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Morphometric studies of subgenus *Bombus* (*s. str.*) (Hymenoptera: Apidae) from Indian Himalaya

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Abstract

The subgenus *Bombus s. str.* is represented by two valid species from the Indian Himalayan region viz., *Bombus jacobsoni* Skorikov and *Bombus tunicatus* Smith. Both species of bumblebees are widely distributed and plays a very significant role in conserving the highland ecosystems. The species including the representatives of each caste, viz., Queen, workers and males were collected from different habitats of Indian Himalayan region. Sixteen morphological parameters of this sub-genus covering 2 species viz., *B. jacobsoni* and *B. tunicatus* were investigated and measured relating to their body size, tongue length and antennae, number of hamuli, fore - wings, hind-wings and legs. Later on, total measurements were evaluated to know the differences for identifying their different castes viz., queen, worker and male. Data generated from total measurements of all castes of two species of bumblebees indicate significant morphometric differences. Present studies will thoroughly help in identification of different castes of *Bombus jacobsoni* and *Bombus tunicatus* and equally support in understanding the classification of bumblebees.

Keywords: Bombus, Himalaya, Hymenoptera, Morphometrics, Sub-Genus

Introduction

Bumblebees (*Bombus* Latreille, 1802) are large, multi-coloured and widespread insect pollinators mostly dominant in alpine, temperate and arctic habitats of the globe (Williams 1994; Cameron and Williams, 2003; Hines, 2008). They are among the few bees that are comparatively managed for pollination services they deliver and are considered to be the most liberal pollinators of crops in temperate ecosystems, generating annually worth billions of dollars for their pollination services (Dias, *et al*., 1999; Winter, *et al*., 2006; Goulson, 2010). There are approximately 265 species of bumblebees reported globally (Williams, *et al*., 1998). Currently, the genus *Bombus* is represented by 62 species of bumblebees from Himalaya, of which 29 have been reported from Kashmir Himalaya (Williams, 2004; Williams, *et al*., 2008; Saini *et al*., 2012). The sub-genus *Bombus s. str.* is morphologically distinguished by medium to very big body size, often with apparent banding patterns (Williams, 2007). It was found resemblance of brightly colored hair pattern between many species of bumblebees or particularly abundant variability within a single species of bumblebees, the separation of bumblebees based on colored hair patterns is unreliable (Williams, 2007; Terzo, *et al*., 2007). Total morphological measurements of insects have had a long history of use particularly the eusocial insect species such as bees (Huxley, 1972). Morphometric studies permitted to analyse the shape of body parts as well as the whole organism (Rohlf and Marcus, 1993; Adams, *et al*., 2004). Some workers outside India are identifying bumblebees traditionally by using only morphological characters mainly the male genitalia (Richards, 1968; Alford, 1975;

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Rasmont, 1983; Ito, 1985; Pawlikowski, 2001; Aytekin and Cagatay 2003). Bumblebees were also identified by applying traditional and geometric morphometrics methods (Ito, 1987; Aytekin and Cagatay, 2003; Wappler, *et al*., 2012) as well as phylogenetically, they were also identified (Williams, 1985; Williams, 1994; Cameron and Williams, 2003; Cameron, *et al*., 2007). Furthermore, molecular procedures were applied to give comprehensive understanding of phylogeny of the bumblebees (Cameron and Williams, 2003; Hines, *et al*., 2006; Cameron, *et al*., 2007). Morphometric techniques with respect to studies pertaining to bumblebees is considered not just as an efficient tool but also beneficial one while it delivers results rapidly, moreover it is relevant even on aged museum items (Aytekin, *et al*., 2007). During the last two decades, the advancement of computer technology had led to the development of various new tools, which significantly facilitate the record of morphometric data (Rohlf, 1990). There are different taxonomic and evolutionary questions regarding the bumblebees, which can be answered by using morphometric techniques. Therefore, alternative approaches like morphometric techniques were adopted to solve the problems in identification and classification of bumblebee species respectively. Thus, the purpose of this study was to examine the species falling under sub-genus *Bombus sensu stricto* by identifying and examining the applicability of morphometric techniques for distinguishing the different castes of *B. jacobsoni* and *B. tunicatus* respectively.

Materials and Methods

Morphometric studies of subgenus *Bombus* (*s. str.*) were made by consisting of queens, workers and males of *B. jacobsoni* and *B. tunicatus*. Samples were collected during September-October, 2020, from different localities of Jammu and Kashmir (33.2778^{0}N, 75.3412^{0}E) and Himachal Pradesh (31.1048^{0}N, 77.1734^{0}E) having an altitudinal range from 2400 to 3300 meters amsl covering different ecological habitats (Figures 1&2). The sampling was made by sweep nets and instantly killed using ethyl acetate in a killing jar and some of the samples were put in to 90% ethanol. Later on, the collected material was transferred and deposited in the National Zoological Collection at Zoological Survey of India, Desert Regional Centre, Jodhpur, Rajasthan, India. The samples preserved in 90% Ethanol were then dissected into different body parts according to the technique of Ruttner, *et al*., 1978. All the body parts to be measured were mounted on clean slides which were then examined using a high-resolution scanner (1100 lp/mm) connected to a desktop computer system supported with NIS-Elements Software D.5.21.03, 64-bit. Thus, total, sixteen different morphometric characters were chosen in the current study allied to body size, tongue length, length of Antenna, number of hamuli, length of fore and hind wings and legs of all respective castes of each species, according to the methods earlier used by Dayal and Rana, 2007; Devanesan, *et al*., 2004; Chauhan, *et al*., 2016.

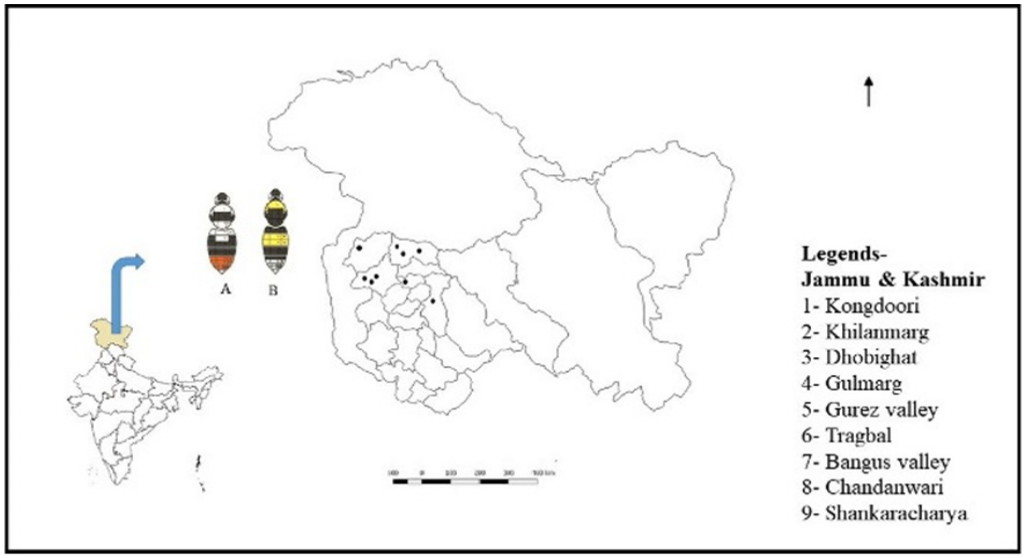

Figure 1. Map showing the survey areas in Union Territory of Jammu and Kashmir.

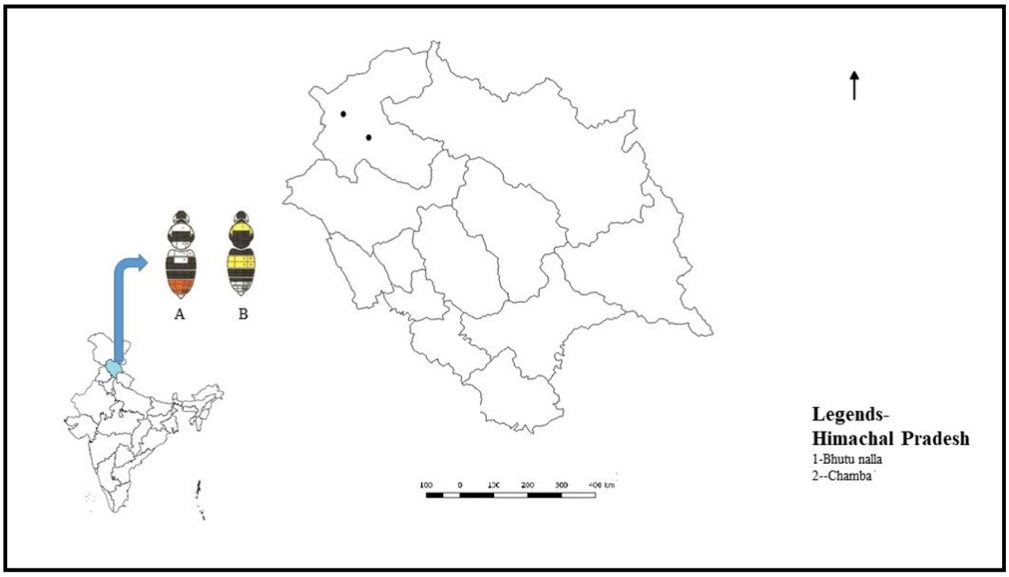

Figure 2. Map showing the survey areas of Himachal Pradesh.

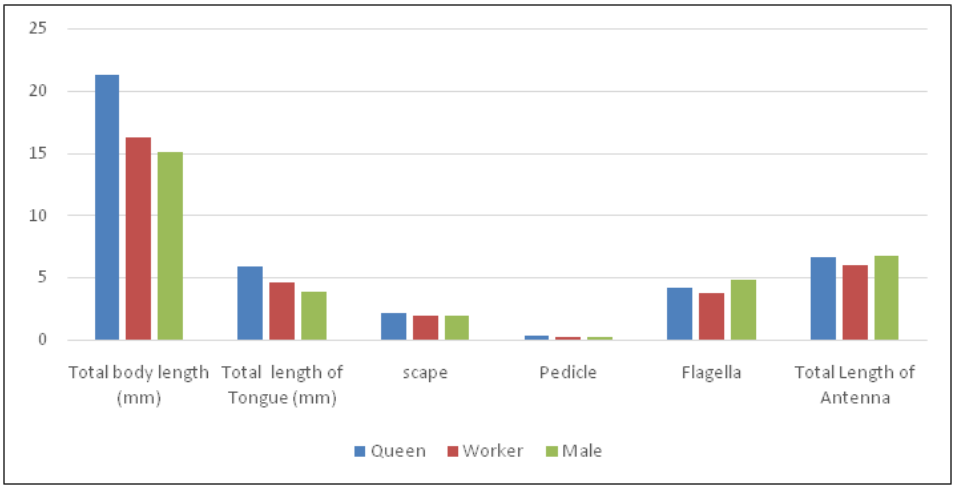

Figure 3. Morphometric parameters of *Bombus tunicatus.*

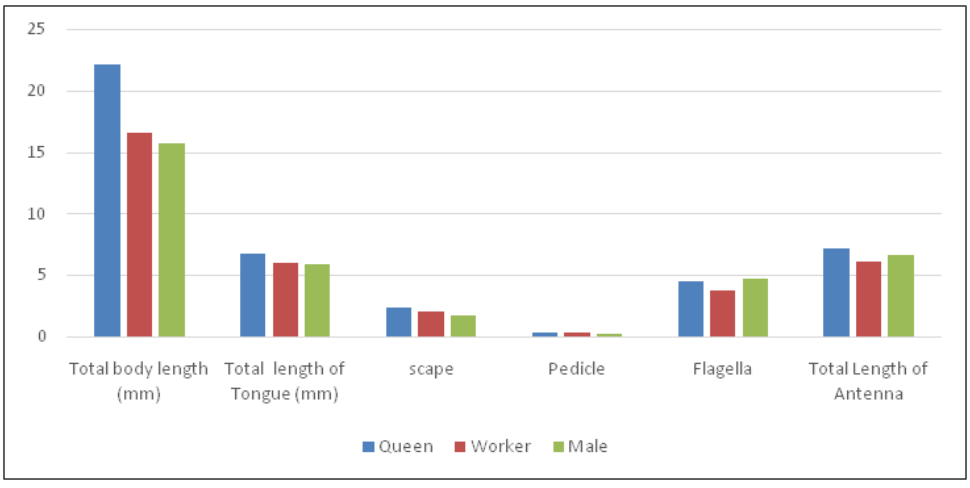

Figure 4. Morphometric parameters of *Bombus jacobsoni.*

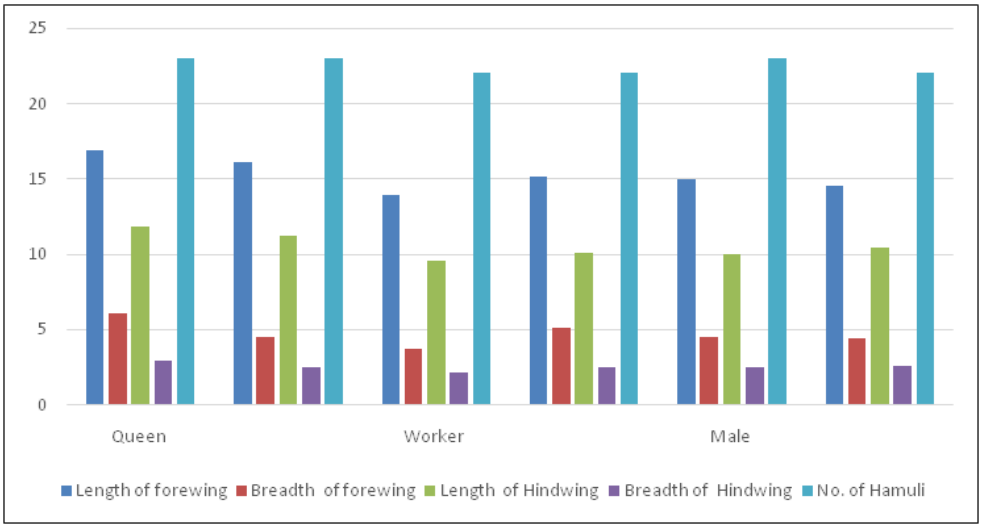

Figure 5. Comparative morphometric parameters of wings of *Bombus tunicatus* and *Bombus jacobsoni.*

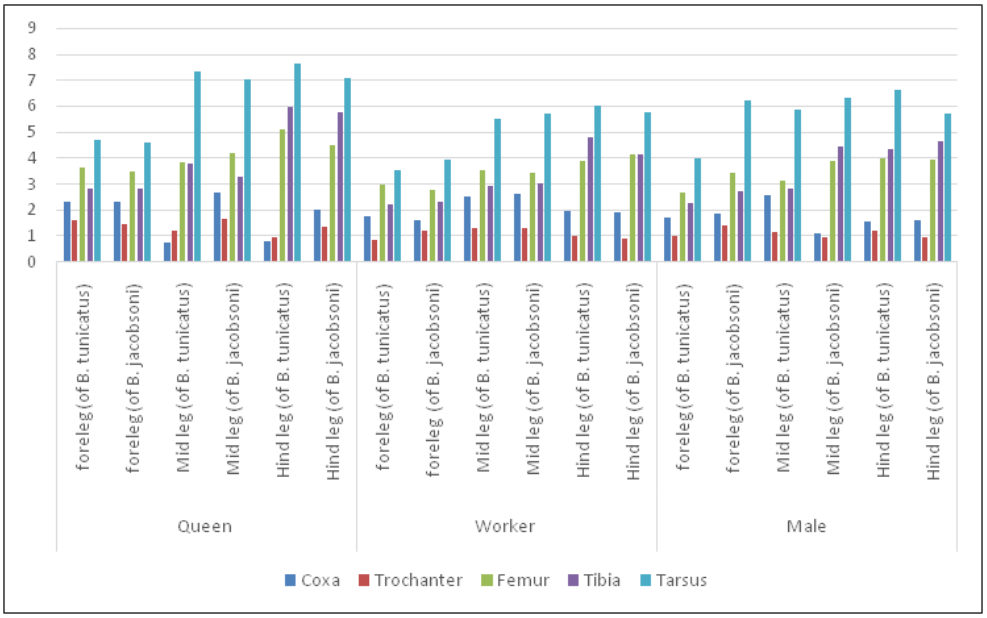

Figure 6. Comparative morphometric parameters of legs of *Bombus tunicatus* and *Bombus jacobsoni.*

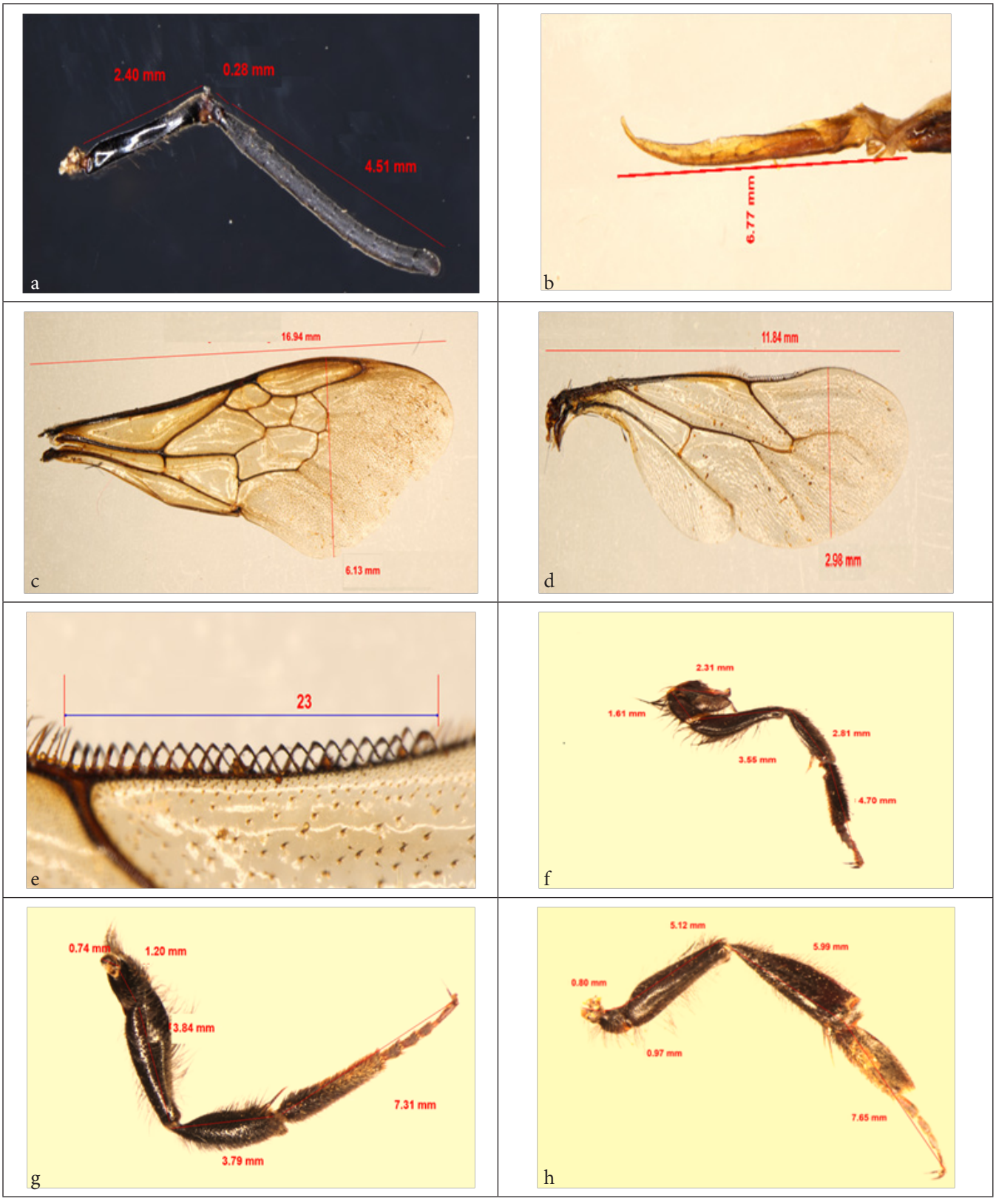

Figure 7. Showing different Taxonomic characters of ***B. tunicatus*** **Queen**: **(a)** Antenna, **(b)** Tongue length, **(c)** fore-wing, **(d)** hind-wing, **(e)** hamuli, **(f)** fore-leg, **(g)** mid-leg, **(h)** hind-leg.

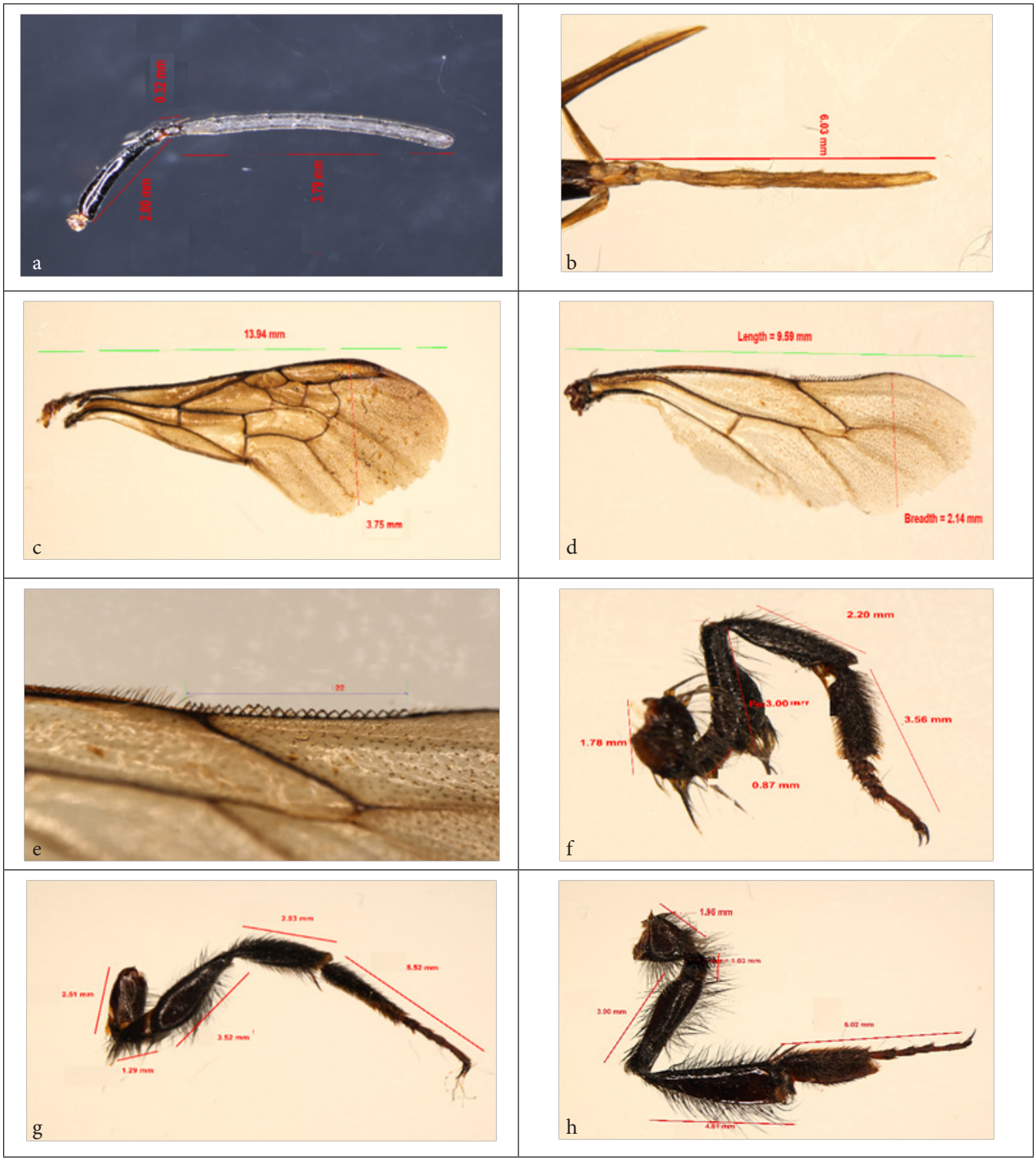

Figure 8. Showing different Taxonomic characters of ***B. tunicatus*** **Worker**: **(a)** Antenna, **(b)** Tongue length, **(c)** fore-wing, **(d)** hind-wing, **(e)** hamuli, **(f)** fore-leg, **(g)** mid-leg, **(h)** hind-leg.

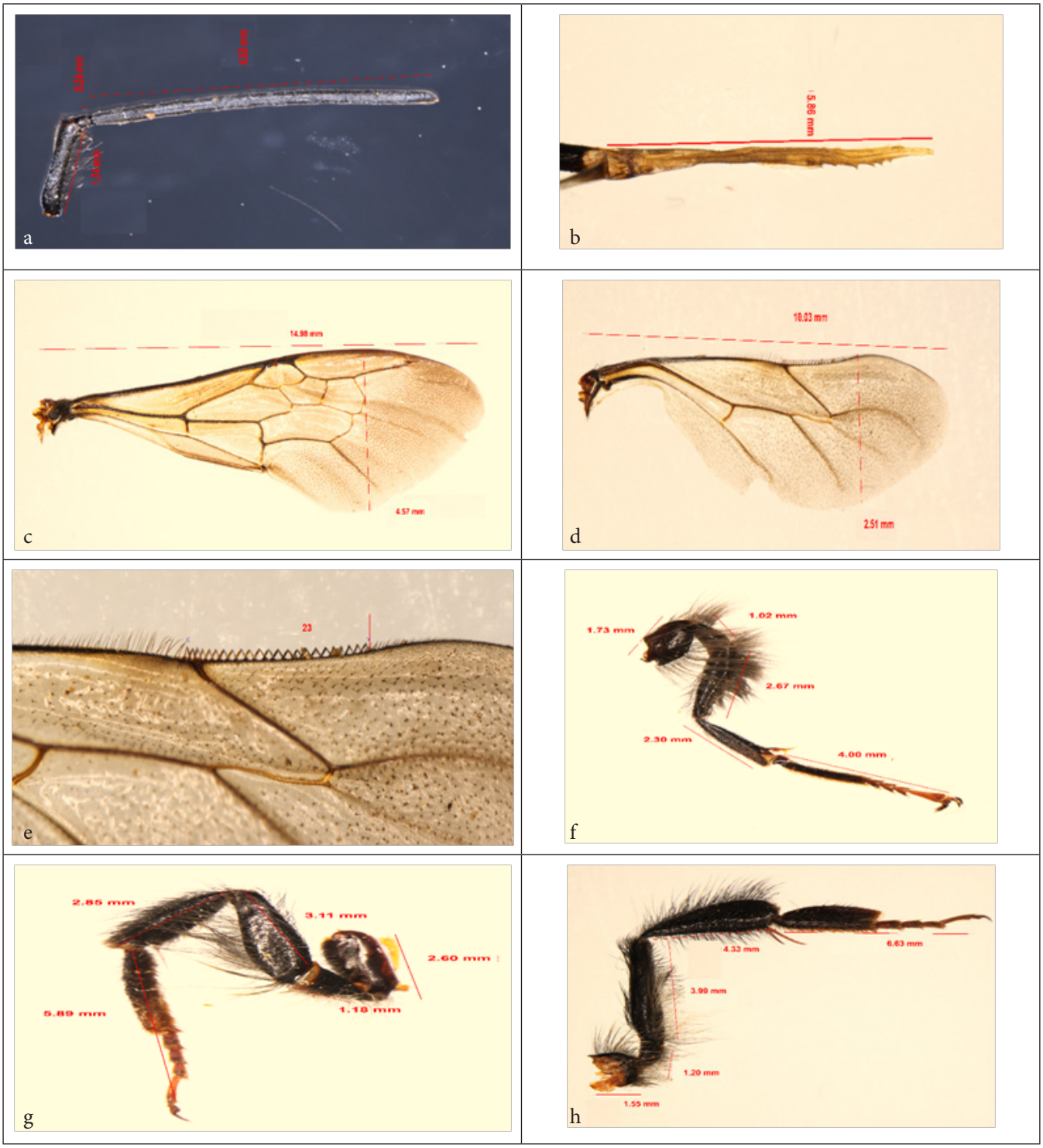

Figure 9. Showing different Taxonomic characters of ***B. tunicatus*** **Male**: **(a)** Antenna, **(b)** Tongue length, **(c)** fore-wing, **(d)** hind-wing, **(e)** hamuli, **(f)** fore-leg, **(g)** mid-leg, **(h)** hind-leg.

Figure 10. Showing different Taxonomic characters of ***B. jacobsoni*** **Queen**: **(a)** Antenna, **(b)** Tongue length, **(c)** fore-wing, **(d)** hind-wing, **(e)** hamuli, **(f)** fore-leg, **(g)** mid-leg, **(h)** hind-leg.

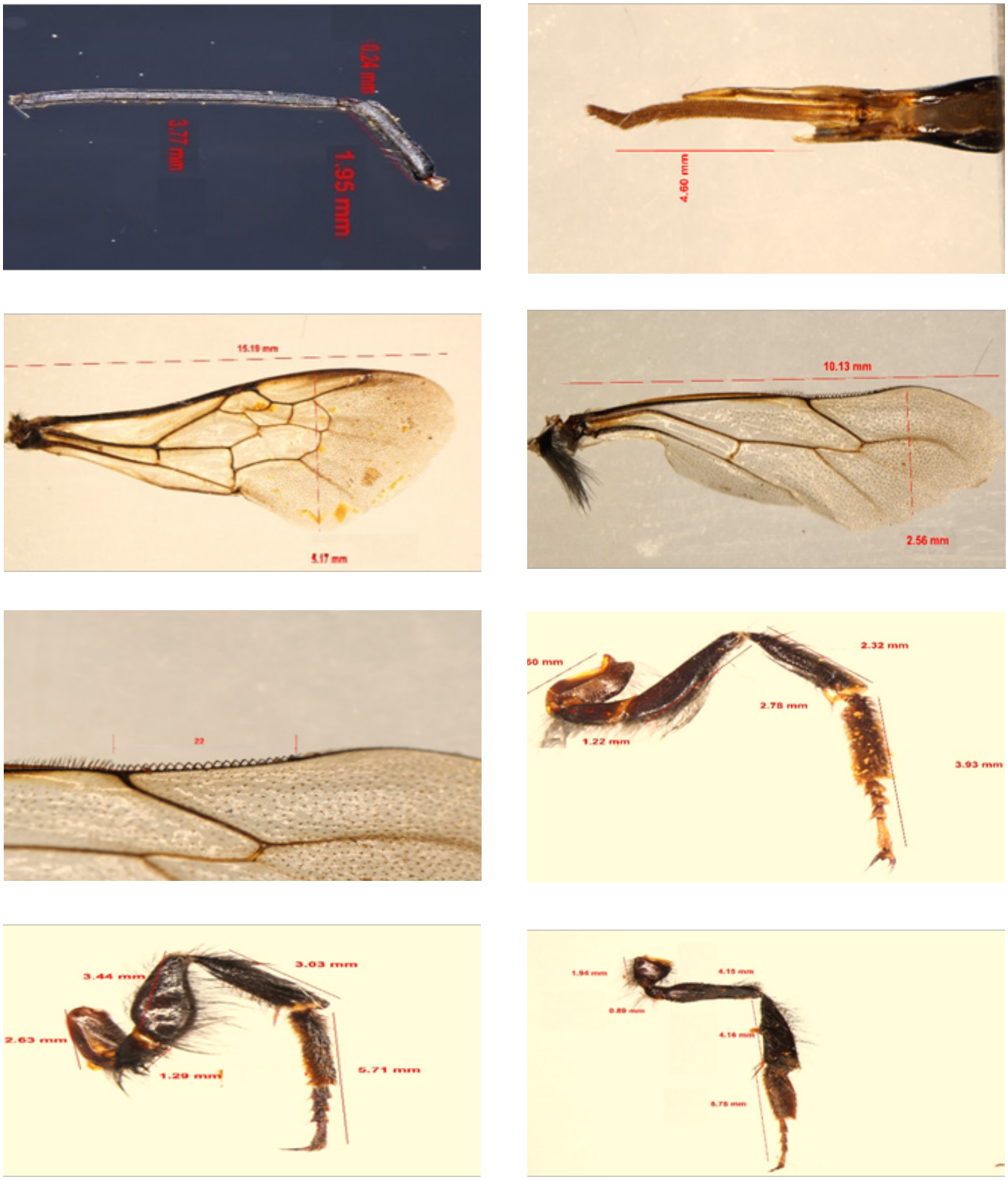

Figure 11. Showing different Taxonomic characters of ***B. jacobsoni*** **Queen**: **(a)** Antenna, **(b)** Tongue length, **(c)** fore-wing, **(d)** hind-wing, **(e)** hamuli, **(f)** fore-leg, **(g)** mid-leg, **(h)** hind-leg.

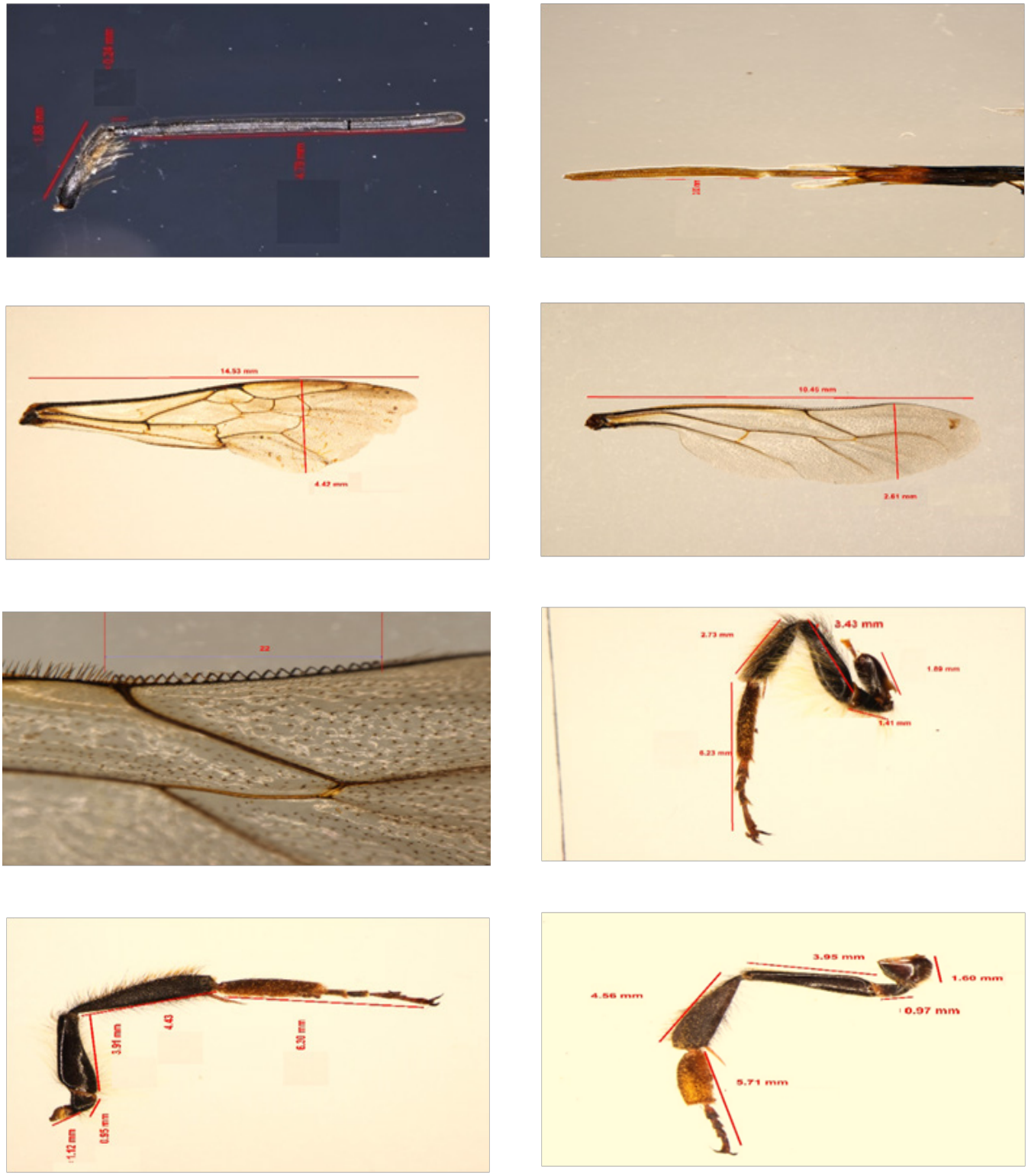

Figure 12. Showing different Taxonomic characters of ***B. jacobsoni*** **Male**: **(a)** Antenna, **(b)** Tongue length, **(c)** fore-wing, **(d)** hind-wing, **(e)** hamuli, **(f)** fore-leg, **(g)** mid-leg, **(h)** hind-leg.

Results

The result of the morphometric measurements of the different castes *viz*., Queen, Worker and male of *Bombus tunicatus* and *Bombus jacobsoni* are summarised in Tables 1 and 2 and also see images from Figure 7 to 12. The different morphometric parameters of *B. tunicatus* describing the body size of queen, worker and male are 22.14 mm, 16.59 mm and 15.74 mm, similarly in *B. jacobsoni*, the body size of queen, worker and male are 21.29 mm, 16.23mm and 15.03 mm respectively. The tongue length in all the castes of *B. tunicatus* were recorded as 6.77 mm, 6.03 mm and 5.86 mm while the tongue length of different castes of *B. jacobsoni* were 5.86 mm, 4.60 mm and 3.83 mm respectively. The result of the measured structures exhibited the length of antenna in queen, worker and male were 7.19 mm, 6.11 mm and 6.66 mm whereas in *B. jacobsoni* the antenna length in queen, worker and male were 6.64 mm, 5.96 mm and 6.69 mm. The flagellum of antenna had 10 segments in queen and worker of both species while the flagellum of antenna in drone caste of respective species had 11 segments. In *B. tunicatus* and *B. jacobsoni* the length of forewings in their three different castes were measured as 16.94 mm; 13.94 mm, 14.98 mm and 16.14 mm., 15.19 mm., 14.53 mm correspondingly, similarly the hindwing measurements were as 11.84 mm; 9.59 mm, 10.03 mm and 11.20 mm; 10.13 mm; 10.45 mm. In addition to this, there were presence of 23 hamuli in hindwing ofqueen, 22 in worker and 23 in male of *B. tunicatus* similarly 23 hamuli in queen, 22 in worker and 22 in male of *B. jacobsoni*. The morphometric techniques were also employed on legs of bees and found constantly diverse variations in legs of all castes of *B. tunicatus* and *B. jacobsoni* respectively as given in Table 2. The *B. tunicatus* queen bee (22.14 mm) was bigger in size than worker (16.59) and male (15.74). Moreover the *B. jacobsoni* queen bee (21.29) was also bigger than its respective castes viz., worker (16.23 mm) and male (15.03 mm).

Discussion

The morphometric analyses of the tongue length in all the castes of *B. tunicatus* were recorded larger than the tongue length of different castes of *B. jacobsoni* respectively. While as morphometric analyses of the legs showed that the hind-legs are larger in respective castes of *B. tunicatus* as compared to *B. jacobsoni*. The hind-legs of queen and workers bear pollen basket or corbicula surrounded by pollen brushes that helps in efficient pollen collection and passively transport them among conspecfic flowers and thus helps in pollination. There is a positive correlation between the size of the bumblebees and the average amount of nectar obtained per flower as predicted by (Schoener, 1969). Thus, *B. tunicatus* will harvest more nectar from flowers than *B. jacobsoni*.These structures were found to play significant role in the activities of the

Tables 1&2. Relative morphometrics statistics of different castes of *B. tunicatus* and *B. jacobsoni*

Table 1						
Characters	**Total body length (mm)**		**Antenna (mm) length**			
Species		**Total length of Tongue (mm)**	**Scape**	**Pedicle**	**Flagella**	**Total Length**
B. tunicatus Smith, 1852						
queen (♀)	22.14	6.77	2.40	0.28	4.51	7.19
worker (♀)	16.59	6.03	2.00	0.32	3.79	6.11
Male (♂)	15.74	5.86	1.74	0.24	4.68	6.66
B. jacobsoni Skorikov, 1912						
queen (♀)	21.29	5.86	2.11	0.36	4.17	6.64
worker (♀)	16.23	4.60	1.95	0.24	3.77	5.96
Male (♂)	15.03	3.83	1.88	0.24	4.79	6.69

Table 2

Characters	Fore-wing (mm)		Hind-wing (mm)			Legs (mm) length				
Species	*	**	*	**	No. of Hamuli	Coxa	Tro-chanter	Femur	Tibia	Tarsus
B. tunicatus Smith, 1852										
queen (♀)	16.94	6.13	11.84	2.98	23	Fore-leg	2.31	1.61	3.65	2.81	4.70
						Mid-leg	0.74	1.20	3.84	3.79	7.31
						Hind-leg	0.80	0.97	5.12	5.99	7.65
worker (♀)	13.94	3.75	9.59	2.14	22	Fore-leg	1.78	0.87	3.00	2.20	3.56
						Mid-leg	2.51	1.29	3.52	2.93	5.52
						Hind-leg	1.96	1.03	3.90	4.81	6.02
Male (♂)	14.98	4.57	10.03	2.51	23	Fore-leg	1.73	1.02	2.67	2.30	4.00
						Mid-leg	2.60	1.18	3.11	2.85	5.89
						Hind-leg	1.55	1.20	3.99	4.33	6.63
B. jacobsoni Skorikov 1912										
queen (♀)	16.14	4.51	11.20	2.50	23	Fore-leg	2.35	1.48	3.50	2.82	4.62
						Mid-leg	2.69	1.66	4.20	3.30	7.02
						Hind-leg	2.03	1.39	4.51	5.78	7.08
worker (♀)	15.19	5.17	10.13	2.56	22	Fore-leg	1.60	1.22	2.78	2.32	3.93
						Mid-leg	2.63	1.29	3.44	3.03	5.71
						Hind-leg	1.94	0.89	4.15	4.16	5.75
Male (♂)	14.53	4.42	10.45	2.61	22	Fore-leg	1.89	1.41	3.43	2.73	6.23
						Mid-leg	1.12	0.95	3.91	4.43	6.30
						Hind-leg	1.60	0.97	3.95	4.66	5.71

Note: * Length, ** Breadth

bumblebees. Hence, morphometric studies has established to have an essential and valuable set of methods that are particularly useful in understanding the morphometric variations in different castes of bumblebees for their identification and classification. The results highlight the importance of different species of sub-genus *Bombus* (*s. str.)* in morphometric measurements and analysis.

Our study concluded that bumblebee and the floral resources they visit a well-matched tongue length is important to a booming association. Some bumblebees and host plants are very closely harmonized with tongue size to the flower intensity while as other bees are generalists and flitting amid different flower species to gulp nectar and collect pollen from the diversity of host plants. Morphometric data on the tongue length, body size, and other parameters taken on this study can help ecologists to understand and envisage the behaviour and resilience of bee populations. Bumblebees are generalists in their choice of food plants and in most species, the tongue is guarded by elongated bilateral, beak of a sheath, which creases under the body when it flies. The tongue length of bees can be entangled with species risk of extinction and specialization.

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