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ARTICLE

Morphometric analysis of cavernicolous adult *Idiophlebotomus asperulus* Quate and Fairchild, 1961 female sand flies in Southern Thailand

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Abstract
Sand flies are potential carriers of various diseases that are transmittable to humans and animals. In this study, United States Centers for Disease Control light traps were set up in four tourist caves in the Thai provinces of Surat Thani, Nakhon Si Thammarat, Satun, and Chumphon to capture *Idiophlebotomus asperulus* sand flies. Over a period of three months, April to June, in 2017, a total of 181 female *Idiophlebotomus asperulus* sand flies were captured during nightly operations. The sand flies were dissected into 23 external and internal parts to identify their morphological characteristics. Statistical analysis was then conducted on these morphological characteristics, involving both univariate analysis (one-way analysis of variance and the Kruskal–Wallis test) and multivariate analysis (canonical discriminant analysis). Levene's, the Kolmogorov–Smirnov, and Box's *M* tests were used for the preliminary statistical screening of the data. The test results revealed significant morphological differences in the sand flies from the four provinces with regard to their antenna segments 5, palpal segments 3, pharynxes, hindlegs, femurs, and spermathecae. These morphological differences in the southern Thai *Idiophlebotomus asperulus* sand fly population suggest the possibility that at least three morphologically different populations are found in this region.

Introduction

Seventy species of sand flies (Diptera, Psychodidae), which can transmit diseases to humans and animals, have been identified (Pothirat *et al.* 2014). Among the diseases carried by sand flies is leishmaniasis (*e.g.*, *Leishmania tropica*, *L. major*, and *L. aethiopica*), a zoonotic disease with the capacity for cross-transmission between humans and animals (Lemma *et al.* 2009; Eshetu and Bassa 2016). Although leishmaniasis infections caused by *L. martiniquensis* have been reported to

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occur in both the northern and southern regions of Thailand, the infections appear to occur more often in the southern region (Thisyakorn *et al.* 1999; Sukmee *et al.* 2008; Chusri *et al.* 2014; Osatakul *et al.* 2014). This may be attributed to this region's abundance of ecotourism locations, such as parks, wildlife sanctuaries, and caves, that provide ideal sand fly breeding grounds. Sand flies, which live in moist environments, can draw blood from vertebrates, including reptiles and mammals, that live in the surrounding area (Apiwathnasorn *et al.* 1993; World Tourism Organisation 2012). According to the results from a survey of sand fly habitats, it was reported that the various species of sand flies prefer caves over other habitats (Apiwathnasorn *et al.* 1989). Taking this into account, this investigation focuses on the cave area in Southern Thailand.

The sand fly genus *Idiophlebotomus* can be distinguished by the insects' parameres, which are single, bifurcated, and covered with scales. However, *Idiophlebotomus asperulus* Quate and Fairchild, 1961, *Idiophlebotomus pholetor* Quate and Fairchild, 1961, *Idiophlebotomus erebicolus* Quate, 1965, *Idiophlebotomus longiforceps* Wang *et al.*, 1974, *Idiophlebotomus frondifer* Lewis and Lane, 1976, *Idiophlebotomus wellingsae* Lewis and Dyce, 1983, *Idiophlebotomus dispar* Lewis, 1987, and *Idiophlebotomus boucheti* Léger and Pesson, 1994 exhibit a single paramere on the male reproductive organ (Ilango 2010; Léger *et al.* 2014). These species may have similar external characteristics but are incapable of breeding even with sibling species. This circumstance gives rise to taxonomy problems. Although an approach for the general identification of sand fly gender is currently lacking, in the context of medical entomology, more emphasis should be placed on efforts to successfully identify the females of the species. Recently, Buatong *et al.* (2022) identified female sand flies located in cavernicolous localities in four provinces in Southern Thailand based on morphology. Morphological variations often stem from evolutionary processes associated with random events, particularly those affecting populations, including the founder effect or genetic drift (Vignon *et al.* 2023).

Sand fly classification calls for expertise and experience in morphology (Nzelu *et al.* 2015). For the most part, sand fly classification entails the use of a microscope for the scrutiny of the insect's head and genitalia (Galati 2010; Maneeroth *et al.* 2020). The close similarity of some sand fly species or groups can complicate the classification process (Kumar *et al.* 2012). The standard procedure for sand fly classification, presented in 1979, requires additional preclassification studies regarding the characteristics to be considered during classification (Lewis 1979). An approach that sidesteps these issues is the morphometric analysis of each species (Campbell *et al.* 1970; Gebre-Michael and Medhin 1997; Godoy *et al.* 2014).

Previous studies have identified a variety of sand fly species inhabiting the caves of Southern Thailand (Maneeroth *et al.* 2020). The emphasis of our research is on the morphometric analysis of the physical characteristics of the adult female sand fly *Idiophlebotomus asperulus* found in the limestone caves of Southern Thailand.

Materials and methods

Sample collection

Sand fly population samples for this investigation were sourced from limestone caves located in Southern Thailand (Fig. 1) during the rainy season, April–June, in 2017 (Table 1). Five Centers for Disease Control (United States of America) light traps (John W. Hock, Co., Florida, United States of America) were installed inside each cave. The light traps were positioned 1.0–1.5 m from the ground and remained operational from dawn to dusk – specifically, from 06:00 to 18:00 local time. The procedures used to collect and prepare specimens for this study are certified by the Institutional Animal Care and Use Committee, Prince of Songkla University, Hat Yai, Songkhla, Thailand (Reference no. 2561-10-021).

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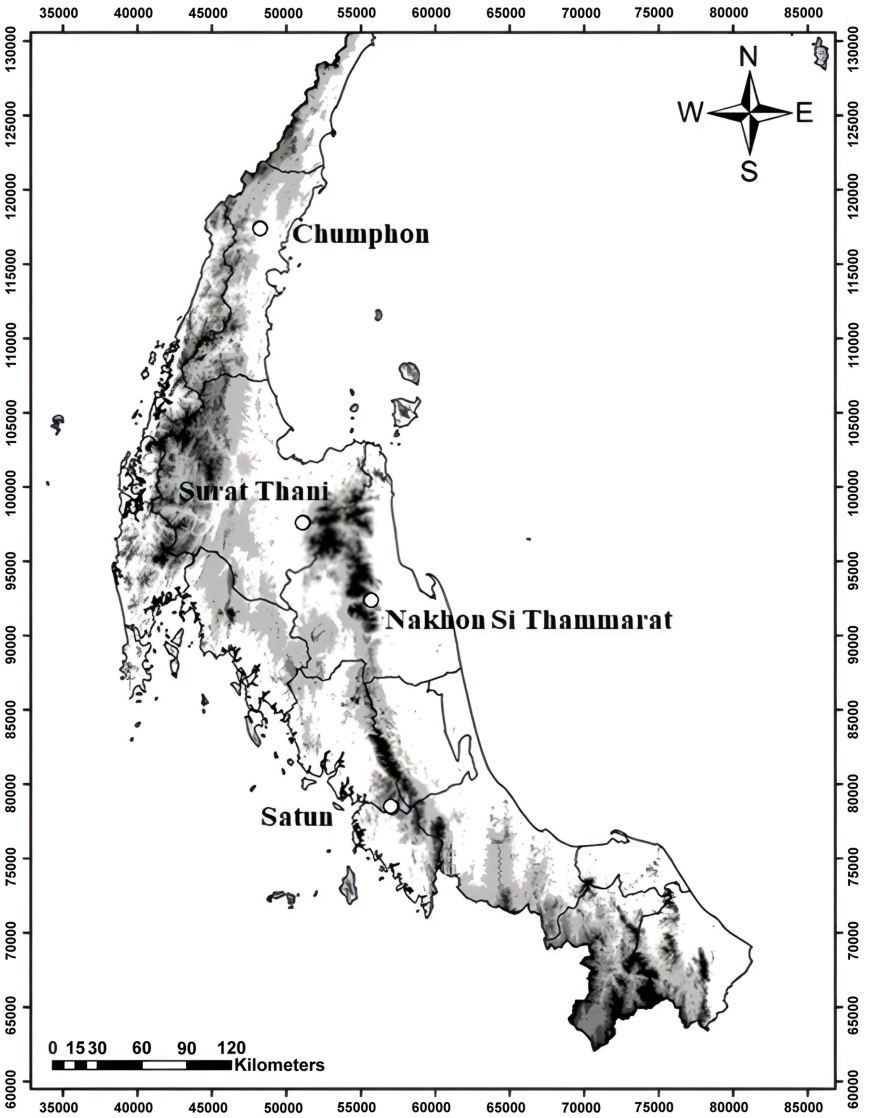

Figure 1. Map of Southern Thailand showing the localities where the specimens were collected in Chumphon, Surat Thani, Nakhon Si Thammarat, and Satun provinces.

Sample preparation

A total of 181 female sand flies were collected for the present study. The specimens were euthanised with 95% ethyl acetate and then were transferred to a conical tube containing 70% ethanol. The female sand fly specimens were dissected to separate the head, thorax, wings, and hind legs (Lewis and Lane 1976; Maneeroth *et al.* 2020). The organs were then treated with Hoyer's medium (Anderson 1954), placed on a glass slide, covered with a coverslip, and placed in a 45 °C oven for seven days. Species identification involved examination of the abdomen, where the genitalia are located, and head, using taxonomic keys (Abonnenc 1972; Lewis and Lane 1976; Léger and Pesson 1994; Léger *et al.* 2014; Loyer *et al.* 2016). Sand flies identified as *Idiophlebotomus asperulus* based on their morphological characteristics were then measured using

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Table 1. The geographic locations and details of the sand fly collection sites, which were caves in Southern Thailand

Province	Geographic coordinates	Temperature (°C)	Humidity (%)	Collection date
Chumphon	10° 37′ 22.5″ N, 99° 06′ 48.1″ E	29	80	27 April 2017
Surat Thani	8° 49′ 47.6″ N, 99° 22′ 44.0″ E	28.6	76.5	30 June 2017
Nakhon Si Thammarat	8° 21′ 40.7″ N, 99° 47′ 06.4″ E	25.9	89.5	25 May 2017
Satun	7° 05′ 39.3″ N, 99° 54′ 58.1″ E	26.8	89	15 June 2017

an Olympus CX31 RBSF microscope (Olympus Corp., Tokyo, Japan) connected to an Olympus DP21 camera (Olympus Corp.).

Morphometric analysis

Morphological characteristics of *Idiophlebotomus asperulus* were measured, based on 23 aspects: (1) ascoid on flagellomere 2 (length of longest ascoid on flagellomere 2), (2) ascoid on flagellomere 3 (length of longest ascoid on flagellomere 3), (3) pharynx length (length of the pharynx), (4) pharynx width (width of the posterior part of the pharynx), (5) pharyngeal armature (length of the teethed area at the posterior end of the pharynx), (6) epipharynx (length from the anterior margin of the clypus to the tip of the labral teeth), (7) femur on hind leg (longest measurement of hind leg femur), (8) tibia on hind leg (longest measurement of hind leg tibia), (9) palpal segment 1 (length of palpal segment 1), (10) palpal segment 2 (length of palpal segment 2), (11) palpal segment 3 (length of palpal segment 3), (12) palpal segment 4 (length of palpal segment 4), (13) palpal segment 5 (length of palpal segment 5), (14) antennal segment 3 (length of antennal segment 3), (15) antennal segment 4 (length of antennal segment 4), (16) antennal segment 5 (length of antennal segment 5), (17) cibarium length (length of cibarium from the chitinous arch at the posterior junction with pharynx, to the anterior junction with the clypus), (18) cibarium width (width of the cibarium measured at the widest part), (19) spermathecae length (length of spermathecae), (20) spermathecae width (width of spermathecae measured at the widest part), (21) head of spermathecae length (length of the teethed area at the posterior end of the pharynx), (22) tarsal segment 1 on hind leg (longest measurement of hind leg tarsal segment 1), and (23) tarsus on hind leg (longest measurement of hind leg tarsus). The morphological results derived from these measurements are displayed in Figure 2. The digital image of the abdomen part of female and male sand fly are shown in Figure 3.

Statistical analyses

Univariate analysis. The missing data for the morphological characters were imputed through the mean substitution of each individual group, with a total sample size of 147. Following an assessment of the normality distribution, a descriptive statistical analysis was carried out to compute the mean and standard deviation. A one-way analysis of variance and a *post hoc* test using Tukey's pairwise test, with a significance level of $P < 0.05$, were used to compare the mean of the morphological characters among specimens according to the four provinces. Before the tests, homogeneity of variances was verified by way of Levene's test and normality distribution was verified by way of the Kolmogorov–Smirnov test, with a significance level of $P > 0.05$.

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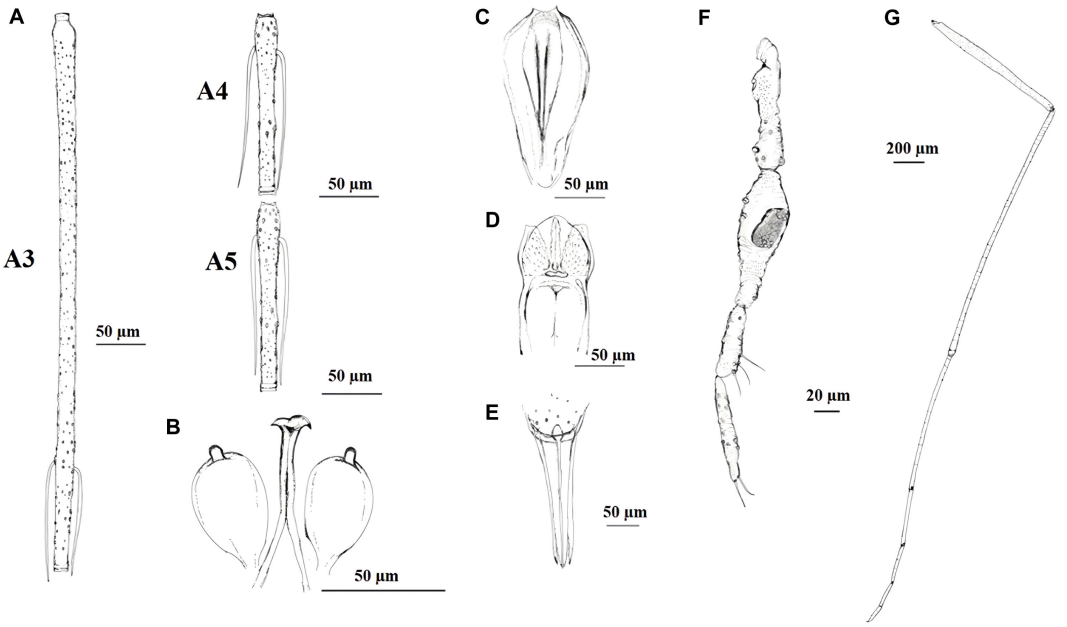

Figure 2. *Idiophlebotomus asperulus* female: **A**, antennal segments A3 to A5; **B**, spermathecae; **C**, pharynx; **D**, cibarium; **E**, epipharynx; **F**, palpal segment 1 to 5; and **G**, hindleg.

A nonparametric analysis with the Kruskal–Wallis test was performed for the morphological characters that did not meet the assumption of normality ($P < 0.05$).

Multivariate analysis. *Z*-score transformation was conducted for all the raw data to ensure the feasibility of the morphological characters for multivariate analysis. Box's M test was used to assess the morphological characteristics for the multivariate assumption of covariance matrix equality. Following its verification, the data were scrutinised using canonical discriminant analysis. The canonical discriminant score was applied to generate a graph, and the canonical discriminant function was used to group the findings. The significance of the discriminant function was calculated by means of Wilk's lambda and the Chi-square statistic. The results from the canonical discriminant analysis were confirmed *via* leave-one-out cross validation. A confusion matrix was then used to gradually regroup the data by excluding one specimen over time. The remaining grouped data was then used to identify that specimen.

Results

Four populations, amounting to a total of 147 individual *Id. asperulus*, were used in the present study, and the multivariate analysis of the 23 morphological characters is tabulated in Table 2. Nine of the 23 morphological characters met the one-way analysis of variance assumptions, and nine morphological characteristics with statistically significant differences among the *Id. asperulus* populations were identified. They were the ascoid on flagellomere 3 ($F = 12.1$, $P < 0.05$), pharynx width ($F = 43.93$, $P < 0.05$), the epipharynx ($F = 28.71$, $P < 0.05$), palpal segment 4 ($F = 9.523$, $P < 0.05$), palpal segment 5 ($F = 12.49$, $P < 0.05$), antennal segment 3 ($F = 23.01$, $P < 0.05$), antennal segment 4 ($F = 32.74$, $P < 0.05$), spermathecae length ($F = 45.92$, $P < 0.05$), and spermathecae width ($F = 28.69$, $P < 0.05$).

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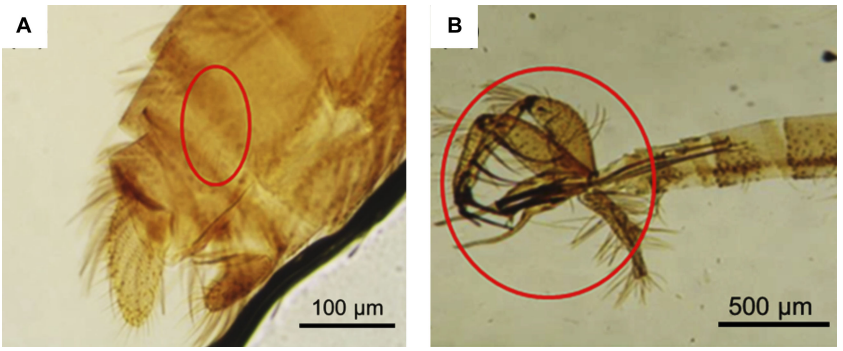

Figure 3. The abdomen of sand flies: **A**, spermathecae in a female sand fly and **B**, styles of a male sand fly.

The Tukey's pairwise *post hoc* analysis indicated two major subgroups – the Surat Thani and Chumphon subgroup and the Nakhon Si Thammarat and Satun subgroup – for seven of the morphological characteristics – specifically, for pharynx length, femur on hind leg, tibia on hind leg, antennal segment 3, antennal segment 4, antennal segment 5, and tarsal segment 1 on hind leg. The Surat Thani and Chumphon subgroup exhibited significantly different morphologies for the ascoid on both flagellomeres 2 and 3, pharynx width, pharyngeal armature, epipharynx, femur on hind leg, cibarium width, spermathecae width, and head of spermathecae length. More specifically, the average ascoids on flagellomeres 2 and 3 were considerably shorter in the Surat Thani subgroup than in any of the other three subgroups. Also, the measurements for pharynx width, pharynx armature, femur on hind leg, and cibarium width derived from specimens in the Chumphon subgroup differed markedly from those derived from specimens from the other provinces. The average spermathecae length (Sperm.L) was significantly longer in the Nakhon Si Thammarat subgroup ($M = 60.83$, standard deviation = 6.58) than in populations from the other provinces (Surat Thani subgroup: $M = 48.52$, standard deviation = 5.53, $P < 0.05$; Satun subgroup: $M = 46.11$, standard deviation = 6.82, $P < 0.05$; and Chumphon subgroup: $M = 47.15$, standard deviation = 5.90, $P < 0.05$). Because no discernible differences were observed in the palpal segment 1 and cibarium length of the samples collected from the four provinces, these characteristics were excluded from the concluding stage of the morphometric multivariate analysis.

The canonical discriminant analysis multivariate analysis results of the *Id. asperulus* sample populations gave rise to three different classification functions. The main characteristics in the structure matrix were pharynx widths, femur on hind leg, antennal segment 5, and spermathecae length (Table 3). Functions 1 and 2 were used for the canonical discriminant analysis, with Function 1 accounting for 58.4% of the variance (chi-square = 432.647, multivariate analysis of variance test yields Wilk's Lambda = 0.041, $df = 693$, $P < 0.001$), and Function 2 accounting for 31.24% of the variance. The scattered plot of the discriminant scores for Functions 1 and 2, with a convex hull, are shown in Figure 4. The morphological characters pharynx width, femur on hind leg, and spermathecae length presented high scores in Function 1, whereas the significantly negative score for spermathecae length in Function 2 indicates a greater length than in specimens from the other provinces. Spermathecae length was therefore construed to be a significant indicator of variation. By looking into Function 3, three significantly positive score characters were identified (epipharynx, palpal segment 5, and spermathecae length); however, these characters do not differ among sand fly populations captured in Satun and Surat Thani provinces. The canonical discriminant analysis analysis revealed that the morphological characters of sand fly populations from Satun and Surat Thani provinces are closely related and the sand fly populations from Chumphon and Nakhon Si Thammarat provinces are distinct from the other provinces. The

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Table 2. A comparison of the means and standard deviations of the morphometric characteristics of female *Idiophlebotomus asperulus*. The significant *P*-values were based on the results of the analysis of variance (*) and Kruskal–Wallis (§) test

	Locations				
Characteristic	Surat Thani ($n = 18$)	Nakhon Si Thammarat ($n = 37$)	Satun ($n = 20$)	Chumphon ($n = 72$)	*P*-values
fII	106.3 ± 6.4^{a}	118.7 ± 6.1^{b}	117.5 ± 7.1^{b}	115.7 ± 6.9^{b}	< 0.001§
fIII	107.3 ± 5.4^{a}	118.5 ± 6.5^{b}	116.7 ± 6.4^{b}	115.1 ± 6.9^{b}	< 0.001*
Ph.L	182.4 ± 7.3^{a}	191.4 ± 9.7^{b}	193.3 ± 11.4^{b}	175.2 ± 11.6^{a}	< 0.001§
Ph.W	101.5 ± 7.1^{a}	110.6 ± 8.4^{b}	107.3 ± 8.0^{a,b}	91.5 ± 9.7^{c}	< 0.001*
Ph.A	24.3 ± 2.2^{a}	25.6 ± 4.8^{a}	30.5 ± 5.7^{b}	19.3 ± 3.2^{c}	< 0.001§
Epi	189.7 ± 9.8^{a}	188.8 ± 7.7^{a}	192.9 ± 12.8^{a}	175.7 ± 9.4^{b}	< 0.001*
Fem	935.2 ± 59.5^{a}	979.8 ± 37.8^{b}	986.9 ± 41.6^{b}	901.8 ± 33.2^{c}	< 0.001§
Tib	1740.2 ± 147.4^{a}	1849.6 ± 107.5^{b}	1901.5 ± 129.5^{b}	1665.7 ± 128.9^{a}	< 0.001§
Palp 1	33.5 ± 3.3^{a}	32.4 ± 3.5^{a}	34.7 ± 4.1^{a}	32.1 ± 3.5^{a}	0.027
Palp 2	51.8 ± 3.7^{a}	55.0 ± 3.3^{a,b}	56.6 ± 6.5^{b}	53.0 ± 4.6^{a}	0.004§
Palp 3	108.8 ± 6.7^{a}	108.8 ± 6.2^{a}	115.1 ± 7.2^{b}	107.7 ± 6.0^{a}	0.001§
Palp 4	57.7 ± 3.7^{a}	58.7 ± 4.6^{a}	61.7 ± 3.4^{b}	56.4 ± 4.0^{a,c}	< 0.001*
Palp 5	82.9 ± 7.3^{a}	87.9 ± 5.9^{b}	86.4 ± 5.2^{a,b}	81.0 ± 5.8^{a}	< 0.001*
A3	521.5 ± 24.7^{a}	553.7 ± 34.3^{b}	566.6 ± 31.4^{b}	517.9 ± 25.7^{a}	< 0.001*
A4	148.2 ± 7.0^{a}	157.3 ± 7.9^{b}	162.8 ± 7.4^{b}	147.3 ± 7.0^{a}	< 0.001*
A5	152.9 ± 7.2^{a}	162.2 ± 8.2^{b}	166.8 ± 8.6^{b}	150.2 ± 7.1^{a}	< 0.001§
Ci.L	159.0 ± 7.9^{a}	159.3 ± 8.9^{a}	163.3 ± 11.2^{a}	158.3 ± 7.0^{a}	0.364
Ci.W	70.84 ± 5.53^{a}	68.4 ± 7.4^{a}	73.8 ± 4.6^{a,b}	64.7 ± 4.5^{c}	< 0.001§
Sperm.L	48.5 ± 5.5^{a}	60.8 ± 6.5^{b}	46.1 ± 6.8^{a}	47.2 ± 5.9^{a}	< 0.001*
Sperm.W	36.8 ± 4.3^{a}	34.9 ± 3.5^{a}	34.4 ± 3.3^{a}	29.3 ± 4.2^{b}	< 0.001*
Sperm.Hea	6.86 ± 0.91^{a}	5.78 ± 0.91^{b}	6.29 ± 0.85^{a,b}	5.65 ± 1.01^{b}	< 0.001§

(*Continued*)

Table 2. (*Continued*)

Characteristic	Locations				
	Surat Thani ($n = 18$)	Nakhon Si Thammarat ($n = 37$)	Satun ($n = 20$)	Chumphon ($n = 72$)	*P*-values
Tar.1	930.3 ± 50.2[a]	1024.5 ± 60.9[b]	1048.5 ± 118.5[b]	950.9 ± 118.9[a]	$< 0.001^{\S}$
Tar	1868.8 ± 95.6[a]	2012.5 ± 78.4[b]	2005.5 ± 189.5[a,b]	1836.5 ± 212.7[a]	$< 0.001^{\S}$

Note: The various superscript alphabets are the subgroups of the Tukey's pairwise test.
fII, ascoid on flagellomere 2; fIII, ascoid on flagellomere 3; Ph.L, pharynx length; Ph.W, pharynx width; Ph.A, pharyngeal armature; Epi, epipharynx; Fem, femur on hind leg; Tib, tibia on hind leg; Palp 1, palpal segment 1; Palp 2, palpal segment 2; Palp 3, palpal segment length of palpal segment 3; Palp 4, palpal segment 4; Palp 5, palpal segment 5; A3, antennal segment 3; A4, antennal segment 4; A5, antennal segment 5; Ci.L, cibarium length; Ci.W, cibarium width; Sperm.L, spermathecae length; Sperm.W, spermathecae width; Sperm.Hea, head of spermathecae length; Tar.1, tarsal segment 1 on hind leg; and Tar, tarsus on hind leg.

Table 3. The structure matrix of the morphometric features of female *Idiophlebotomus asperulus* chosen using canonical discriminant analysis. The samples were collected from tourist caves in four provinces, namely, Surat Thani, Nakhon Si Thammarat, Satun, and Chumphon, in Southern Thailand

	Function		
Character	1	2	3
fII	–0.0869	–0.3197	–0.2306
fIII	–0.1740	–0.4402	–0.2833
Ph.L	0.1435	–0.1826	–0.0073
Ph.W	0.5393	–0.1062	0.0887
Ph.A	0.4850	0.3003	–0.6510
Epi	0.0600	0.5284	0.8803
Fem	0.5822	–0.2926	0.1462
Tib	0.2685	0.2862	–0.3680
Palp 2	–0.0560	–0.1838	–0.2493
Palp 3	–0.5589	0.3571	–0.2439
Palp 4	0.0423	0.0887	–0.3222
Palp 5	–0.0901	–0.2326	0.3093
A3	–0.0324	–0.1549	0.1654
A4	–0.0079	0.1000	–0.5322
A5	0.5171	0.0967	–0.0126
Ci.W	0.0431	0.4701	0.0465
Sperm.L	0.7505	–1.0121	0.3789
Sperm.W	0.1342	0.5989	0.3044
Sperm.Hea	–0.2391	0.3053	0.0191
Tar.1	–0.0269	0.0862	–0.2830
Tar	0.0494	–0.2403	0.0982

fII, ascoid on flagellomere 2; fIII, ascoid on flagellomere 3; Ph.L, pharynx length; Ph.W, pharynx width; Ph.A, pharyngeal armature; Epi, epipharynx; Fem, femur on hind leg; Tib, tibia on hind leg; Palp 1, palpal segment 1; Palp 2, palpal segment 2; Palp 3, palpal segment length of palpal segment 3; Palp 4, palpal segment 4; Palp 5, palpal segment 5; A3, antennal segment 3; A4, antennal segment 4; A5, antennal segment 5; Ci.L, cibarium length; Ci.W, cibarium width; Sperm.L, spermathecae length; Sperm.W, spermathecae width; Sperm.Hea, head of spermathecae length; Tar.1, tarsal segment 1 on hind leg; and Tar, tarsus on hind leg.

group classification equation categorised 79% of the sand fly populations accurately. According to the scatter plot of discriminant scores, with regard to the two functions, the populations of *Id. asperulus* in two locations are close to each other. The confusion matrix analysis (Table 4), based on the canonical discriminant analysis results, revealed that 97.3% of the originally grouped cases and 88.4% of the cross-validated grouped cases are accurately classified.

Discussion

The findings derived through the present study reveal variations in terms of internal and external morphological features among sand fly populations in Southern Thailand. These morphological variations likely stem from the environmental conditions in which the insects exist (Thongsripong *et al.* 2013). It is debatable whether caves with more resources are endowed with greater biological complexity than are caves with fewer resources. Local climate and ecology also

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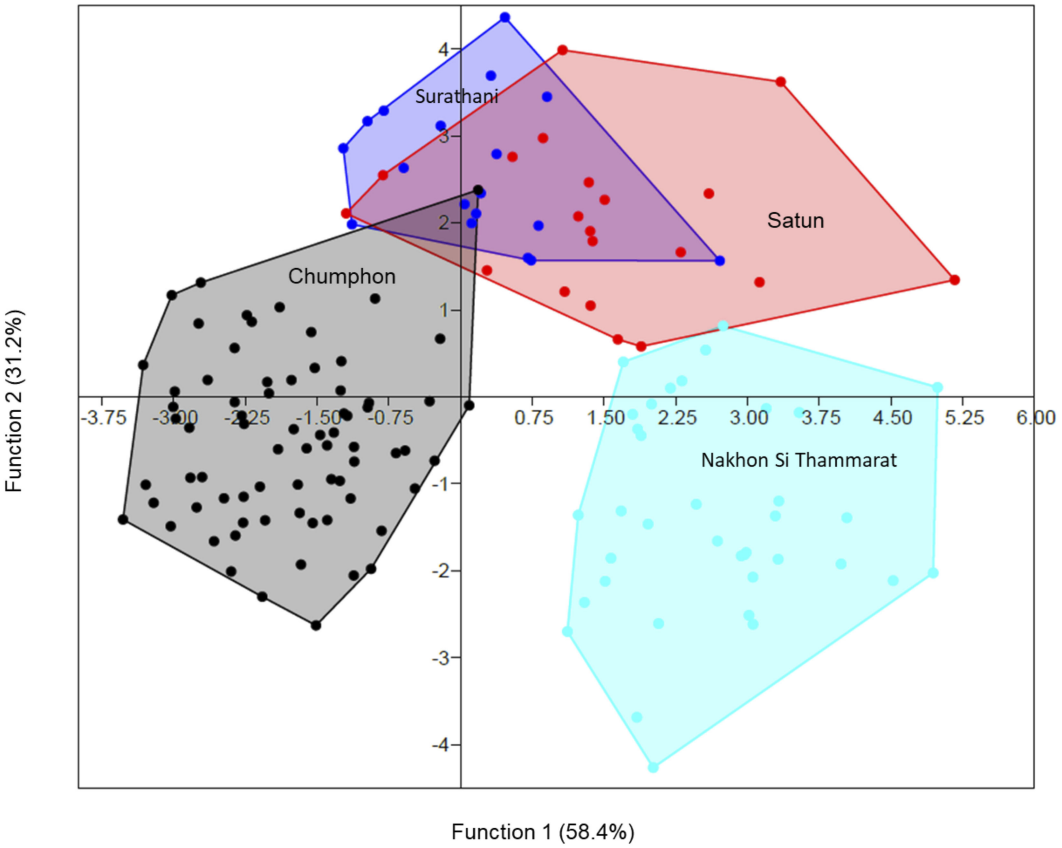

Figure 4. The canonical discriminant analysis results of the measured characteristics of *Idiophlebotomus asperulus* collected from Chumphon (black), Nakhon Si Thammarat (teal), Satun (red), and Surat Thani (blue).

may influence morphological change, which may occur in the form of a genetic drift or directional selection. Some studies have suggested that larval development is limited to an area within the maximum flight distance of adult sand flies (Claborn 2010) and that the poor flying ability of sand flies restricts their maximum flying distance to no more than 100 m (Killick-Kendrick 1999). Based on this, sand fly populations may be restricted to each local area, and genetic differences may occur not only among different species but also among populations of the same species in different areas (Belen *et al.* 2004).

Differences in sand fly morphological features can be attributed to the geographical locations of the caves, which influence habitat environmental factors, such as temperature, physical barriers, precipitation, latitude, and altitude, and the abundance and distribution of vertebrate hosts. The *Idiophlebotomus* sand fly species feeds on the blood of mammals, reptiles, and amphibians living in caves (Srisuton *et al.* 2019; Toontong *et al.* 2022). The highest temperature of any of the present study's sand fly sampling sites was recorded as 29 °C in Chumphon, and the lowest was recorded as 25.9 °C in Nakhon Si Thammarat. According to Rafatbakhsh-Iran *et al.* (2016), the density of sand fly populations is influenced by relative humidity. The relative humidity of the sampling sites ranges between 75 and 90%. The findings of Rafatbakhsh-Iran *et al.*'s (2016) study, which was conducted in central Morocco, showed that higher environmental temperatures promote increases in sand fly populations, which may explain why the more female sand flies were collected from Chumphon than from the other three sampling locations. The results from our investigation

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Table 4. The leave-one-out cross-validation of all the samples used in the canonical discriminant analysis of the morphological characteristic measurements of *Idiophlebotomus asperulus* collected from tourist caves in four provinces, namely, Surat Thani, Satun, Chumphon, and Nakhon Si Thammarat, in Southern Thailand

			Predicted group membership				
		Location	Surat Thani	Nakhon Si Thammarat	Satun	Chumphon	Total
Original	Count (%)	Surat Thani	17 (94.4)	0 (0)	1 (5.6)	0 (0)	18 (100)
		Nakhon Si Thammarat	0 (0)	36 (97.3)	1 (2.7)	0 (0)	37(100)
		Satun	0 (0)	1 (5)	19 (95)	0 (0)	20 (100)
		Chumphon	1 (1.4)	0 (0)	0 (0)	71 (98.6)	72 (100)
Cross-validated	Count (%)	Surat Thani	14 (77.8)	1 (5.6)	2 (11)	1 (5.6)	18 (100)
		Nakhon Si Thammarat	0 (0)	34 (91.9)	3 (8.1)	0 (0)	37 (100)
		Satun	1 (5)	4 (20)	15 (75)	0 (0)	20 (100)
		Chumphon	2 (2.8)	1 (1.4)	2 (2.8)	67 (93)	72 (100)

show that density of sand fly populations is affected by temperature in the four caves. This suggests the sand fly has less capacity to adapt to cave environments with lower temperatures.

The present study's results indicate a certain degree of variability in the sand fly pharynx. This morphological feature presents a possible approach for sand fly classification. However, the two morphological characteristics with low variations in the populations of sand flies investigated are the posterior segment of the pharynx (pharynx width, pharynx length) and the width of the cibarium. Based on appearance, it was difficult to differentiate the pharynx, antennal segment 3, and ascoid among *P. argentipes*, *P. annandalei,* and *P. glacus*, which share similar habitats and external morphology (Ilango 2010). This could be due to the fact that the samples for this study were collected from near the cave's entrances, where the environment is similar to that above ground. Cave entrances typically experience daily fluctuations in sunlight and temperature, which support the growth of green plants (Lee *et al.* 2012). The use of these areas by animals to eat, sleep, or nest means cave entrances are appropriate food-source locations for sand flies.

Conventional morphometrics is a cost-effective method for the characterisation and classification of organisms (Bhat *et al.* 2022). In the present study, a statistical morphometric analysis was performed to identify variations in the interior and exterior morphology of *Id. asperulus* sand fly populations that live in the caves of Southern Thailand that are visited by tourists. Sand fly morphology can be used to identify comparable species or to detect changes in sand fly populations. Additionally, it can be used to monitor sand fly populations for improved management and prevention of leishmaniasis.

Conclusion

The present study identified 23 morphological characteristics of sand fly, 14 of which had high variation. These highly varied characteristics were the ascoid on antennal segment 4, the ascoid on antennal segment 5, the epipharynx, the femur on hindleg, the tibia on hindleg, palpal segment 2, palpal segment 3, palpal segment 4, palpal segment 5, antennal segment 3, cibarium length, spermathecae length, tarsal segment 1 on hindleg, and tarsus on hindleg. None of the species exhibited low-variation characteristics. Canonical discriminant analysis indicated that, for the most part, morphology differences among the four sand fly populations in each study area occurs within eight characteristics: the longest ascoid on antennal segment 5; palpal segment 2, 3, and 5; the lengths of antennal segments 3 and 4; cibarium; and the width of the spermathecae. Low variations were detected for two sand fly morphology characteristics, the pharynx and cibarium. Therefore, morphological comparisons facilitate the detection of comparable sand fly species and changes within populations when monitoring sand fly populations to reduce leishmaniasis incidence.

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