Vespa* and *Provespa* (Figure 2).

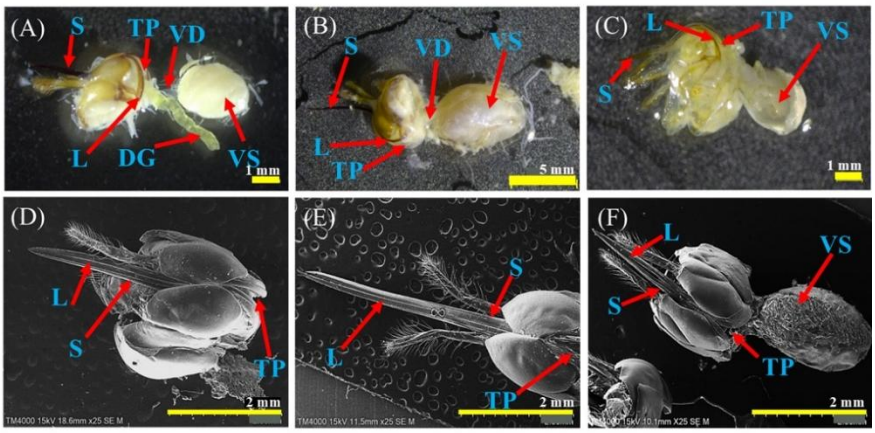

Figure 2 Sting apparatus of 3 wasp species. (A-C) *Vespa affinis, Vespa tropica* and *Provespa barthelemyi*, respectively. (D-F) SEM *Vespa affinis, Vespa tropica* and *Provespa barthelemyi*, respectively. (S= Stylet, TP= Triangular plate, L=Lancet, DG= Dufour's gland, VD = Venom duct, VS = Venom sac, VG = Venom gland).

The stylet morphology was investigated in the 3 wasp species, and it was found that *V. tropica* has the most curved stylet tip followed by *V. affinis* and very slightly curved observed in *P. barthelemyi* (Figure 3). The width and length of stylet were largest in *V. tropica* followed by *V. affinis* and *P. barthelemyi*, respectively (Table 2). Their small diameters and hollow structures can be resulted in sting flexibility [4]. ANOVA analysis showed significantly

different stylet sizes among the 3 species (P<0.05). The sizes of sting parts were correlated to body size of each wasp species.

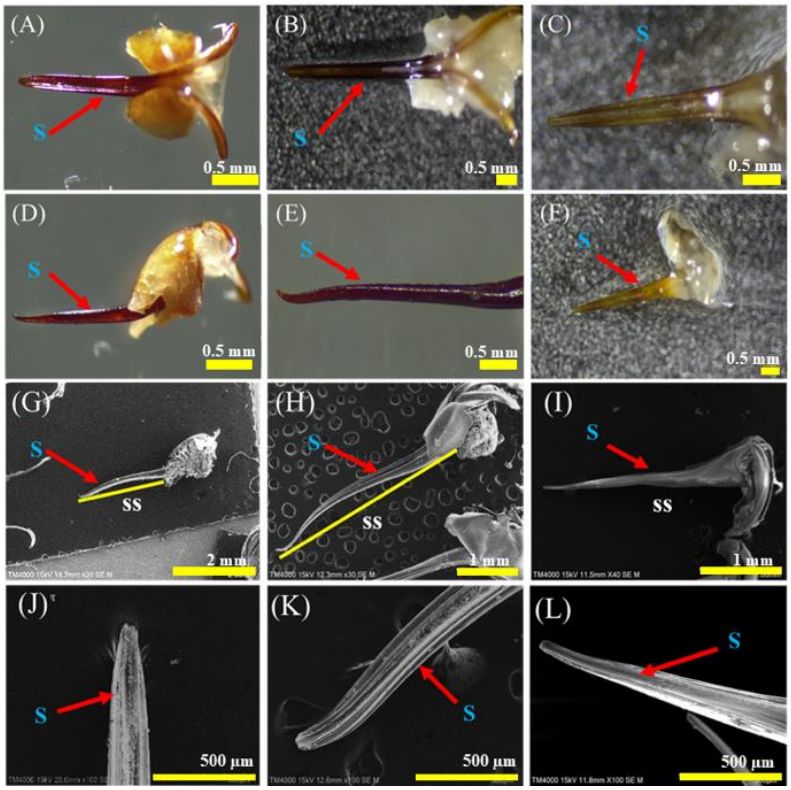

Figure 3 Comparisons of the stylet characteristics in 3 species of wasps: (A, D, G, J) *Vespa affinis* (B, E, H, K) *Vespa tropica* and (C, F, I, L) *Provespa barthelemyi*. (A-F) are images from stereoscopic microscope. (G-L) are images from scanning electron microscope (S = Stylet, SS = the curvature of sting shaft).

Table 2 Average width and length of the stylet in 3 wasp species: *Vespa affinis*, *Vespa tropica*, and *Provespa barthelemyi.*

Species (N = 30)	Stylet width (µm)			Stylet length (µm)		
	Mean	±SD	Min-Max	Mean	±SD	Min-Max
V. affinis	259.42[a]	21.55	215.67-306.33	3199.24[a]	183.56	2458.00-3444.33
V. tropica	320.60[b]	17.29	286.33-358.33	3634.77[b]	443.08	2702.33-4232.33
P. barthelemyi	233.18[c]	10.10	214.33-263.00	2272.74[c]	72.68	2149.00-2475.33

P < 0.05 *different letters in the same column indicate significant difference of means at the 95 % confidence level.

Barbs were found on the lancet of stings in the 3 wasp species (Figure 4). There were 7, 8, and 11 barbs on the lancets of *V. affinis*, *V. tropica*, and *P. barthelemyi*, respectively. The distance between each barb was determined to be significantly different among the 3 species (Table 3). Significant differences were found among sizes of left and right lancet. The largest size of lancet was *V. tropica* followed by *V. affinis* and *P. barthelemyi* (Table 4). The barbs of these wasp stings are small, and lancets assume a spiral shape to avoid being anchored by victim's tissue fibers [4].

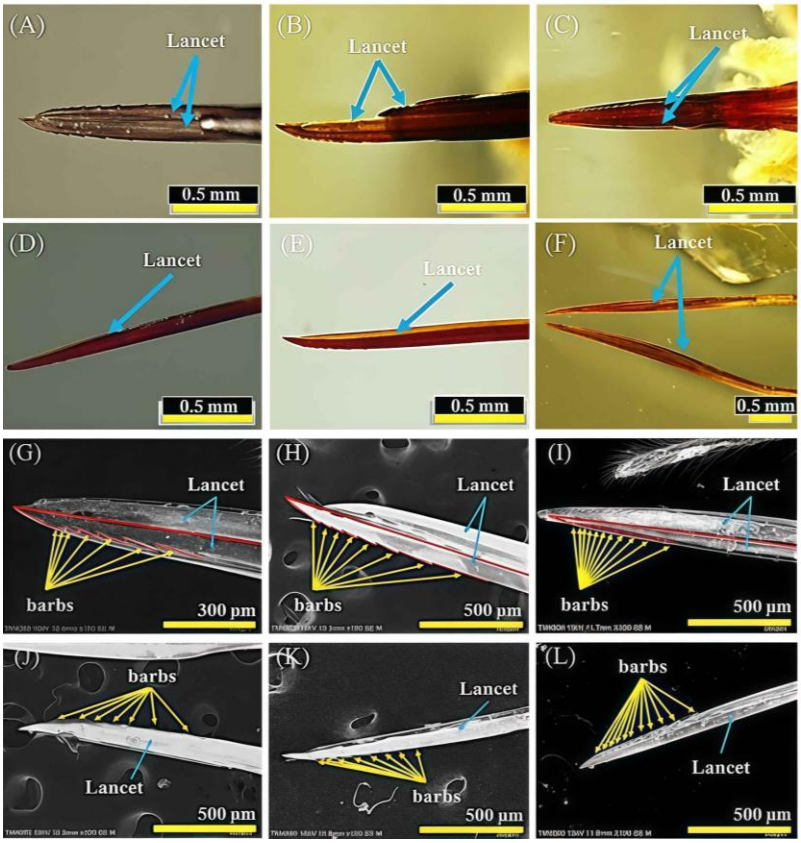

Figure 4 Comparisons of lancet characteristics in 3 wasp species: (A, D, G, J) *Vespa affinis* (B, E, H, K) *Vespa tropica* and (C, F, I, L) *Provespa barthelemyi*. (A-F) are images from a stereo microscope. (G-L) are images from a scanning electron microscope.

Table 3 Number of barbs and distance between each barb of 3 wasp species (A) *Vespa affinis* (B) *Vespa tropica* and (C) *Provespa barthelemyi*.

Samples	Mean (µm)			±SD			Min-max (µm)		
(N = 30)	A	B	C	A	B	C	A	B	C
Barbs 1	175.011[a]	206.922[b]	132.733[c]	9.774	7.053	13.316	159.67-212.67	187.67-223.33	112.00-158.00
Barbs 2	54.500[a]	59.800[b]	31.788[c]	7.339	5.239	3.109	35.00-66.00	48.33-70.00	23.67-35.67
Barbs 3	71.155[a]	78.044[b]	40.077[c]	8.835	4.648	2.728	58.67-98.00	65.33-85.33	33.33-46.00
Barbs 4	91.722[b]	91.055[b]	48.455[a]	7.131	5.043	4.510	74.67-102.67	77.33-96.33	42.67-57.67
Barbs 5	114.111[a]	100.377[b]	52.466[c]	8.606	17.757	5.059	96.00-135.33	78.00-132.00	46.67-65.67
Barbs 6	144.966[a]	127.055[b]	63.566[c]	6.594	15.894	6.722	134.33-163.33	103.67-161.33	38.67-70.00
Barbs 7	182.077[a]	165.566[b]	69.788[c]	12.791	25.735	3.932	162.00-203.00	131.33-205.67	62.67-77.33
Barbs 8	-	225.022[b]	76.333[c]	-	50.653	4.906	-	168.00-288.67	68.00-86.00
Barbs 9	-	-	82.577[c]	-	-	7.01	-	-	71.67-97.00
Barbs 10	-	-	93.53[c]	-	-	3.09	-	-	84.33-97.67
Barbs 11	-	-	112.622[c]	-	-	8.71	-	-	94.33-122.00

P < 0.05 *different letters in the same row indicate significant difference of means at the 95 % confidence level.

Table 4 Average width and length of lancet of 3 wasp species: *Vespa affinis*, *Vespa tropica*, and *Provespa barthelemyi.*

Samples (N = 30)		Lancet width (µm)			Lancet length (µm)		
		Mean	±SD	Min-max	Mean	±SD	Min-max
Left	*V. affinis*	140.14[a]	11.79	112.67-156.00	2059.65[a]	85.14	1901.00-2240.67
	V. tropica	154.70[b]	11.38	124.00-185.67	3426.73[b]	174.67	3093.67-3751.67
	P. barthelemyi	84.41[c]	5.29	74.00-100.33	2133.00[c]	52.82	1966.00-2213.33
Right	*V. affinis*	140.566[a]	6.646	124.33 -151.33	2061.11[a]	96.757	1933.67-2253.00
	V. tropica	147.611[b]	4.635	135.00 - 154.67	3421.90[b]	170.468	3093.67-3713.33
	P. barthelemyi	84.044[c]	4.461	73.00 - 95.00	2093.41[a]	61.956	1894.00-2231.67

P < 0.05 *different letters in the same column indicate significant difference of means at the 95 % confidence level.

The results of this study are consistent with the reports by [1, 5, 6]. Comparative sting morphology of wasps in *Crabro scutellatus* von Scheven, 1781 and *Oxybelus quatuordecimnotatus* Jurine, 1807 (Crabronidae) indicated possible relationship between the shape and similarity of the sting of the two species [1]. However, the sting differed significantly in the curvature and surface of the sting. Moreover, the shape of the hook on the lancet were significantly different.

The stinging mechanisms of bee and paper wasp species have been previously investigated and it was found that each species had a stylet and two lancets, which were connected by a sliding joint mechanism. The lancet of the bee had a hook protruding from the stylet, while this hook is absent in wasps. Moreover, the sting of the paper wasp could be easily pulled out from the surface of the prey, while the bee had difficulty pulling out the sting because of the hook protruding from the outside [4].

3.3 Morphology of venom gland, venom duct, and venom sac

The venom gland of 3 species had 2 long white cloudy lines separated from each other at the end of the venom sac (Figure 5). Because the samples of venom gland were small, thin, and fragile, the samples were not adequate for measuring the size. Moreover, the end of the venom gland was twisted together, so the actual length could not be known. The venom gland plays an important role in producing venom for the stinging mechanism, which works together with several parts; initially the venom gland produces venom which is then stored in the venom sac. When the stinging mechanism starts, the muscles around the venom sac contract to send the venom stored in the venom sac along the venom duct to the sting and inject it into the tissue of the prey [6, 7], [9–11], [14-23].

The average sizes of venom duct (Figure 5, Table 5) and venom sac (Figure 5, Table 6) suggested that the length and width of venom duct and venom sac were related to the body size of wasps. *V. tropica* had the largest venom apparatus followed by *V. affinis* and *P. barthelemyi*. ANOVA analysis indicated significant differences in sizes of venom duct and venom sac among different wasp species ($P<0.05$) (Table 5, 6).

The venom duct is an important part of the stinging mechanism of the wasp. Venom produced by venom gland is sent for storage at the venom sac which has thin muscle bundles arranged transversely around it. When stinging, the muscles contract and the venom is sent along the venom duct to the sting to inject into the prey. This process takes up to 1 minute to deliver the venom to the prey tissue [24]. Moreover, we found different patterns of muscle arrangement on the venom sac (Figure 6). Muscle bundles lining the venom gland of *V. affinis* were more curved than *V. tropica*, which had muscle bundles in a straight line. *P. barthelemyi* showed a straight muscle bundle line with slightly or no curvature. This is consistent with [25], which reported on differences of the venom gland of 25 social wasp species in the subfamily Polistinae by measuring the morphological characteristics such as the length of the venom gland, the length of the venom sac, the width of the venom sac, the length of the main venom duct, the length of the Dufour's gland, and the length of the sting. It was found that the characteristic venom gland was a long fiber-like duct separated from the venom sac. However, the morphology of the venom gland was of similar pattern, while the length of the venom gland fiber of each species differed statistically.

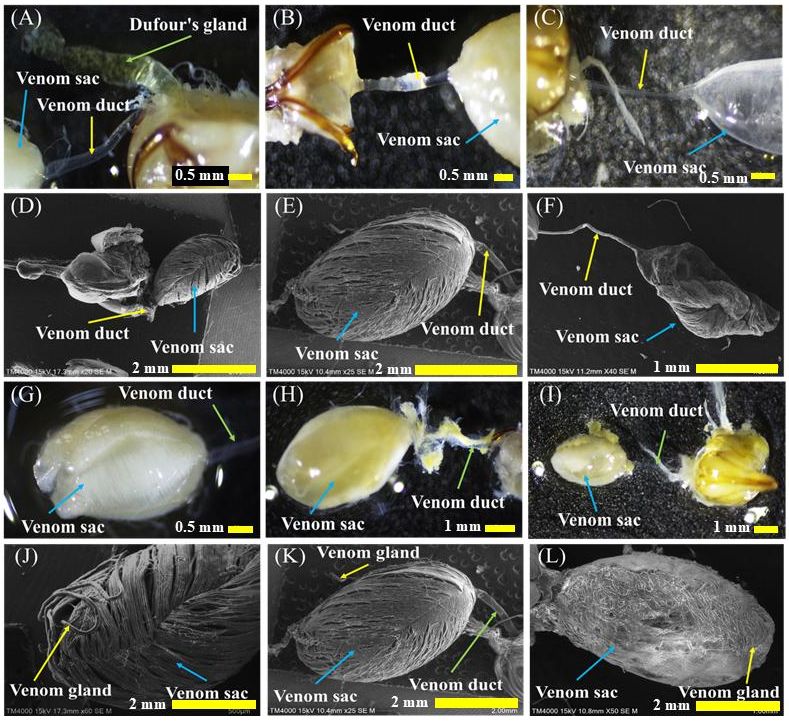

Figure 5 (A-F) Comparisons of venom duct characteristics of 3 wasp species: (A, D) *Vespa affinis* (B, E) *Vespa tropica* and (C, F) *Provespa barthelemyi*. (A-C) are images from a stereo microscope. (D-F) are images from scanning electron microscope. (G-L) Comparisons of venom sac characteristics of 3 wasp species: (G, J) *Vespa affinis* (H, K) *Vespa tropica* and (I, L) *Provespa barthelemyi*. (G-I) are images from a stereo microscope. (J-L) are images from scanning electron microscope.

Table 5 Width and length of venom duct of 3 wasp species: *Vespa affinis*, *Vespa tropica*, and *Provespa barthelemyi.*

Species (N=30)	Venom duct width (μm)			Venom duct length (μm)		
	Mean	±SD	Min-max	Mean	±SD	Min-max
V. affinis	188.756[ab]	38.603	147.00-266.33	3609.90[a]	275.753	3128.67-4029.33
V. tropica	206.433[b]	33.829	147.00-246.00	4225.222[b]	389.140	3555.00-4761.00
P. barthelemyi	170.022[ac]	24.302	81.67-209.33	3099.10[c]	380.984	2376.67-3590.33

P < 0.05 *different letters in the same column indicate significant difference of means at the 95 % confidence level.

Table 6 Width and length of venom sac of 3 wasp species: *Vespa affinis*, *Vespa tropica*, and *Provespa barthelemyi.*

Species (N=30)	Venom sac width (μm)			Venom sac length (μm)		
	Mean	±SD	Min-max	Mean	±SD	Min-max
V. affinis	2611.322[a]	279.272	1959.33-3020.67	4381.688[a]	528.898	3381.33-6069.33
V. tropica	3226.500[b]	187.917	2895.33-3557.33	5486.500[b]	456.665	4753.67-6321.33
P. barthelemyi	1786.844[c]	101.142	1602.67-1958.33	3563.533[c]	624.022	2600.33-4392.33

P < 0.05 *different letters in the same column indicate significant difference of means at the 95 % confidence level.

Cluster analysis based on the morphological characteristics of number of barbs on lancet, sizes of lancet, sizes of stylet, sizes of venom sac, and sizes of venom duct are shown in Figure 6. The wasp samples were resolved into two groups. Group 1 consisted of *V. tropica* and *V. affinis*, and group 2 was *P. barthelemyi*. The cluster analysis results were matched with phylogenetic relationships of Vespidae based on molecular and phenotypic data sets stating that the wasps in the genus *Vespa* and *Provespa* were closely related within the evolutionary line [1, 5, 6].

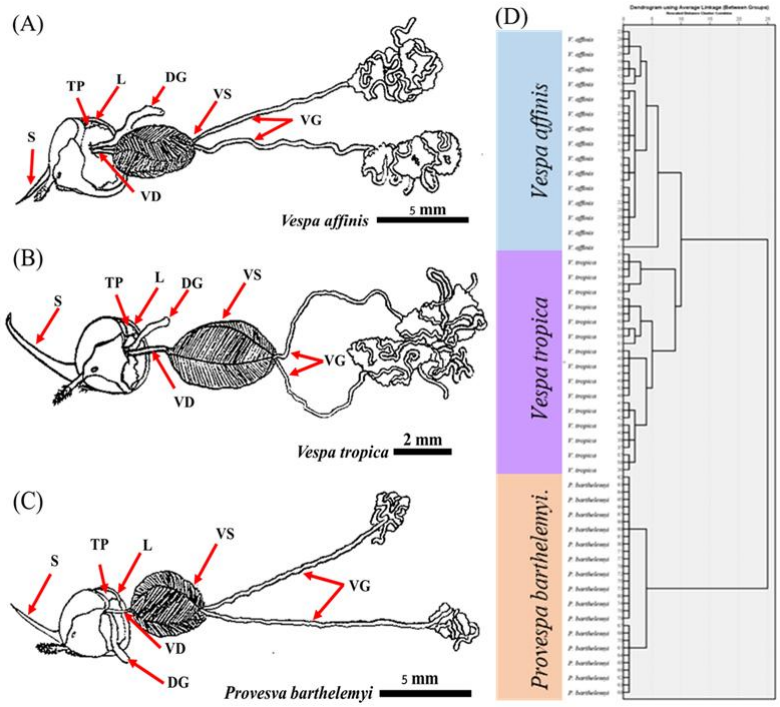

Figure 6 The illustration shows comparison of venom gland characteristics of 3 wasp species (A) *Vespa affinis* (B) *Vespa tropica* and (C) *Provespa barthelemyi* (S= Stylet, TP= Triangular plate, L=Lancet, DG= Dufour's gland, VD = Venom duct, VS = Venom sac, VG = Venom gland). (D) Diagram of the evolutionary relationships of 3 wasp species: *Vespa affinis*, *Vespa tropica*, and *Provespa barthelemyi*.

4. Conclusions

In conclusion, our findings show that morphology of sting and venom apparatus of the 3 wasp species were similar in the same genus (*Vespa*). In contrast, they were clearly different between the genus *Vespa* and *Provespa*. Sting and venom apparatus characters including sizes of sting parts, number of barbs, sizes of venom duct and venom sac in 3 species were significantly different. Sting morphology is unique in each wasp species which can be used in various aspects including species identification, evolution and sting mechanisms. More studies on the functional morphology of the sting apparatus are required to provide possible function use in the future.

5. Ethical approval

The research project had been reviewed and approved by the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU). The approval number is IACUC-MSU-009/2020.

6. Acknowledgements

This research project was financially supported by Mahasarakham University. We thank to the Faculty of Science, Mahasarakham University for an international conference participation fund. We also thank Dr. Adrian R. Plant for English assistance on the manuscript.

7. Conflicts of interest

The authors declare no conflict of interest.

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