

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

### DIVERSITY OF STINGLESS BEES, *Tetragonula* sp. (HYMENOPTERA: APIDAE) IN TAMIL NADU, INDIA

#### Hindistan Tamil Nadu'da İğnesiz Arılar, *Tetragonula* Sp. (Hymenoptera: Apidae) Çeşitliliği

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#### ABSTRACT

The present study was conducted to find the diversity of stingless bees (*Tetragonula* sp.) in Coimbatore, Madurai, Dindigul, and Tiruchirappalli districts in Tamil Nadu, India. Adult worker bees were collected from feral stingless bee colonies from 16 locations, four from each district. The collected stingless bees were preserved in 70% alcohol (ethanol) and examined under a Leica stereo-zoom microscope. Seventeen morphometric key characteristics were measured to know the diversity of stingless bees. The results revealed that two species of stingless bees, *Tetragonula iridipennis* and *T. laeviceps* were prevalent in the four selected districts of Tamil Nadu. Four vital morphometric characteristics of the worker bees namely the head width, the forewing length, the hind tibial length, and the whole body length helped to distinguish the two *Tetragonula* species. Among all the locations, the bees collected from L1- Insectary, TNAU (Coimbatore) had much higher values of the above four morphometric characters ( $1.67\text{mm} \pm 0.04$ ,  $3.69\text{mm} \pm 0.02$ ,  $1.53\text{mm} \pm 0.01$  and  $3.98\text{mm} \pm 0.14$  respectively) and were identified to be *T. laeviceps* based on these key morphometric characters. The bees from the rest of the 15 locations which had the morphometric values 1.59 to 1.61 mm, 3.20 to 3.41mm, 1.34 to 1.43mm and 3.51 to 3.65mm respectively were categorized as *T. iridipennis*. As a result, *T. iridipennis* was the most commonly prevalent stingless bee species in Coimbatore, Madurai, Dindigul and Trichy districts while *T. laeviceps* was found only to be present in Coimbatore.

Key words: *Tetragonula* sp., Distribution, Morphometrics, Species categorization, PCA

#### ÖZ

Bu çalışma, Hindistan'ın Tamil Nadu kentindeki Coimbatore, Madurai, Dindigul ve Tiruchirappalli bölgelerindeki iğnesiz arıların (*Tetragonula* sp.) çeşitliliğini bulmak amacıyla gerçekleştirilmiştir. Yetişkin işçi arılar, her bölgeden dörder adet olmak üzere 16 lokasyondaki yabani iğnesiz arı kolonilerinden toplanmıştır. Toplanan iğnesiz arılar %70'lik alkol (etanol) içinde muhafaza edildi ve Leica stereo zoom mikroskobu altında incelenmiştir. İğnesiz arıların çeşitliliğini bilmek için on yedi morfometrik temel özellik ölçülmüştür. Sonuçlar, iki iğnesiz arı türünün, *Tetragonula iridipennis* ve *T. laeviceps*'in Tamil Nadu'nun seçilen dört bölgesinde yaygın olduğunu ortaya çıkarmaktadır. İşçi arıların dört önemli morfometrik karakteri (baş genişliği, ön kanat uzunluğu, arka bacak uzunluğu ve

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tüm vücut uzunluğu), iki *Tetragonula* sp'.yi ayırt etmeye yardımcı olmuştur. Tüm lokasyonlar arasında L1- Insectary, TNAU, Coimbatore'den toplanan arılar yukarıdaki dört morfometrik karakterin çok daha yüksek değerlerine sahip olduğu belirlenmiş olup (sırasıyla 1,67 mm ± 0,04, 3,69 mm ± 0,02, 1,53 mm ± 0,01 ve 3,98 mm ± 0,14) ve bu önemli morfometrik karakterlere dayanarak *T. laeviceps* olduğu kaydedilmiştir. Morfometrik değerleri sırasıyla 1,59 ila 1,61 mm, 3,20 ila 3,41 mm, 1,34 ila 1,43 mm ve 3,51 ila 3,65 mm olan 15 lokasyonun geri kalanındaki arılar ise *T. iridipennis* olarak kategorize edilmiştir.

**Anahtar kelimeler:** *Tetragonula* sp., Dağılım, Tamil Nadu, Morfometri, Tür sınıflandırması, PCA.

### GENİŞLETİLMİŞ ÖZET

**Çalışmanın amacı:** Bu çalışma, Hindistan'ın Tamil Nadu kentindeki Coimbatore, Madurai, Dindigul ve Tiruchirappalli bölgelerindeki iğnesiz arıların (*Tetragonula* sp.) çeşitliliğini bulmak amacıyla gerçekleştirilmiştir. Bu çalışmada iğnesiz arı örneklerinin tüm işçi sınıfı için standart morfometrik karakterleri incelenmiştir. Sonuçlar standart taksonomik anahtarlar ve önceki literatürle karşılaştırılmıştır.

**Gereç ve yöntemler:** İğnesiz arıların işçi sınıfı, 2023 yılında her bölge için dört konum olmak üzere Hindistan'ın Tamil Nadu, Coimbatore, Madurai, Tiruchirappalli ve Dindigul olmak üzere dört bölgesinde tanımlanan yabancı kolonilerden toplanıp her konum (L)1- L16 olarak kodlanmıştır. On altı farklı lokasyonun her birinden on iğnesiz arı örneği toplanmıştır. Morfometrik çalışmalar için örnekler %70'lik alkolde muhafaza edilerek numunelerden antenler, kanatlar, bacaklar, kafa, mesosoma ve metasoma disekte edilmiştir. Leica M205C yakınlaştırmalı stereomikroskop yardımıyla, anten uzunluğu, başın uzunluğu ve genişliği, hamuli sayısı, arka tibial uzunluk, ön, orta ve arka bacak uzunluğu, vücut uzunluğu, mesosoma ve metasoma uzunluk ve genişliklerine ilişkin ölçümler (mm) alınmıştır. Genel tanımlamalar, geçerli literatür ve taksonomik anahtarlar, örnekleri ayırt etmek için kullanılmıştır.

**Bulgular:** L1'den (Insectary, TNAU, Coimbatore) toplanan işçi iğnesiz arı örneklerinin *T. laeviceps* olduğu, geri kalan örneklerin ise *T. iridipennis* olduğu önceki literatürdeki morfometrik değerlerle karşılaştırılarak tespit edilmiştir. PCA'da fark edilen ayırt edici karakterler kafa genişliği ile ön kanadın ve arka bacağın uzunluğu olmuştur. Veri matrisi, 17 morfometrik karakter ve 16 konum içeren 272 ölçümden oluşmuştur. Ana bileşenler, L2'den L16'ya kadar olan konumlar arasında herhangi bir değişiklik göstermedi ancak tek başına L1'deki numuneler diğer konumlardan sapmıştır. Daha fazla doğrulama için moleküler çalışmalar yapılabilir.

**Sonuç:** Hindistan Tamil Nadu'nun farklı bölgelerinde iğnesiz arıların (*Tetragonula* sp.) çeşitliliği ve dağılımı üzerine yapılan araştırmada, Coimbatore, Madurai, Dindigul ve Tiruchirappalli bölgelerinde en yaygın iğnesiz arı türünün *T. iridipennis* olduğu, *T. laeviceps*'in ise sadece Coimbatore'da mevcut olduğu bulunduğu belirlenmiştir. Türleri ayırt etmek için iğnesiz arıların işçi sınıfının morfometrik analizi kullanıldı. Bununla birlikte, daha ileri moleküler karakterizasyon tür kimliğinin doğrulanmasında önemli bir rol oynayabilir.

### INTRODUCTION

In the year 1999, Heard reported that Stingless bees (Hymenoptera: Apidae) eusocial insects belong to five different genera *Melipona*, *Trigona*, *Meliponula*, *Dectylurina*, and *Lestrimelitta* and have significant pollination function. However stingless bees belonging to many other genera have been reported subsequently including *Paratrigona*, *Nogueirapis*, *Oxytrigona*, *Scaptotrigona*, *Lestrimelitta*, *Friesella*, *Tetragonisca*, etc. (Rahman et al., 2015). Bees belong to these genera collect propolis, which they use in construction and sealing of their hives. Propolis is a combination of pollen, plant resins, and beeswax. Meliponini, usually form large colonies, attaining 10,000 to more than 100,000 individuals in number. (Michener, 2007). *Trigona iridipennis* was the type species of the subgenus *Tetragonula*, that contains 12 species which was the most prevalent (Rasmussen, 2013; Viraktamath and Roy 2022). *Tetragonula iridipennis*, a stingless bee species, was frequently observed in India (Raakhee and Devanesan, 2000; Swaminathan, 2000; Danaraddi and Viraktamath, 2009). Seven other *Tetragonula* species from India were also reported, in addition to *T. iridipennis* (Rasmussen, 2013). In the southern region of India, some research on the geographic distribution and morphometric analysis of *T. iridipennis* was conducted (Devanesan et al., 2013). One of the most used methods for classifying

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organisms is morphometric taxonomy. Several previous attempts have been made to categorize stingless bees based on their body size, number of hamuli, length of forewing and hind wing, and cephalic characteristics (Vijaykumar and Jeyaraj, 2013). In southern regions of Tamil Nadu, there was no more report other than *T. iridipennis*. Hence, the study on diversity of *Tetragonula* sp. in these regions has to be crucial. The goal of the present research was based on their morphometric characteristics to explore and describe *Tetragonula* sp. and their distribution in Tamil Nadu.

### MATERIALS AND METHODS

#### Experimental Location

Worker caste of the stingless bees were collected from the identified feral colonies (Fig. 1) from four districts (Coimbatore, Madurai, Tiruchirappalli, and Dindigul) of Tamil Nadu, India with four locations for each district in 2023, and each location was named and denoted by the codes Location (L)1 to L16.

**Table 1.** GPS (Global Positioning System) data for sampling locations

District	Sampling location	Geographical position	
		Latitude	Longitude
Coimbatore	L1-Insectary, TNAU	11.016925°	76.929063°
	L2-Botanical garden	11.015313°	76.932123°
	L3-Thondamuthur	10.992892°	76.843539°
	L4-P. N. Pudhur	11.009005°	76.929158°
Madurai	L5-Narasingam	9.975217°	78.208031°
	L6-Vowal thottam	9.964481°	78.201628°
	L7-Melur	9.970307°	78.204831°
	L8-Insectary, AC&RI.	9.961482°	78.202803°
Dindigul	L9-Oddanchatram	10.475583°	77.733461°
	L10-Nilakottai	10.234328°	77.896929°
	L11-Athoor	10.320013°	77.902908°
	L12-Thadiyankudisai	10.296814°	77.708788°
Tiruchirappalli	L13-Navalurkottapattu	10.752106°	78.602454°
	L14-Woraiyur	10.828859°	78.691849°
	L15-Manapparai	10.609503°	78.423773°
	L16-Muthukulam	10.755965°	78.601856°

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a. Colony inside the wooden box



b. Colony inside the iron rod



c. Colony inside the tree trunk



d. Colony inside the mud wall



e. Colony inside the mud pot



f. Colony inside the bamboo cavity

**Figure 1.** Identified feral colonies of stingless bees, *Tetragonula* sp.

### Preservation and Morphometric analysis

Ten stingless bee specimens were collected from each of the sixteen different locations. The specimens were preserved in 70% alcohol for morphometric studies. The antennae, wings, legs, head, mesosoma, and metasoma were dissected from the specimen. The measurements (mm) on antennal length, length and width of head, compound eyes, forewing, number of hamuli, hind tibial length, length of fore, middle and hind leg, body length, length and width of mesosoma and metasoma were taken with the help of Leica M205C zoom stereomicroscope. In total, seventeen morphometric key characteristics (Table 2) were measured to find out the distribution of stingless bees in different districts of Tamil Nadu.

The stingless bee specimens' morphometric measurements on the head include antennal length, length and width of the head and compound eyes. The antennal length was measured from the scape to the last flagellomere. The head length from the head's base to the mandibles' summit straight along the median lines and head width from the most significant distance across the eyes was measured. Measuring the compound eyes' length from the head's apex to the base and width at the most significant width point of compound eyes was done. Morphometric measurements on wings of stingless bee specimens include the length and width of the forewing and the number of hamuli on the hind wing. The length of the forewing from the wing base to the apex and the width at the greatest width of the forewing were measured. The number of hamuli present on the hind wing was recorded.

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Morphometric measurements on legs include the length of the hind tibia, hind leg, middle leg, and foreleg. The hind tibial length was measured vertically, and the three pairs of legs were measured segment-wise. The mesosoma's length was recorded along the straight line from the cervix to the propodeum, the first segment of the metasoma, and the maximum width of the mesosoma was also measured. The metasoma's length was recorded

from the propodeum to the apical point, and the maximum width of the metasoma was measured. The total body length was measured from the tip of the head to the abdominal end. The overall descriptions were used to distinguish the specimen with the help of prevailing pieces of literature and taxonomic keys (Sakagami, 1978,; Rasmussen, 2013,; Rahman *et al.*, 2015).

**Table 2.** List of morphometric characters examined

<b>Morphometric key characters</b>	<b>Abbreviation</b>
Head length	HL
Head width	HW
Antennal length	AL
Compound eye length	CL
Compound eye width	CW
Forewing length	FWL
Forewing width	FWW
Number of hamuli	NH
Hind tibial length	HTL
Hind leg length	HLL
Middle leg length	MLL
Foreleg length	FLL
Mesosomal length	MSL
Mesosomal width	MSW
Metasomal length	MTSL
Metasomal width	MTSW
Body length	BL

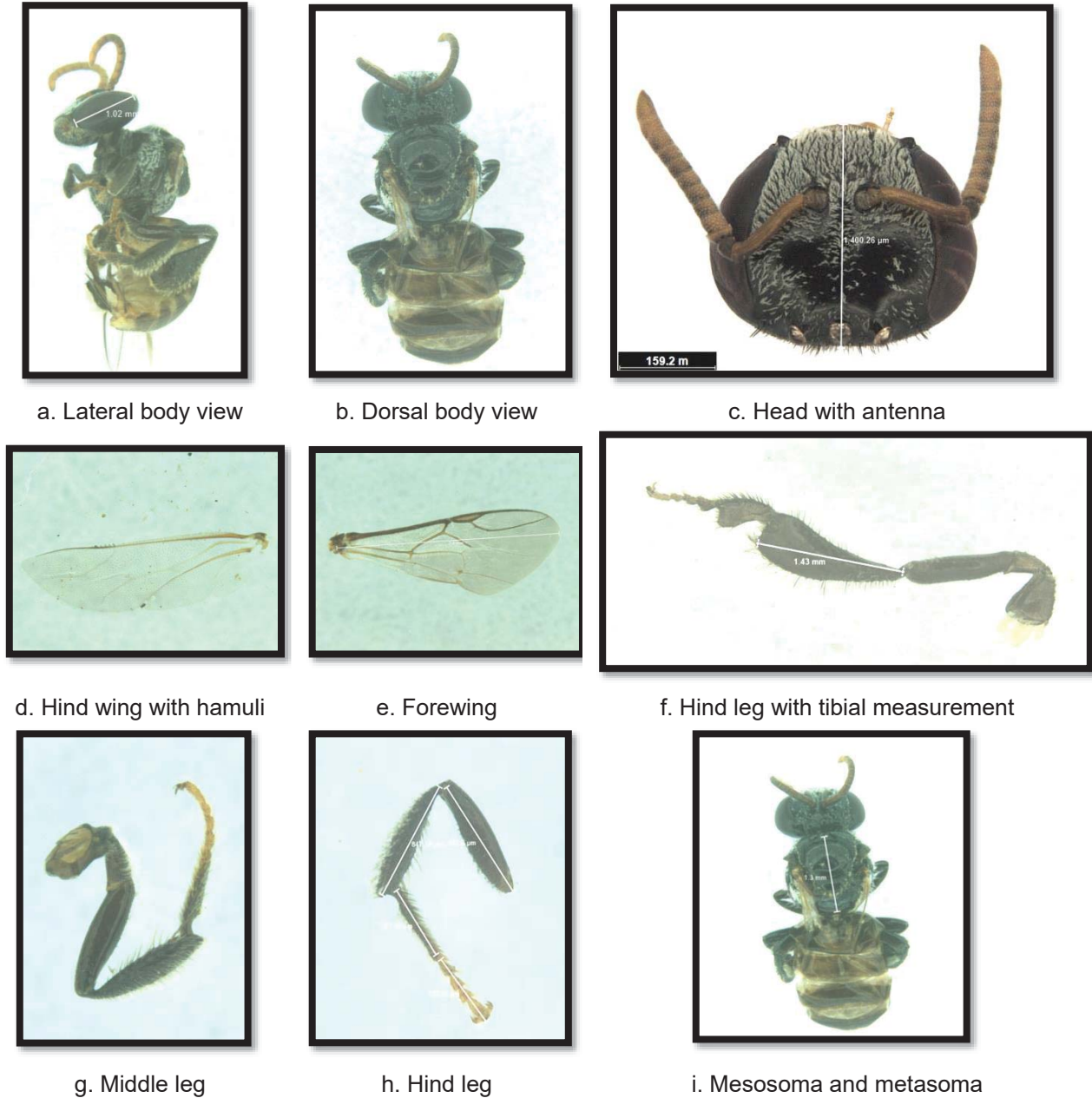
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### Statistical Analysis

Mean and Standard deviation were calculated for the replicated samples. Principal Component Analysis (PCA) was done to study the variation in the morphometric values of *Tetragonula* sp. based on the covariance matrix. PCA was worked out by using Python programming software.

### RESULTS

This study classified key characters (Fig.2) into head morphometry, wing morphometry, leg morphometry, mesosomal, and metasomal morphometry. These measurements were compared with standard taxonomic keys and the prevailing literature to identify the species variation among the collected specimens.



**Figure 2.** Magnified images of stingless bee species under Leica stereo zoom microscope

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### Head morphometry (Table 3)

Among the 16 locations studied, the specimens collected from L1 (Insectary, TNAU, Coimbatore), measured with the most significant length of the head ( $1.46\text{mm} \pm 0.05$ ) followed by L13 (Navalurkottapattu, Tiruchirappalli) ( $1.38\text{mm} \pm 0.06$ ). In all other specimens, the head length ranged from 1.20 to 1.34 mm. The greatest width of the head was also recorded from L1 ( $1.67\text{mm} \pm 0.04$ ) followed by L2 ( $1.62\text{mm} \pm 0.09$ ), both belonging to the Coimbatore district. All other specimens ranged from

1.59 to 1.61mm of head width. The maximum antennal length was observed from L1 ( $1.89\text{mm} \pm 0.02$ ), and others ranged from 1.80 to 1.86mm. The length of the compound eye was the greatest ( $1.06\text{mm} \pm 0.05$ ) of the specimens collected from L1 and L5 (Narasingam, Madurai), and others ranged from 0.98 to 1.05mm in length of their compound eye. The width of the compound eye was also greater ( $0.48\text{mm} \pm 0.03$ ) in L1, followed by L13 ( $0.44\text{mm} \pm 0.03$ ), and for other specimens, ranged from 0.35 to 0.43mm.

**Table 3.** Head morphometric measurements of examined stingless bee specimens

Location/key characters	HL $\pm$ SD	HW $\pm$ SD	AL $\pm$ SD	CL $\pm$ SD	CW $\pm$ SD
L1	$1.46 \pm 0.05$	$1.67 \pm 0.04$	$1.89 \pm 0.02$	$1.06 \pm 0.05$	$0.48 \pm 0.03$
L2	$1.32 \pm 0.03$	$1.62 \pm 0.09$	$1.81 \pm 0.49$	$1.04 \pm 0.60$	$0.38 \pm 0.22$
L3	$1.29 \pm 0.05$	$1.60 \pm 0.01$	$1.83 \pm 0.05$	$0.98 \pm 0.02$	$0.37 \pm 0.02$
L4	$1.30 \pm 0.02$	$1.60 \pm 0.04$	$1.82 \pm 0.06$	$1.02 \pm 0.04$	$0.35 \pm 0.01$
L5	$1.32 \pm 0.03$	$1.61 \pm 0.05$	$1.86 \pm 0.01$	$1.06 \pm 0.05$	$0.35 \pm 0.03$
L6	$1.30 \pm 0.02$	$1.60 \pm 0.07$	$1.81 \pm 0.02$	$1.04 \pm 0.03$	$0.41 \pm 0.01$
L7	$1.29 \pm 0.03$	$1.59 \pm 0.04$	$1.86 \pm 0.01$	$1.04 \pm 0.02$	$0.39 \pm 0.02$
L8	$1.32 \pm 0.02$	$1.60 \pm 0.05$	$1.84 \pm 0.01$	$1.05 \pm 0.04$	$0.35 \pm 0.03$
L9	$1.20 \pm 0.04$	$1.59 \pm 0.01$	$1.81 \pm 0.02$	$1.02 \pm 0.04$	$0.43 \pm 0.01$
L10	$1.25 \pm 0.05$	$1.57 \pm 0.03$	$1.86 \pm 0.07$	$1.05 \pm 0.02$	$0.38 \pm 0.02$
L11	$1.20 \pm 0.01$	$1.60 \pm 0.01$	$1.80 \pm 0.04$	$1.02 \pm 0.01$	$0.39 \pm 0.02$
L12	$1.29 \pm 0.01$	$1.59 \pm 0.03$	$1.81 \pm 0.03$	$0.99 \pm 0.03$	$0.41 \pm 0.01$
L13	$1.38 \pm 0.06$	$1.60 \pm 0.04$	$1.82 \pm 0.02$	$0.98 \pm 0.02$	$0.44 \pm 0.03$
L14	$1.30 \pm 0.06$	$1.59 \pm 0.03$	$1.80 \pm 0.07$	$1.02 \pm 0.01$	$0.42 \pm 0.01$
L15	$1.32 \pm 0.01$	$1.60 \pm 0.02$	$1.86 \pm 0.01$	$1.04 \pm 0.31$	$0.39 \pm 0.02$
L16	$1.34 \pm 0.02$	$1.57 \pm 0.06$	$1.82 \pm 0.07$	$0.98 \pm 0.02$	$0.41 \pm 0.01$

**Note:** Values are the mean of three replications for each location. SD: Standard Deviation. The mean values were measured in millimeters (mm).

### Wing morphometry (Table 4)

Maximum length of forewing among all the examined specimens was recorded from L1 ( $3.69\text{mm} \pm 0.02$ ), followed by L3 ( $3.41 \pm 0.11$ ), L12 ( $3.41\text{mm} \pm 0.10$ ), and L14 ( $3.41\text{mm} \pm 0.08$ ), belonged to Coimbatore, Dindigul and Tiruchirappalli districts respectively. Others measured from 3.20 to 3.40mm in their length

of forewings. The greatest width of forewing was observed from L1 ( $1.67\text{mm} \pm 0.07$ ) followed by L5 ( $1.38\text{mm} \pm 0.01$ ) and L7 ( $1.38\text{mm} \pm 0.02$ ) which belonged to the locations of Narasingam (Madurai) and Melur (Madurai) respectively. The rest of the specimens measured from 1.27 to 1.35mm of the forewing width. The number of hamuli on their hind wing was 5 for all the examined specimens.

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**Table 4.** Wing morphometric measurements of examined stingless bee specimens

Location/key characters	FWL ± SD	FWW ± SD	NH ± SD
L1	3.69 ± 0.02	1.67 ± 0.07	5 ± 0.00
L2	3.40 ± 0.15	1.31 ± 0.01	5 ± 0.00
L3	3.41 ± 0.11	1.32 ± 0.01	5 ± 0.00
L4	3.35 ± 0.03	1.32 ± 0.03	5 ± 0.00
L5	3.29 ± 0.15	1.38 ± 0.01	5 ± 0.00
L6	3.32 ± 0.04	1.32 ± 0.02	5 ± 0.00
L7	3.20 ± 0.04	1.38 ± 0.02	5 ± 0.00
L8	3.35 ± 0.02	1.35 ± 0.03	5 ± 0.00
L9	3.40 ± 0.01	1.27 ± 0.04	5 ± 0.00
L10	3.36 ± 0.01	1.32 ± 0.03	5 ± 0.00
L11	3.32 ± 0.02	1.28 ± 0.01	5 ± 0.00
L12	3.41 ± 0.10	1.30 ± 0.03	5 ± 0.00
L13	3.35 ± 0.03	1.29 ± 0.05	5 ± 0.00
L14	3.41 ± 0.08	1.31 ± 0.06	5 ± 0.00
L15	3.38 ± 0.15	1.32 ± 0.05	5 ± 0.00
L16	3.35 ± 0.14	1.35 ± 0.04	5 ± 0.00

Note: Values are the mean of three replications for each location. SD: Standard Deviation. The mean values were measured in millimeters (mm).

### Leg morphometry (Table 5)

The greatest hind tibial length was recorded on the specimens collected from L1 (1.53mm ± 0.01), followed by L3 (Thondamuthur, Coimbatore), L5 (Narasingam, Madurai), and L8 (AC&RI, Madurai) and the measurements were 1.43mm ± 0.02, 1.43mm ± 0.10 and 1.43mm ± 0.01 respectively. The remaining specimens varied from 1.34 to 1.42mm of their tibial length. The hind leg's length was the

maximum in L1 (3.72mm ± 0.03), followed by L4 (P.N. Pudhur, Coimbatore) (3.69mm ± 0.04). Other specimens ranged from 3.35 to 3.64mm of the hind leg's length. The maximum length of the middle leg (2.96mm ± 0.03) and foreleg (2.82mm ± 0.04) was recorded from L1. The other specimens recorded from 2.61 to 2.87mm of middle leg length and 2.74 to 2.81mm of foreleg length.



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**Table 5.** Leg morphometric measurements of examined stingless bee specimens

Location/key characters	HTL ± SD	HLL ± SD	MLL ± SD	FLL ± SD
L1	1.53 ± 0.01	3.72 ± 0.03	2.96 ± 0.03	2.82 ± 0.04
L2	1.42 ± 0.03	3.64 ± 0.05	2.85 ± 0.04	2.79 ± 0.05
L3	1.43 ± 0.02	3.63 ± 0.01	2.81 ± 0.01	2.81 ± 0.07
L4	1.40 ± 0.04	3.69 ± 0.04	2.86 ± 0.07	2.76 ± 0.06
L5	1.43 ± 0.10	3.51 ± 0.05	2.69 ± 0.08	2.77 ± 0.12
L6	1.39 ± 0.06	3.49 ± 0.03	2.61 ± 0.10	2.74 ± 0.10
L7	1.35 ± 0.05	3.50 ± 0.08	2.66 ± 0.03	2.79 ± 0.09
L8	1.43 ± 0.01	3.48 ± 0.14	2.69 ± 0.02	2.77 ± 0.08
L9	1.36 ± 0.02	3.35 ± 0.02	2.73 ± 0.11	2.80 ± 0.03
L10	1.41 ± 0.01	3.41 ± 0.12	2.69 ± 0.08	2.79 ± 0.11
L11	1.38 ± 0.05	3.38 ± 0.05	2.71 ± 0.11	2.77 ± 0.10
L12	1.42 ± 0.01	3.44 ± 0.06	2.73 ± 0.05	2.79 ± 0.06
L13	1.34 ± 0.02	3.42 ± 0.11	2.72 ± 0.05	2.77 ± 0.09
L14	1.41 ± 0.02	3.48 ± 0.02	2.81 ± 0.07	2.74 ± 0.01
L15	1.38 ± 0.03	3.49 ± 0.01	2.87 ± 0.11	2.81 ± 0.10
L16	1.42 ± 0.05	3.42 ± 0.03	2.79 ± 0.02	2.79 ± 0.11

Note: Values are the mean of three replications for each location. SD: Standard Deviation. The mean values were measured in millimeters (mm).

### Morphometry of mesosoma and metasoma (Table 6)

The maximum mesosomal length ( $1.36\text{mm} \pm 0.02$ ) was recorded from L1, followed by L9 (Oddanchatram, Dindigul) and L14 (Woraiyur, Tiruchirappalli) measuring  $1.30\text{mm} \pm 0.04$ . Other specimens ranged from 1.19 to 1.29mm of mesosomal length. The specimens collected from L6 (Vowal Thottam, Madurai) measured the maximum mesosomal width ( $1.31\text{mm} \pm 0.04$ ), and others ranged from 1.21 to 1.29mm. The maximum

metasomal length was measured by L5 ( $1.24\text{mm} \pm 0.03$ ) and L15 ( $1.24\text{mm} \pm 0.05$ ), and the remaining specimens ranged from 1.18 to 1.23mm. The width of metasoma was comparatively higher on the specimens collected from L1, measuring  $1.17\text{mm} \pm 0.01$  followed by L16 ( $1.16\text{mm} \pm 0.04$ ), and the rest ranged from 1.10 to 1.14mm. The body length was comparatively greater on the specimens collected from L1, measured at about  $3.98\text{mm} \pm 0.14$  and other specimens varied from 3.51 to 3.65mm of body length.

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**Table 6.** Morphometric measurements of examined stingless bee specimens on their mesosoma, metasoma and their body length

Location/key characters	MSL ± SD	MSW ± SD	MTSL ± SD	MTSW ± SD	BL ± SD
L1	1.36 ± 0.02	1.28 ± 0.05	1.18 ± 0.05	1.17 ± 0.01	3.98 ± 0.14
L2	1.21 ± 0.03	1.29 ± 0.01	1.20 ± 0.01	1.13 ± 0.02	3.65 ± 0.05
L3	1.24 ± 0.04	1.21 ± 0.02	1.23 ± 0.03	1.10 ± 0.02	3.58 ± 0.08
L4	1.22 ± 0.01	1.24 ± 0.01	1.20 ± 0.04	1.12 ± 0.01	3.51 ± 0.10
L5	1.20 ± 0.05	1.29 ± 0.02	1.24 ± 0.03	1.12 ± 0.03	3.59 ± 0.07
L6	1.22 ± 0.04	1.31 ± 0.04	1.19 ± 0.01	1.12 ± 0.01	3.61 ± 0.11
L7	1.19 ± 0.04	1.29 ± 0.05	1.19 ± 0.03	1.13 ± 0.02	3.56 ± 0.15
L8	1.20 ± 0.01	1.27 ± 0.01	1.21 ± 0.02	1.12 ± 0.03	3.58 ± 0.05
L9	1.30 ± 0.04	1.29 ± 0.02	1.18 ± 0.01	1.11 ± 0.02	3.56 ± 0.09
L10	1.22 ± 0.05	1.28 ± 0.06	1.21 ± 0.03	1.14 ± 0.03	3.59 ± 0.03
L11	1.24 ± 0.02	1.21 ± 0.02	1.19 ± 0.01	1.12 ± 0.03	3.62 ± 0.04
L12	1.20 ± 0.01	1.29 ± 0.01	1.20 ± 0.02	1.12 ± 0.01	3.65 ± 0.05
L13	1.24 ± 0.05	1.21 ± 0.01	1.21 ± 0.04	1.10 ± 0.02	3.56 ± 0.04
L14	1.30 ± 0.03	1.24 ± 0.05	1.18 ± 0.04	1.12 ± 0.04	3.61 ± 0.09
L15	1.22 ± 0.02	1.21 ± 0.02	1.24 ± 0.05	1.13 ± 0.01	3.59 ± 0.03
L16	1.29 ± 0.01	1.26 ± 0.05	1.21 ± 0.02	1.16 ± 0.04	3.65 ± 0.06

**Note:** Values are the mean of three replications for each location. SD: Standard Deviation. The mean values were measured in millimeters (mm).

### DISCUSSION

The recorded values were correlated with standard taxonomic keys and prevailing literature. The key parameters which have significant difference in morphometric characteristics between the specimens were preferred to study the diversity of *Tetragonula* sp. Among the 17 morphometrics, HW, AL, FWL, NH, HTL, HLL, MSL, MTSW and BL were chosen for their significance. And some of the existing literature also accounted the key morphometrics which was in concurrence with Trianto *et al.* (2020) who reported that *T. laeviceps* on the basis of Head width, forewing length, hind tibial length and body length.

Based on the results (Fig. 3), the head width ( $1.67 \pm 0.04$ ) of specimens collected from L1 (Insectary, Coimbatore) was in line with Trianto *et al.* (2020), who reported that the head width of *T. laeviceps* was 1.68mm. Except for L1, all other specimens ranged from 1.59 to 1.61mm in head width, which concurs with Rasmussen (2013), who stated that the head width of *T. iridipennis* was 1.60mm. Also, the antennal length, which ranged from 1.80 to 1.86mm, is in concurrence with the results of Kishan *et al.* (2017), who found that the antennal length of *T. iridipennis* was from 1.78 to 1.87mm.

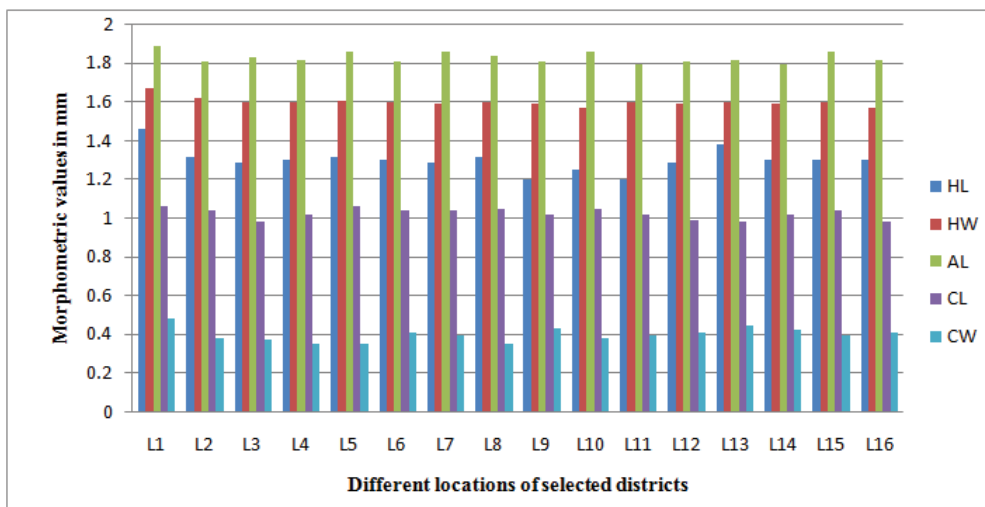


Figure 3. Head morphometry of stingless bee specimens

In the present study (Fig. 4), the length of the forewing was found to be  $3.69\text{mm} \pm 0.02$  for the specimens collected from L1 (Insectary, Coimbatore) which is on par with Trianto *et al.* (2020) who reported the forewing length of *T. laeviceps* was

$3.76\text{mm}$ . The number of hamuli for all the specimens was 5, which is in line with Smith (2012), Rasmussen (2013), and Trianto *et al.* (2020) who stated that the number of hamuli for *Tetragonula sp.* was 5.

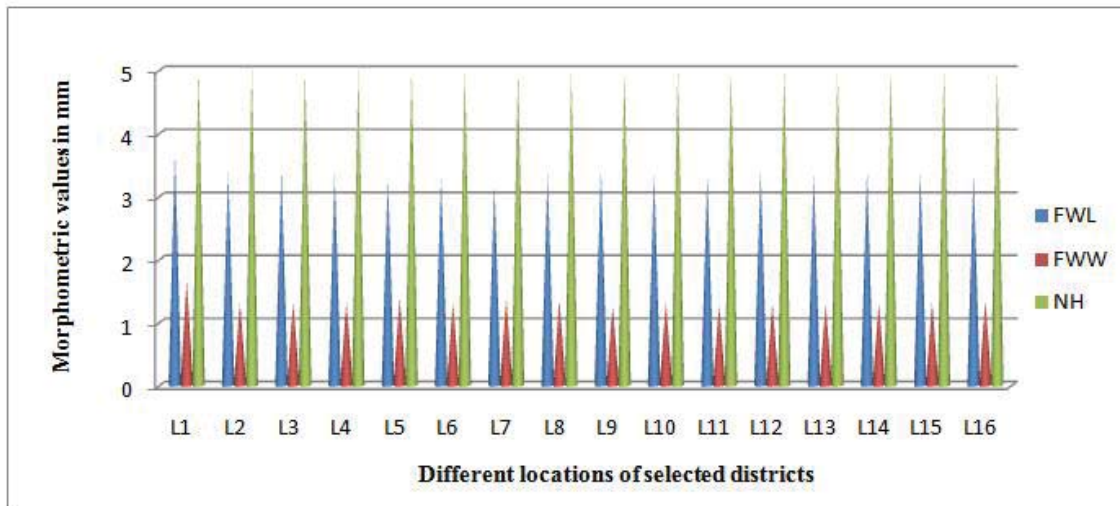


Figure 4. Wing morphometry of stingless bee specimens

According to the results of the present research (Fig. 5), the hind tibial length of L1 (Insectary, Coimbatore) specimens recorded about  $1.53\text{mm} \pm 0.01$ , which is in concurrence with Trianto *et al.* (2020) reported that the hind tibial length of *T. laeviceps* was measured about  $1.55\text{mm}$  and Danaraddi and Viraktamath (2009) stated that the

hind tibial length of *T. iridipennis* was ranged from  $1.32$  to  $1.39\text{mm}$ . The hind leg length of all the specimens except the Coimbatore district ranged from  $3.41$  to  $3.51\text{mm}$ , which corroborates with Kishan *et al.* (2017), who reported that the length of the hind leg varied from  $3.26$  to  $3.40\text{mm}$  in *T. iridipennis*.

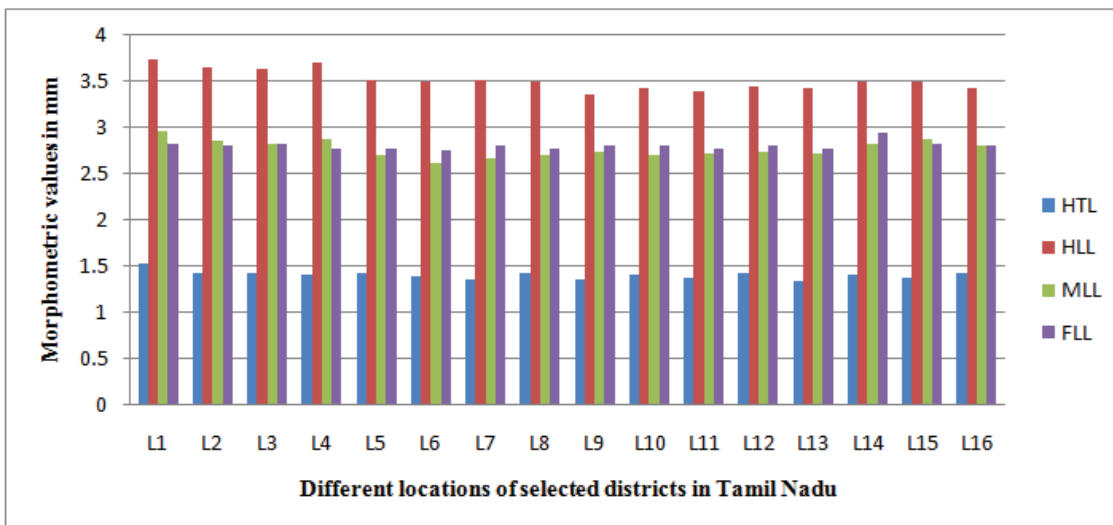


Figure 5. Leg morphometry of stingless bee specimens

Based on the present study (Fig. 6), the mesosoma length of L1 (Insectary, Coimbatore) was found to be  $1.36\text{mm} \pm 0.02$ , and that of other locations measured from 1.19 to 1.30 mm, which is in corroboration with Rahman *et al.* (2015) who reported that the mesosoma length of *T. iridipennis* was 1.24mm. The width of metasoma for the specimens collected from L1 was recorded at about  $1.17\text{mm} \pm 0.01$ , which is

in line with Rahman *et al.* (2015) who stated that the metasomal width of *T. laeviceps* was 1.16mm. The body length of all the specimens except L1 ranged from 3.51 to 3.65mm, and L1 was measured at about  $3.98\text{mm} \pm 0.14$ , which follows Rasmussen (2013), who reported that the whole body length of *T. iridipennis* was 3.55mm and Rahman *et al.* (2015) stated the body length of *T. laeviceps* was 4.04mm.

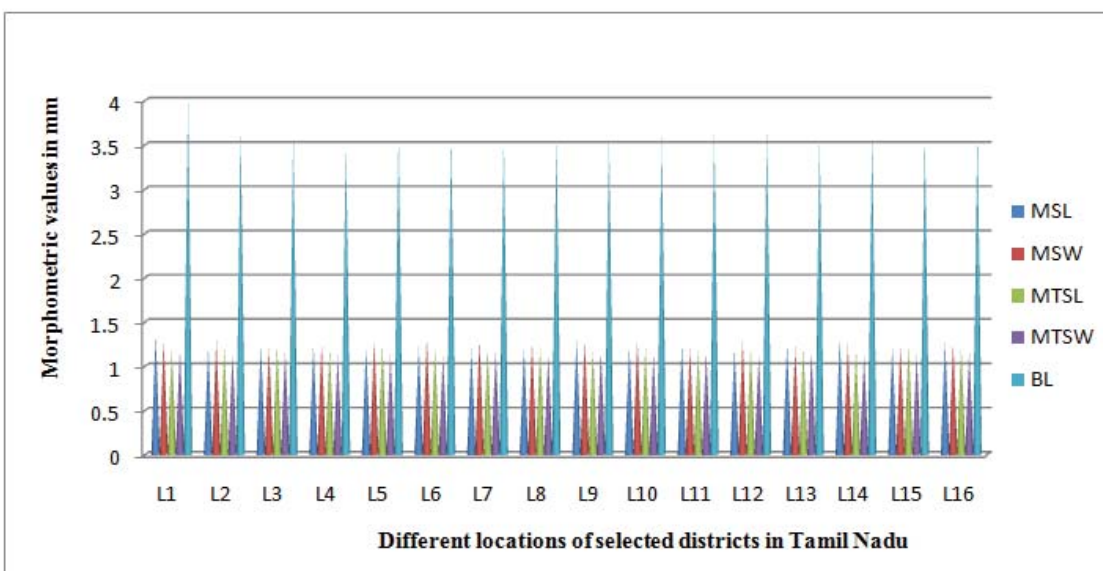


Figure 6. Morphometry of mesosoma and metasoma of stingless bee specimens

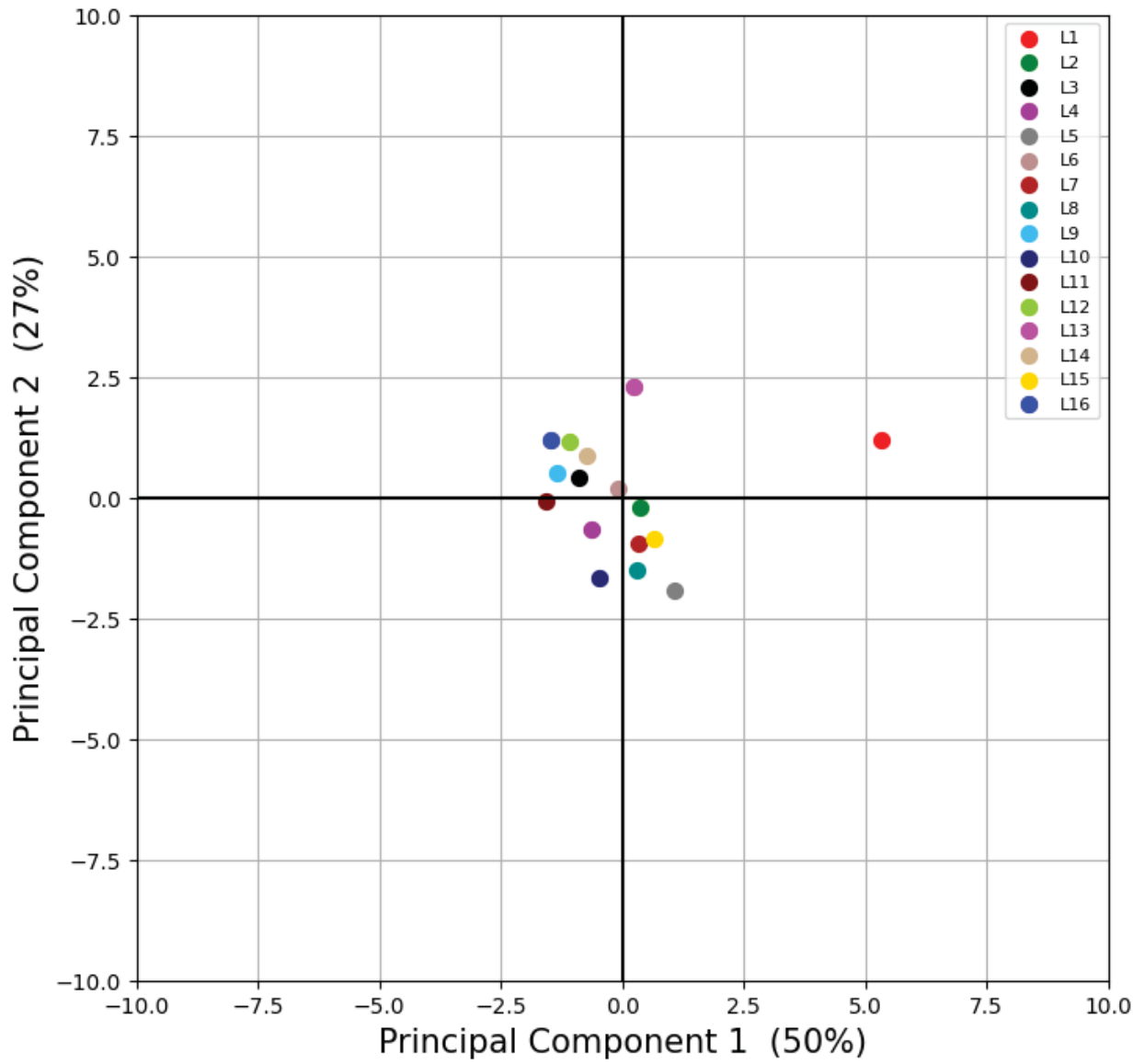
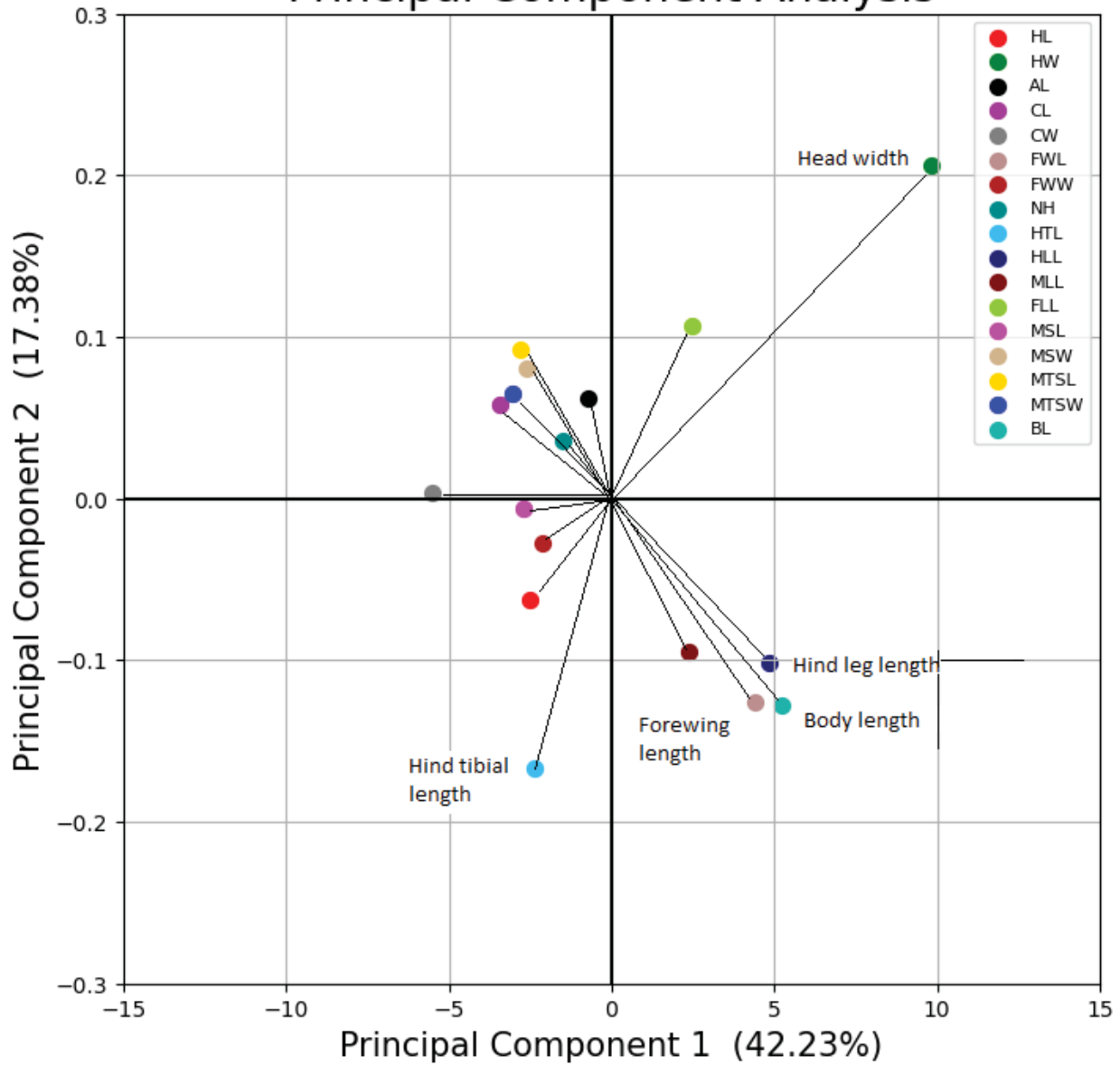


Figure 7. Principal Component Analysis (PCA) for the samples collected from 16 different locations

## Principal Component Analysis



**Figure 8.** Discriminative characters recorded by Principal Component Analysis

The data matrix consisted of 272 measurements which include 17 morphometric characters and 16 locations. Based on the result of PCA (Fig. 7), the cumulative variance of principal components such as PC1 and PC2 were 50.0% and 27.0% respectively. The discriminative characters (Fig. 8) recorded 42.23% (PC1) and 17.38% (PC2) which

was noticed in PCA were the head width, body length, forewing length, hind leg length and hind tibial length. The principal components exhibited no variation among the locations from L2 to L16 but the specimens from L1 alone deviated from the other locations.

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**Table 7.** Key morphometric characters examined to distinguish two species of stingless bees, *Tetragonula* sp.

Morphometric characters (mm)	<i>T. iridipennis</i>	<i>T. laeviceps</i>
Head width	1.59 to 1.61 mm	1.67mm ± 0.04
Forewing length	3.20 to 3.41mm	3.69mm ± 0.02
Hind tibial length	1.34 to 1.43mm	1.53mm ± 0.01
Body length	3.51 to 3.65mm	3.98mm ± 0.14

The worker stingless bee specimens collected from L1 (Insectary, TNAU, Coimbatore) were found to be *T. laeviceps* and the rest of the specimens were found to be *T. iridipennis* by comparing with the morphometric values of previous literature. Further confirmation can be done through molecular studies.

### Conclusion

The investigation on the diversity and distribution of stingless bees (*Tetragonula* sp.) in different districts of Tamil Nadu, India revealed *T. iridipennis*, the most commonly prevalent stingless bee species in Coimbatore, Madurai, Dindigul and Tiruchirappalli districts while *T. laeviceps* was found only in Coimbatore district. Morphometric analysis of the worker bee caste of the stingless bees was used to differentiate the species. Nevertheless, further molecular characterization can play an essential role in confirming the species identity.

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### Author contribution

Study design, data collection, analysis and result interpretation: P. Sabatina, Study conception and manuscript correction: Dr. M.R. Srinivasan, Dr. M. Murugan and V.R. Saminathan, Manuscript editing: Dr. M.R. Srinivasan, Dr. M. Murugan and V.R. Saminathan.

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**Data availability:** Not applicable

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