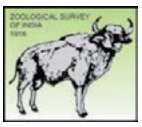
ISSN (Online) : 2581-8686
ISSN (Print) : 0375-1511

Rec. zool. Surv. India: Vol. 123(iS2)/185-200, 2023
DOI: 10.26515/rzsi/v123/iS2/2023/172515



Comparative Morphometric Studies of The *Habrobracon hebetor-brevicornis* and *Bracon lefroyi-greeni* Complexes of Braconid Wasps (Hymenoptera: Braconidae: Braconinae)

Longjam Roni Kumar Singh[1]* and Dulal Chandra Ray[2]

[1]Zoological Survey of India, M- Block, New Alipore, Kolkata 700053
[2]Department of Ecology and Environmental Science, Assam University, Silchar, Assam 788801; raydulal@yahoo.co.in

Abstract

The accurate identification of parasitoids is of immense importance to biological control programs. Despite their economic importance and commonly occurring, the taxonomy of *Habrobracon hebetor-brevicornis* and *Bracon lefroyi-greeni* complexes is difficult and currently confused being cryptic and occurring as distinct populations as well as groups of populations. Thus, there exists confusion in species recognition and the diagnosis of the appropriate host can dramatically affect the outcome of a biological control program. This study focuses on the morphometric variations in females of five biotypes in each complex associated with different hosts and localities. Statistical analyses, such as Unweighted Pair-Group Method Arithmetic Average (UPGMA) cluster analysis, Canonical Discriminant Analysis and Predictive Discriminant Analysis were used to analyse the complexes, and these also enabled the identification of taxonomically important characters in differentiating the population and grouping. The evidence presented here rationalizes the confusion between the species complexes, and the additional morphological characters brought out here will help to resolve the taxonomic complexity.

Keywords: Biocontrol, parasitoid, biotypes, character, complex, identification, morphometrics

Introduction

***Hbrobracon hebetor-brevicornis* Complex**

The species of the *Habrobracon hebetor-brevicornis* and *Bracon lefroyi-greeni* complexes are parasitoids of economically important insect pests and have considerable potential as biological control agents. Despite their economic importance and commonly occurring, the taxonomy of these is difficult and currently confused as these occurred as complexes and distinct populations as well as groups of populations. It is essential to bring out the complexity, delineate, and explore their complexities through detailed study, especially of morphometrics.

The species of the *Habrobracon hebetor-brevicornis* complex are economically important worldwide for the biological control of lepidopteran insect pests. They are polyphagous, larval ectoparasitoids and sometimes overlap among the populations parasitizing the various host species (Puttarudriah and Channa Basavanna, 1956). The complex consists of two morphologically similar species: *H. hebetor* (= *Bracon hebetor* Say, 1836) and *H. brevicornis* (= *Braco brevicornis* Wesmael, 1838), which has led to confusion in species recognition and also in synonymization. Cushman (1922) first attempted to clarify the species identity of *B. juglandis* Ashmead, 1889 (= *H. hebetor*) and *H. brevicornis*, relying upon the variations in antennal segments, i.e., with 13-15 segments in the former and that of later with 17-19 segments. Cushman did not mentioned *H. hebetor* and their relationship with *H. juglandis*. Later, Muesebeck (1925) refer the character used by Cushman (1922) that distinguished *H. juglandis* from *H. brevicornis* and the original description of *H. hebetor*, which led to synonymized *H. juglandis* with *H. hebetor* and provided a key (under the genus *Microbracon*). Lal (1947) studied the identity of the complex species reared from various hosts at the Indian Lac Research Institute (= National Institute of Secondary Agriculture), Namkum,

*Author for correspondence : lucicrohn@gmail.com
Article Received on: 21.04.2023 Accepted on: 15.06.2023

Ranchi, Jharkhand and at the Imperial Agricultural Research Institute (= Indian Agricultural Research Institute), New Delhi. He shows the confusion prevailing and comes to the conclusion that these species can be separated with Muesebeck's key (1925), with a slight modification in the female antennal segments of *H. brevicornis* (16-21 segments). Cherian and Margubandhu (1951) followed Muesebeck's work and retained these as two distinct species. Puttarudriah and Channa Basavanna (1956) studied the species complex by deploying morphology i.e., colour, antennal segments, genitalia, and biology and concluded that the two names should be synonymized. However, Narayanan *et al.* (1958) suggested that it is a complex of more than two species. In the meantime, Chawla and Subba Rao (1965) further studied the identity of *H. hebetor-brevicornis* complex using the paper chromatography technique and suggested that the complex consists of two distinct species.

The species taxonomy of the complex is thus difficult, hardly discernible and currently confused. Some authors believed these are identical and *H. brevicornis* is a junior synonym of *H. hebetor* (Papp, 2008, 2012; Yu *et al.*, 2012; Ameri *et al.*, 2013). At the same time, some treated these as two distinct species (van Achterberg and Polaszek, 1996; van Achterberg and Walker, 1998; Haider *et al.*, 2004; Sheeba and Narendran, 2007; Ehteshami and van Achterberg, 2012). However, most biologists frequently used the name *H. brevicornis* (sometimes under the genera *Bracon* or *Microbracon*) ignoring the existence of complexity at the species level (Krishnamurti and Appana, 1944; Dharmarju, 1952; Subba Rao and Kumar, 1960; Deka, 1969; Sarup *et al.*, 1971; Mathai, 1972; Jacob *et al.*, 1980; Mathew *et al.*, 1980; Narendran *et al.*, 1981; Abbas, 1982; Ram *et al.*, 1982; Sudheendrakumar *et al.*, 1982; Jayanth and Nagarkatti, 1985; Ghosh *et al.*, 1993; Nasser and Abdurahiman, 1998; Mohapatra and Mohapatra, 2003; Venkatesan *et al.*, 2009; Srivastava *et al.*, 1997; Mohanty *et al.*, 2000; Rukhsana and Sebastian 2015). Recently, molecular identification of the two species had been done for the populations from Barbados and the United States by Heimpel *et al.* (1997), Egypt by Bakr *et al.* (2003), India by Rukhsana and Sebastian (2015), Thailand and Japan by Chomphukhiao *et al.* (2018) and from Germany, Egypt, Japan, Spain, Thailand, United States, Uzbekistan, and Barbados by Kittel and Maeto (2019). This study reviewed their status as distinct species.

***Bracon lefroyi-greeni* complex**

Bracon greeni Ashmead, is an important larval parasitoid of lac insect predator, *Eublemma amabilis* Moore (Lepidoptera: Noctuidae) and *Syncola pulverea* (Meyrick) (Lepidoptera: Blastobasidae) (Varshney, 1976). *Bracon lefroyi* (Dudgeon and Gough) is also a larval parasitoid of spotted bollworms of cotton, *Earias insulana* Boisduval, *E. vittella* (Fabricius (Lepidoptera: Noctuidae) and pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Sharma *et al.*, 2000; Naik *et al.*, 2018). *Bracon greeni* and *B. lefroyi* are very similar to each other in general appearance and morphology, and it is often difficult to separate them. The species complexity had been studied using selected morphological characters viz., colour, antennal segment, wing veins, etc. Lal (1939) established that there is not a single constant morphological character by which this complex species can be distinguished and concluded that *B. greeni* and *B. lefroyi* are two biological races of the same species. Till now no valid key for identification is available, however, Sheeba and Narendran (2013) keyed out these as distinct species in their key to the Indian species of *Bracon*, but their description is quite confusing since some taxonomic attributes of *B. greeni* written in *B. lefroyi* (such as ovipositor length) and *B. lefroyi* was considered near *B. albolinetus* Cameron, which had been transferred to the genus *Amyosoma*.

Thus, there exists confusion in species recognition and the diagnosis of appropriate host strains can dramatically affect the outcome of a biological control program against a specific host species. Hence, the present study focuses on the morphometric variations in females of five biotypes in each complex associated with different hosts and localities, on the basis of one meristic and 14 ratio characters.

Materials and Methods

The present study is based on the specimens from the National Pusa Collection (NPC), Division of Entomology, Indian Agricultural Research Institute, New Delhi (studied during 2012-2015). In addition, specimens from the Biological Control Laboratory, Division of Entomology, IARI, New Delhi and Central Institute for Cotton Research (CICR), Nagpur, Maharashtra, received by Dr. V.V. Ramamurthy for observations have been included.

Selection and preparation of samples

Fresh specimens collected and received were examined under a Leica M205FA stereozoom microscope and mounted on pointed triangular cards with a very small amount of water-

soluble glue using a Leica EZ4 stereozoom microscope. Care was taken to spread the legs and wings so as to expose all the taxonomic characters of importance. The specimens were labelled with data of locality, host, date and name of the collector. The museum specimens were individually examined under the stereozoom microscope to confirm their uniformity and suitability for morphometrics. Physical conditions like cleanliness, completeness and amenability for measurement were the criteria utilized for the selection of specimens. Such good specimens of nearly uniform nature and complete with the least variability were randomly selected irrespective of the identity of species and grouped into various biotypes pertaining to the various hosts and localities and their numbers as indicated (Table 1).

Selection and measurement of characters

The general morphological characters illustrated by van Achterberg (1979) were utilized. Both males and females are equally taxonomically important, though females are most relied upon for authentic generic and species identification. Taxonomic characters especially ovipositor sheath length had been earlier relied upon greatly (van Achterberg and Quicke, 1992). Literature on the taxonomic descriptions of Braconidae as a whole was also consulted for the selection of characters (van Achterberg, 1979, 1992, 1993; Quicke, 1982, 1987). Thus, a total of 15 and 13 quantitative characters were measured in 52 specimens from *H. hebetor-brevicornis* (23) and *B. lefroyi-greeni* (29) complexes, respectively, for the analysis of morphometrics. The biotypes with codes NPECT, belonging to the *B. lefroyi-greeni* and DECOR, belonging to *H. hebetor-brevicornis* complex species group, were used as outgroups in the respective species complex analyses. The quantitative characters were of two kinds: meristic and ratios (Table 2) and data were transformed using $\log_{10}(x+1)$.

While studying so, care was taken to ensure the specimens were always in the same plane and angle to ensure uniformity and concordance of values. Individual specimens were examined carefully under the stereozoom microscope at 50× for the selected measurements. The measurements in terms of ocular values were tabulated and these were further calibrated using a stage micrometer, and then used for the statistical analyses.

Statistical analyses

The pattern of morphometrics was analysed using the following multivariate statistical approaches: (i) The average-linkage-between-groups method, often called UPGMA (Unweighted Pair-Group Method Arithmetic Average) using Euclidean distance is a simple agglomerative (bottom-up) hierarchical clustering method; (ii) Canonical Discriminant Analysis (CDA) (or Canonical Discriminant Function Analysis in SPSS) is a dimension-reduction technique. CDA was carried out to assess the canonical variables that provide maximal separation between the groups, and (iii) Predictive Discriminant Analysis (PDA) is a predictive classification technique which is concerned with classifying observations into one of several groups. PDA was used to determine the potential misclassification of specimens or populations. Statistical analysis was performed with SPSS Version 20.0 (SPSS Inc, Chicago, IL, USA) for Windows.

Results

A. *Habrobracon hebetor-brevicornis* complex

(i) UPGMA

The linkage distance among the biotypes of the complex based on morphometrics is indicated in Figure 1 by an UPGMA dendrogram. This shows a main cluster including all the biotypes except NPECT (outgroup) in another cluster. The main cluster was subdivided into two subclusters, the first included two biotypes DELIR and DEANT. In the second subcluster two biotypes NEUBA and DECOR are very closely clustered and DEEAR biotype is separated from the previous two biotypes.

(ii) Canonical discriminant function analysis

The eigenvalues indicated that the first two canonical variate pairs provide a summary of the discrimination abilities with 95.9% of the total variation and the first two canonical correlations (0.99 and 0.99) are very high and these imply that these two correlations are important (Table 3). Individual specimens are projected on the first two canonical axes (CV1 and CV2) which contain the highest percentage of variability. NPECT specimens are clearly separated along the first two canonical axes from the DEARI, FEARI, DBRIN, NPECT and NEUBA and are in the III quadrant (Figure 2). DELIR specimens are in I quadrat with weakly discriminated from the specimens of DEANT. Most of the specimens of the NEUBA and DECOR form a uniform cluster with weakly separated DEEAR and are in IV quadrant.

Biotypes NEUBA, DECOR and DEEAR are clearly separated from DELIR and DEANT specimens along the first two canonical axes. Class mean for NEUBA, DECOR and DEEAR are 9.73, 9.12 and 11.44, and for DELIR and DEANT are 6.58 and 5.72 along the 1st canonical axis (Table 4). And for the 2nd canonical axis, class mean for NEUBA, DECOR and DEEAR are -4.95, -5.93 and -2.50, and for DELIR and DEANT are 7.57 and 10.70, respectively. Taxonomic characters which contribute to the greatest extent to the discrimination of specimens of NEUBA, DECOR and DEEAR from DELIR and DEANT specimens along the 1st canonical axis are: number of antennal segments (ALN), length of 2nd flagellar segment: width of 2nd flagellar segment (F2L/F2W), length of 3rd radius sector: length of 2nd radius sector (SR1/3-SR) and length of ovipositor sheath: length of metasoma (OL/ML) (Table 4). On the 2nd canonical axis are: number of antennal segments (ALN), length of antenna: length of head and mesosoma (AL/HML), transverse diameter of eye: length of temple (EL/TML), length of 1st flagellar segment: width of 1st flagellar segment (F1L/F1W), length of 2nd flagellar segment: width of 2nd flagellar segment (F2L/F2W), length of 2nd radius sector: length of 1st radius sector (3-SR/r), length of 2nd radius sector: length of 1st intercubitus (3-SR/2-SR).

iii) Predictive discriminant analysis

The classification matrix of discriminant analysis in Table 5 shows the number and percentage of correctly placed specimens in the studied populations. All the specimens of biotypes DECOR, DEEAR, DELIR and DEANT are correctly placed. However, it is mixed classified with the specimens of NEUBA biotype, ex. *Eublemma amabilis* from Namkum, Ranchi, Jharkhand biotype. An overall 96.4% of original grouped cases got correctly classified.

B. *Bracon lefroyi-greeni* complex

(i) UPGMA

The UPGMA dendrogram (Figure 3) shows a main cluster including all the biotypes except DECOR (outgroup) in another cluster. The main cluster included four biotypes (DEARI, FEARI, NEUBL and DBRIN) at a considerable distance from NPECT. In this subcluster of the two biotypes, DEARI and FEARI are very closely clustered with NEUBL, and DBRIN biotype is separated from the previous three biotypes.

(ii) Canonical discriminant function analysis

The eigenvalues indicated that the first two canonical variate pairs provide a summary of the discrimination abilities with 96.0% of the total variation and the first two canonical correlations (0.99 and 0.96) are very high and these imply that these two correlations are important (Table 6). Individual specimens are projected on the first two canonical axes (CV1 and CV2) which contain the highest percentage of variability. NPECT and DBRIN are clearly separated along the first two canonical axes from DEARI, FEARI and NEUBL specimens. Class mean for NPECT and DBRIN are 2.074 and 3.244, and for DEARI, FEARI and NEUBL are -5.484, -5.922 and -4.347, along the 1st canonical axis, respectively (Table 7). On the 2nd canonical axis, class mean for NPECT and DBRIN are -5.732 and -4.887, and for DEARI, FEARI and NEUBL are 2.426, 2.562 and 0.381, respectively. Taxonomic characters which contribute to the greatest extent to the discrimination of specimens of NPECT and DBRIN from DEARI, FEARI and NEUBL specimens along the 1st canonical axis: length of 1st flagellar segment: width of 1st flagellar segment (F1L/F1W), length of 2nd flagellar segment: width of 2nd flagellar segment (F2L/F2W), and length of ovipositor sheath: length of metasoma (OL/ML). On the 2nd canonical axis are: length of 1st flagellar segment: width of 1st flagellar segment (F1L/F1W) and length of 2nd flagellar segment: width of 2nd flagellar segment (F2L/F2W).

DECOR specimens are clearly separated along the first two canonical axes from the DEARI, FEARI, DBRIN, NPECT and NEUBL and are in the I quadrant (Figure 4). NPECT specimens are in IV quadrat with weakly discriminated from the specimens of DBRIN. Most of the specimens of the DEARI and FEARI form a uniform cluster with partial overlapping with NEUBL and are in II quadrant.

iii) Predictive discriminant analysis

The specimens of two biotypes NPECT, ex. *Pectinophora gossypiella* from Nagpur and DBRIN from Delhi (NPC) are correctly placed (Table 8). With other three biotypes such as DEARI, ex. *Earias fabia, E. insulana* from Delhi (NPC), FEARI, ex. *E. insulana* from Faisalabad, Pakistan (NPC) and NEUBL, ex. *Eublemma amabilies* from Ranchi (NPC) show the lowest percentage of correctly placed i.e., 42.8, 80.0, 62.5, respectively. An overall 75.8% of original grouped cases were correctly classified.

Discussion

***Habrobracon hebetor-brevicornis* complex**

This species complex consists of two morphologically similar species viz., *Habrobracon hebetor* and *H. brevicornis*. The species taxonomy of the two has been confused for many years. Some authors believed that these are identical but some treated them as distinct species. van Achterberg and Polaszek (1996) treated these as distinct and provided a key for identification, with the morphology viz., number of antennal segments and forewing veins 3-SR and r. But most biologists frequently used the name *B. brevicornis* (sometimes under the genera *Bracon* or *Microbracon*) ignoring the existence of complexity at the species level. This made more confusion about the identity and is not allowed in the case of these parasitoids considering their importance in insect pest control.

The present study focuses on the morphometric variations in females of five biotypes of different hosts and localities. Using numerical methods we showed a clear distinction between the species of *H. hebetor* and *H. brevicornis* reared or bred from the host (see Table 1). The taxonomic character which has the greatest contribution to the distinction of NEUBA, DÉCOR and DEEAR biotypes from the biotypes DELIR and DEANT on both axes are: number of antennal segments (ALN) and length of 2nd flagellar segment: width of 2nd flagellar segment. The number of antennal segments for NEUBA, DÉCOR and DEEAR biotypes has 12-14 segments (= *H. hebetor*, Figure 5a) and that of DELIR and DEANT have 15-21 segments (= *H. brevicornis*, Figure 5b), which was used as an important diagnostic character in *H. hebetor and H. brevicornis* (van Achterberg and Polaszek (1996). Besides this characters: length of antenna: length of head and mesosoma (AL/HML), length of 2nd radius sector: length of 1st radius sector (3-SR/r) has an important contribution to the discrimination of both groups, which support the work of van Achterberg and Polaszek (1996).

***Bracon lefroyi- greeni* complex**

The parasitoids, *Bracon greeni* and *B. lefroyi* are very similar to each other in general appearance and morphology so it is often difficult to separate them. Nevertheless, the complex species were studied using limited selected morphological characters, colours, antennal segments, wings veins, etc. Lal (1939) stated that there is not a single constant morphological character by which this complex species can be distinguished and he believes that these two *B. greeni* and *B. lefroyi* are two biological races of the same species.

The taxonomic character which has the greatest contribution to the distinction of DBRIN and NPECT biotypes from the other biotypes are: length of 1st flagellar segment: width of 1st flagellar segment, length of 2nd flagellar segment: width of 2nd flagellar segment and length of ovipositor sheath: length of metasoma (OL/ML). The length of the ovipositor sheath of DBRIN, ex. pest of brinjal from New Delhi, is shorter than the length of metasoma (i.e. 0.5-0.85), thus supporting the original description of *B. greeni* (Ashmead, 1896; cf. Ramakrishna Ayyar, 1928; Figure 6c). Whereas, NPECT, ex. *Pectinophora gossypiella* from Nagpur, the length of ovipositor sheath is as long as or longer than length of metasoma (i.e. 1.0-1.), showing the character of *B. lefroyi* (Figure 6d), thus supporting the works of Brues (1920) and Ramakrishna Ayyar (1928). However, the other three biotypes such as DEARI, ex. *Earias fabia, E. insulana* from Delhi (NPC), FEARI, ex. *E. insulana* from Faisalabad, Pakistan (NPC) and NEUBL, ex. *Eublemma amabilies* from Ranchi (NPC) show similar affinities with NPECT biotype in terms of length of ovipositor sheath, but differ in having ocello-ocular line: transverse diameter of posterior ocellus = 2.0-2.6 (vs 1.5-1.7 in NPECT biotype); transverse diameter of eye: length of temple = 1.7-2.3 (vs 2.8-3.2).

The evidence presented here rationalizes the confusion between these complexes and shows the aggregation of similar species occurring as distinct populations as well as clusters of populations with the presence of overlapping characters and the utility of the selected characters for the characterization of species or populations. Multivariate analyses enabled the identification of taxonomically important characters, which will help to resolve the taxonomic complexity of the *H. hebetor-brevicornis* and *B. lefroyi- greeni* complexes under studied and other populations as well.

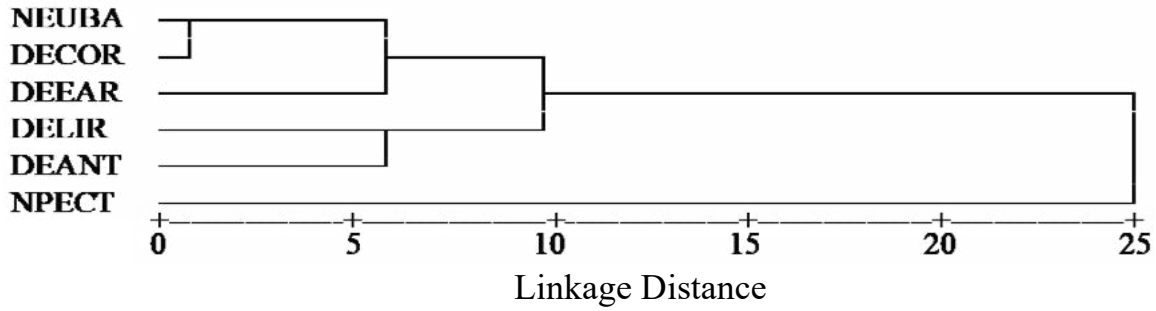


Figure 1 . UPGMA dendrogram of generalized distances between the *H. hebetor-brevicornis* complex.

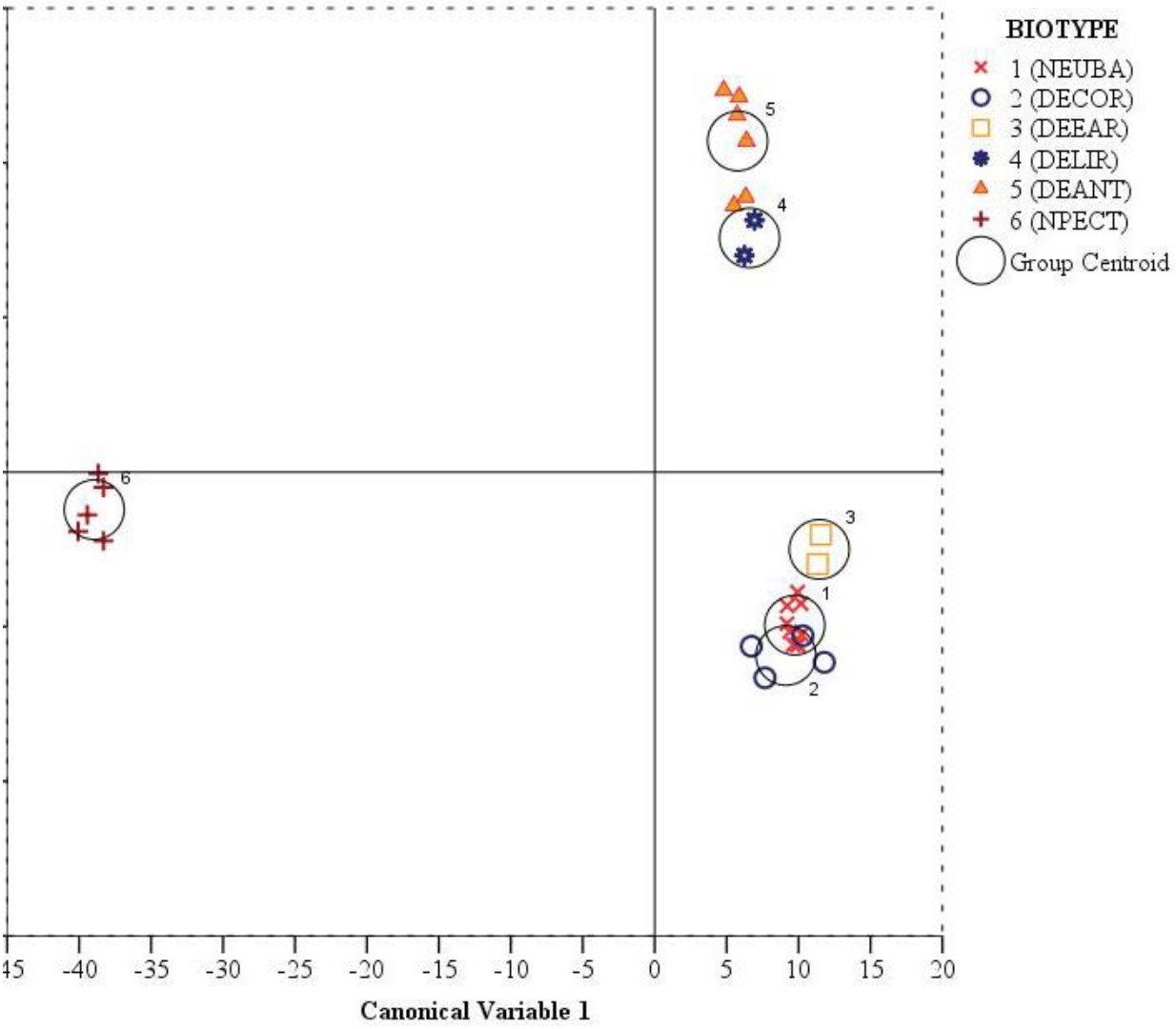


Figure 2. Plot of biotypes on the first two canonical axes of class means (*H. hebetor-brevicornis* complex*).*

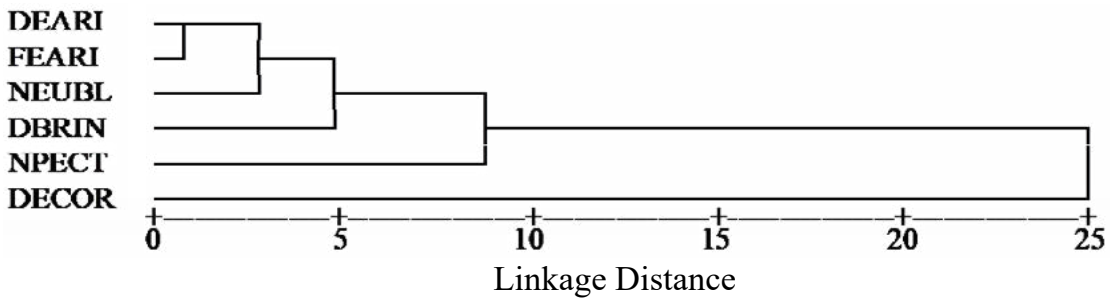


Figure 3. UPGMA dendrogram of generalized distances between the *B. lefroyi-greeni* complex.

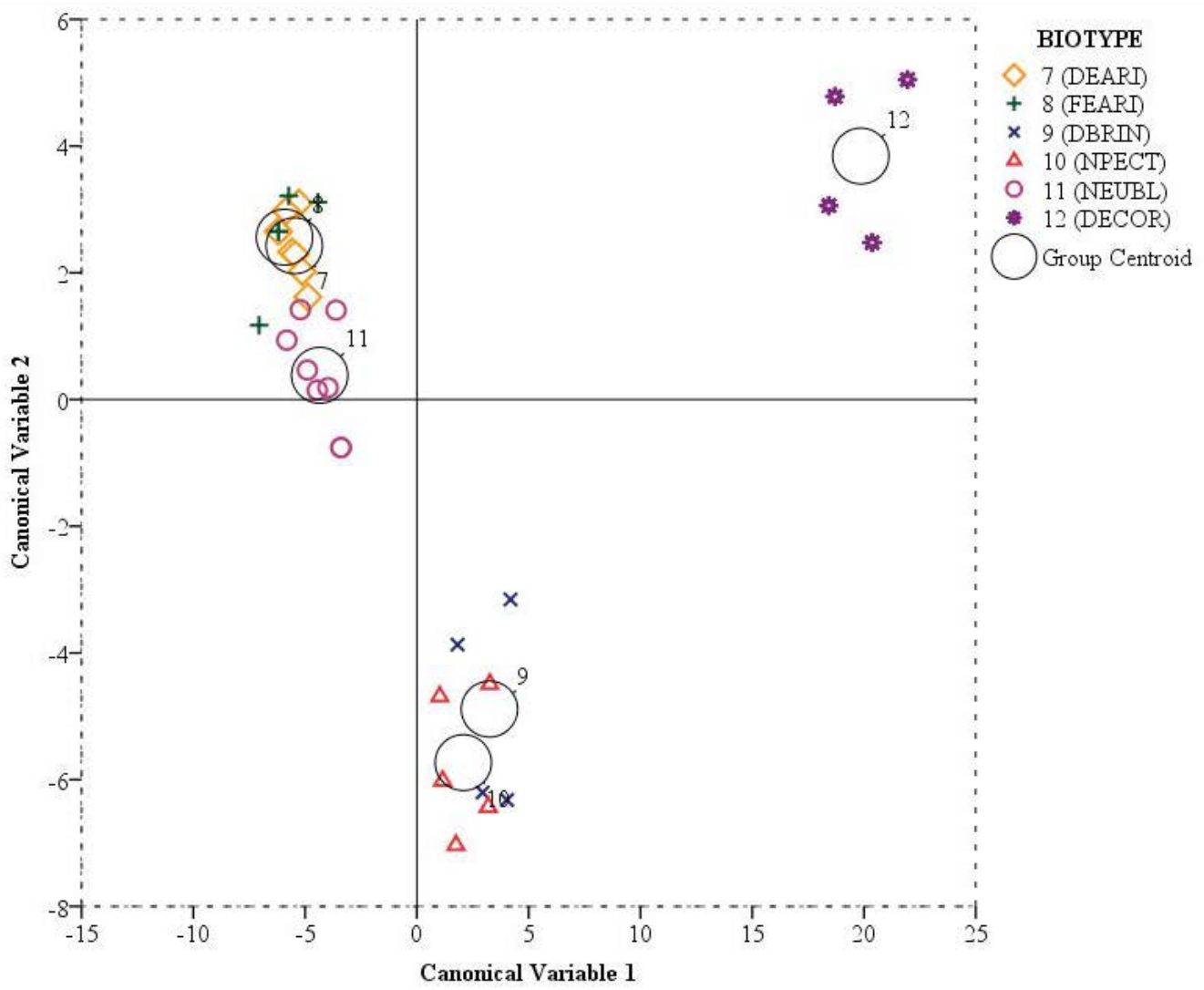


Figure 4. Plot of biotypes on the first two canonical axes of class means (*B. lefroyi-greeni* complex).

Table 1. Details of species, biotype codes, hosts, and localities of the material examined of *Habrobracon hebetor-brevicornis* and *Bracon lefroyi-greeni* complexes

Species	Biotype code	Number of specimens	Host	Locality
B. hebetor-brevicornis complex	NEUBA	9	*Eublemma amabilis* Moore (Lepidoptera: Noctuidae)	Namkum, Ranchi, Jharkhand (NPC)
	DECOR	4	*Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)	I.A.R.I., New Delhi
	DEEAR	2	*Earias* sp. (Lepidoptera: Noctuidae)	New Delhi (NPC)
	DELIR	2	*Liriomyza* sp. (Lepidoptera: Agromyzidae)	Delhi (NPC)
	DEANT	6	*Antigastra catalaunalis* (Lepidoptera: Crambidae)	Delhi (NPC)
B. lefroyi-greeni complex	DEARI	7	*Earias* sp., *E. fabia* (Stoll), *E. insulana* (Boisduval) (Lepidoptera: Noctuidae)	Delhi (NPC)
	FEARI	5	*E. insulana* (Boisduval) (Lepidoptera: Noctuidae)	Faisalabad, Pakistan (=Lyallapur, Punjab) (NPC)
	DBRIN	4	Pest of brinjal, host not defined	Delhi (NPC)
	NPECT	5	*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)	Nagpur, Maharashtra
	NEUBL	8	*Eublemma amabilis* Moore (Lepidoptera: Noctuidae)	Namkum, Ranchi, Jharkhand (NPC)

Table 2. List of characters for morphometrics

Character no.	Character code	Type	Description
C1	ALN	Meristic	Number of antennal segments
C2	AL/HML	Ratio	Length of antenna: length of head and mesosoma
C3	POL/OD	Ratio	Posterior ocellar line: transverse diameter of posterior ocellus
C4	OOL/OD	Ratio	Ocello-ocular line: transverse diameter of posterior ocellus
C5	EL/TML	Ratio	Transverse diameter of eye (in dorsal view): length of temple
C6	F1L/F1W	Ratio	Length of 1stflagellar segment: width of 1stflagellar segment
C7	F2L/F2W	Ratio	Length of 2ndflagellar segment: width of 2ndflagellar segment
C8	3-SR/r	Ratio	Length of 2nd radius sector: length of 1st radius sector
C9	3-SR/2-SR	Ratio	Length of 2nd radius sector: length of 1stintercubitus
C10	SR1/3-SR	Ratio	Length of 3rd radius sector: length of 2nd radius sector
C11	SC+R1/1r-m	Ratio	Length of metacarpella: length of basella
C12	1r-m/2-SC+R	Ratio	Length of basella: length of 2ndcostella sector
C13	1-M/M+CU	Ratio	Length of 2ndmediella sector: length of 1stmediella sector
C14	OL/ML	Ratio	Length of ovipositor sheath: length of metasoma
C15	TBL/BTL	Ratio	Length of hind tibia: length of hind basitarsus

Table 3. Summary of Canonical Discriminant Functions (*H. hebetor-brevicornis* complex)

Sl. No.	Eigenvalues	% of Variance	Cumulative %	Canonical Correlation
1	423.652	85.1	85.1	.999
2	53.806	10.8	95.9	.991
3	12.987	2.6	98.6	.964
4	4.534	0.9	99.5	.905
5	2.666	0.5	100.0	.853

Table 4. Standardized Canonical Discriminant Function Coefficients with class means of canonical variables based on discriminant function for measurements of 15 morphological characters (*H. hebetor-brevicornis* complex)

Character	Canonical variable	
	CV1	CV2
ALN	**-1.265**	**1.243**
AL/HML	0.381	**1.645**
POL/OD	0.277	-0.001
OOL/OD	-0.047	-0.034
EL/TML	-0.121	**-1.071**
F1L/F1W	0.500	**1.959**
F2L/F2W	**1.108**	**-2.113**
3-SR/r	-0.985	**-1.957**
3-SR/2-SR	0.085	**2.221**
SR1/3-SR	**1.306**	0.921
SC+R1/1r-m	0.786	-0.726
1r-m/2-SC+R	0.425	-0.713
1-M/M+CU	-0.189	0.767
OL/ML	**-2.553**	-0.932
TBL/BTL	0.474	-0.772
Biotype	Class means (group centroids)	
NEUBA	9.733	-4.950
DECOR	9.121	-5.935
DEEAR	11.441	-2.503
DELIR	6.589	7.574
DEANT	5.770	10.705
NPECT	-38.953	-1.217

Table 5. Classification matrix of Discriminant Analysis for correctly placed specimens (*H. hebetor-brevicornis* complex)

	Predicted Group Membership						
Biotype	**NEUBA**	**DECOR**	**DEEAR**	**DELIR**	**DEANT**	**NPECT**	**Total**
NEUBA	8	1	0	0	0	0	9
(%)	88.9	11.1	0.0	0.0	0.0	0.0	100.0
DECOR	0	4	0	0	0	0	4
(%)	0.0	100.0	0.0	0.0	0.0	0.0	100.0
DEEAR	0	0	2	0	0	0	2
(%)	0.0	0.0	100.0	0.0	0.0	0.0	100.0
DELIR	0	0	0	2	0	0	2
(%)	0.0	0.0	0.0	100.0	0.0	0.0	100.0
DEANT	0	0	0	0	6	0	6
(%)	0.0	0.0	0.0	0.0	100.0	0.0	100.0
NPECT	0	0	0	0	0	5	5
(%)	0.0	0.0	0.0	0.0	0.0	100.0	100.0

Table 6. Summary of Canonical Discriminant Functions (*B. lefroyi-greeni* complex)

Sl. No.	Eugenvalues	% of Variance	Cumulative %	Canonical Correlation
1	80.663	81.3	81.3	0.994
2	14.594	14.7	96.0	0.967
3	2.206	2.2	98.2	0.829
4	1.333	1.3	99.5	0.756
5	0.463	0.5	100.0	0.562

Table 7. Standardized Canonical Discriminant Function Coefficients with class means of canonical variables (*B. lefroyi-greeni* complex)

Character	Canonical variable	
	CV1	CV2
POL/OD	0.051	0.279
OOL/OD	-0.870	0.211
EL/TML	0.559	-0.921
F1L/F1W	**2.065**	**-2.596**
F2L/F2W	**-2.201**	**2.377**
3-SR/r	0.154	0.112
3-SR/2-SR	-0.593	-0.203
SR1/3-SR	0.957	0.594
SC+R1/1r-m	0.799	0.588
1r-m/2-SC+R	0.552	0.417
1-M/M+CU	0.039	0.927
OL/ML	**-1.092**	0.443
TBL/BTL	0.514	-0.307
Biotype	Class means (group centroids)	
DEARI	-5.484	2.426
FEARI	-5.922	2.562
DBRIN	3.244	-4.887
NPECT	2.074	-5.732
NEUBL	-4.347	0.381
DECOR	19.857	3.842

Table 8. Classification matrix of Discriminant Analysis for correctly placed specimens (*B. lefroyi-greeni* complex)

	Predicted Group Membership						
Biotype	**DEARI**	**FEARI**	**DBRIN**	**NPECT**	**NEUBL**	**DECOR**	**Total**
DEARI	3	2	0	0	2	0	7
(%)	42.8	28.6	0.0	0.0	28.6	0.0	100.0
FEARI	1	4	0	0	0	0	5
(%)	20.0	80.0	0.0	0.0	0.0	0.0	100.0
DBRIN	0	0	4	0	0	0	4
(%)	0.0	0.0	100.0	0.0	0.0	0.0	100.0
NPECT	0	0	0	5	0	0	5
(%)	0.0	0.0	0.0	100.0	0.0	0.0	100.0
NEUBL	2	1	0	0	5	0	8
(%)	25.0	12.5	0.0	0.0	62.5	0.0	100.0
DECOR	0	0	0	0	0	4	4
(%)	0.0	0.0	0.0	0.0	0.0	100.0	100.0

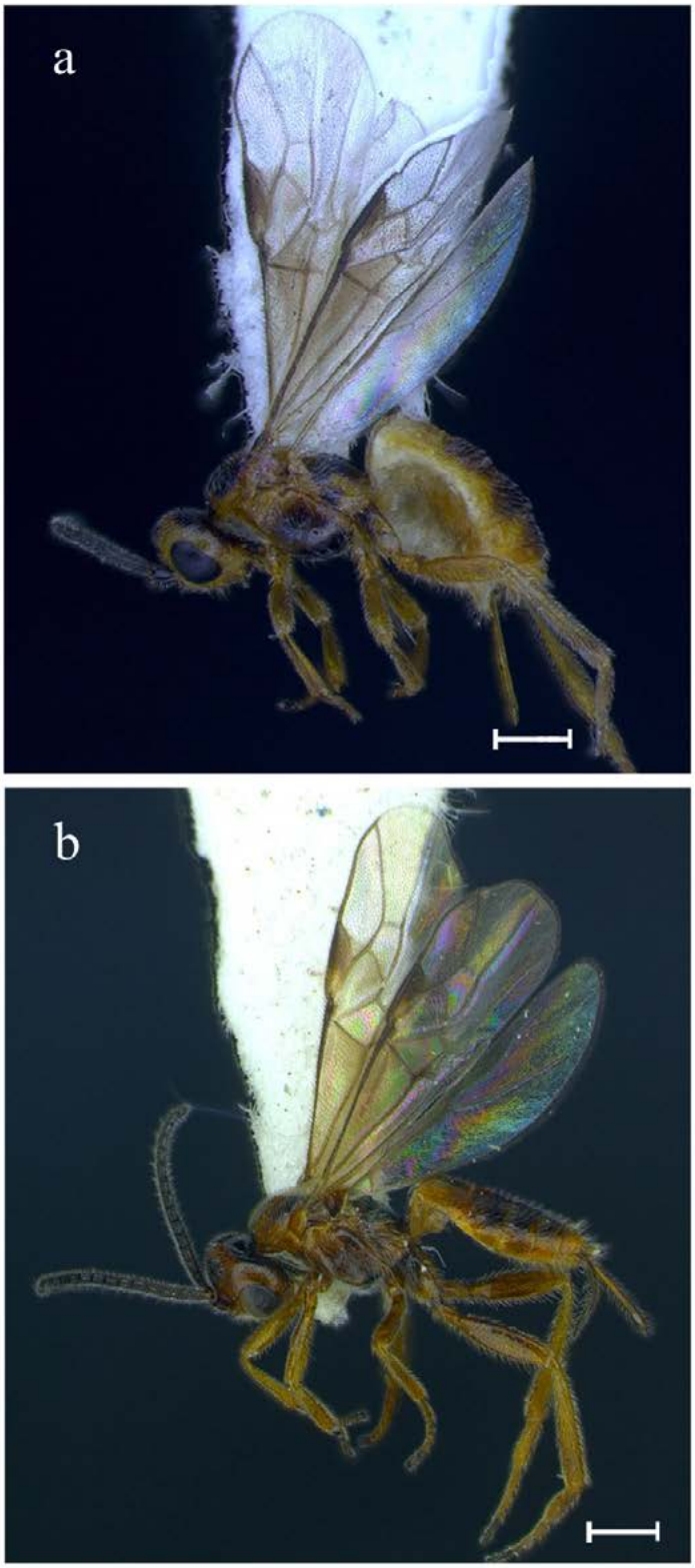


Figure 5(a–b). Lateral habitus, female. a) *Habrobracon hebetor* (Say); b) *Habrobracon brevicornis* (Wesmael); Scale bar = 0.5 mm.

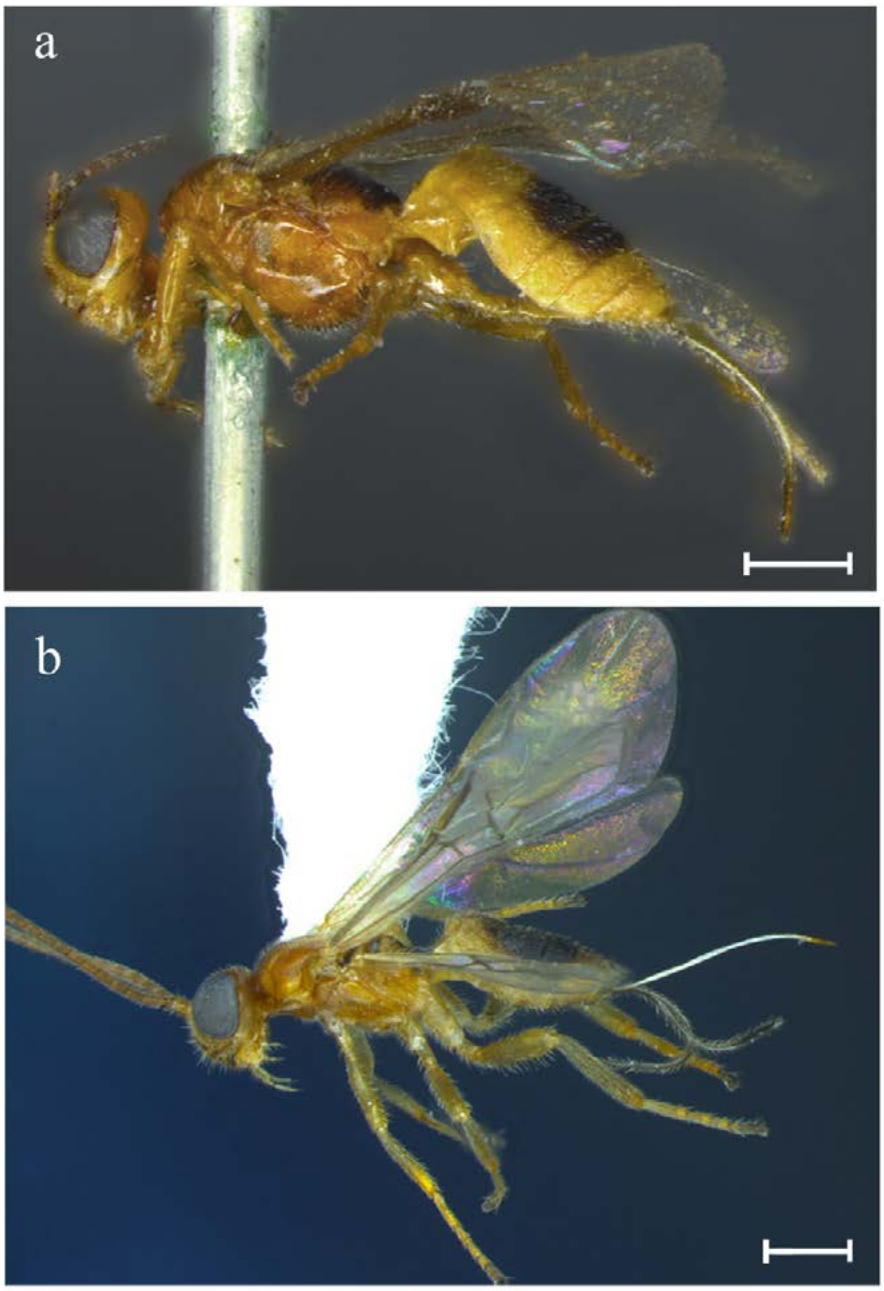


Figure 6(a–b). Lateral habitus, female. a) *Bracon greeni* Ashmead; b) *Bracon lefroyi* (Dudgeon and Gough); Scale bar = 0.5 mm.

Acknowledgements

The authors gratefully acknowledge the Director, Zoological Survey of India, Kolkata for the support. The senior author wishes to extend his gratitude to the Zoological Survey of India, Kolkata for financial assistance through the Post-Doctoral Research Fellowship. This contribution forms part of the PhD thesis of the senior author under the guidance of Dr. D.C. Ray, Professor, Department of Ecology and Environmental Science, Assam University, Silchar. This work was accomplished with the co-supervision of Prof. V.V. Ramamurthy, Ex. National Coordinator, ICAR Network Project on Insect Biosystematics, Division of Entomology, Indian Agricultural Research Institute, New Delhi.

References

Ameri, A., Talebi, A.A., Beyarslan, A., Kamali, K. and Rakhshani, E. 2013. Study of the genus *Bracon* Fabricius, 1804 (Hymenoptera: Braconidae) of Southern Iran with description of a new species. *Zootaxa*, 3754(4): 353–380.

Ashmead, W.H. 1896. On some reared parasitic hymenopterous insects from Ceylon. *Proceedings of the United States National Museum*, 18: 633–648.

Bakr. R.F.A., Gesraha, M.A., Guneidy, N.A.M., Frag, N.A.E., Ebeid, A.R., Elbehery, H.H.A. and Abou-Ellail, M. 2013. Molecular genetic identification of two *Bracon* species based on RAPd-PCR and 16sRNA genes. *Egyptian Academic Journal of Biological Science*, 5(2): 99–107.

Brues, C.T. 1920. On the bollworm parasite described as *Rogas lefroyi* by Dudgeon & Gough. *Report of the Proceedings of the Third Entomological Meeting,* held at Pusa, 3rd to 15th February 1919, Superintendent Government Printing, Calcutta, India, Vol. III. pp. 1026 1028.

Chawla, S.S. and Subba Rao, B.R. 1965: Further studies on the *Bracon hebetor-brevicornis*-complex (Hymenoptera: Braconidae) by paper chromatography. *Beiträge Zur Entomologie* (= *Contributions to Entomology*), 15(1-2): 83–86.

Cherian, M.C. and Margabandhu, V. 1949. 1951. Identity of *Microbracon brevicornis* Wesm. and *Microbracon hebetor* Say. *Journal of Bombay Natural History Society*, 48: 335–337.

Chomphukhiao, N., Takano, S.I., Takasu, K. and Uraichuen, S. 2018. Existence of two strains of *Habrobracon hebetor* (Hymenoptera: Braconidae): a complex in Thailand and Japan. *Applied Entomology and Zoology,* 53: 373–380.

Cushman, R.A. 1922. The identity of *Habrobracon brevicornis* (Wesmael) (Hym., Braconidae). *Proceedings of the Entomological Society of Washington*, 24: 241–242.

Deka, T.C. 1969. The effect of competition between two homologues on the maintenance of their sex-ratio. *Journal of the Assam Science Society*, 12: 134–140.

Dharmarju, E. 1952. The biological control of the black headed caterpillar of coconut (*Nephantis serinopa* M.) in the East Godavari district of Madras state. *Indian Coconut Journal*, 5: 171–176.

Ehteshami, F. and van Achterberg, C. 2012. First record of *Bracon* (*Habrobracon*) *brevicornis* (Wesmael) (Hymenoptera: Braconidae: Braconinae) from Iran. *In*: Conference: The 17th National & 5th International Iranian Biology Confrence, at Shahid Bahonar University of Kerman, Kerman, Iran, Volume: 17th National & 5th International Iranian Biology Confrence. https://www.researchgate.net/publication/303644176

Ghosh, S.M., Chandrasekharan, K. and Abdurahiman, U.C. 1993. The bionomics of *Pediobius imbreus* (Hymenoptera) and its impact on the biological control of the coconut caterpillar. *Journal of Ecobiology*, 5(3): 161–166.

Haider, A.A., Ahmad, Z., Pandey, K. and Shuja-Uddin. 2004. Description of a new species of the genus *Habrobracon* Ashmead (Hymenoptera: Braconidae), along with key to the Indian species. *Journal of Entomological Research* (*New Delhi*), 28(2): 153–156.

Heimpel, G.E., Antolin, M.F., Franqui, R.A. and Strand, M.R. 1997. Reproductive isolation and genetic variation between two "strains" of *Bracon hebetor* (Hymenoptera: Braconidae). *Biological control*, 9(3): 149–156.

Jayanth,K.P. and Nagarkatti, S. 1985. Low temperature storage of adult of *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae). *Entomon,* 10(1): 39–41.

Kittel, R.N. and Maeto, K. 2019. Revalidation of *Habrobracon brevicornis* stat. rest. (Hymenoptera: Braconidae) based on the CO1, 16S, and 28S gene fragments. *Journal of Economic Entomology*, 112(2): 906–911.

Krishnamurti, B. and Appana, M. 1944. *Microbracon brevicornis*, W. in the biological control of the lab-lab podborer. *Current Science* (Bangalore), 13: 135.

Lal, K.B. 1939. Identity of two important parasites hitherto considered as distinct species. *Current Science*, 8: 125–126.

Srivastava, M., Paul, A.V.N., Rengasamy, S., Kumar, J. and Parmar, B.S. 1997. Effect of neem (*Azadirachta indica* A. Juss) seed kernel extracts on the larval parasitoids *Bracon brevicornis* Wesm. (Hym., Braconidae). *Journal of Applied Entomology (= Zeitschrift fur Angewandte Entomologie),* 121(1): 51–57.

Mohanty, J.N., Anand, P. and Jagadiswari, R. 2000. Management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen. by release of *Bracon brevicornis* Wesm. In the field. *Journal of Applied Zoological Researches*, 11(2–3): 96–97.

Ram, A., Tiwari, L.D., Dass, R. and Mehrotra, K.N. 1982. Evidence for the presence of a kairomone in *Corcyra cephalonica* Staint larvae for *Bracon brevicornis* Wesm. (Hymen., Braconidae). *Journal of Applied Entomology (= Zeitschrift fur Angewandte Entomologie),* 93(4): 338–341.

Abbas, M.S.T. 1982. *Bracon brevicornis* Wesm., a larval parasite of the pink bollworm *Pectinophora gossypiella* Saunders. *Agricultural Research Review*, 58(1): 233–238.

Jacob, S., Abraham, C.C. and Joy, P.J. 1980. Regulation of fecundity, progeny production and female-male composition of *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae). *Agricultural Research Journal of Kerala*, 18(2): 191–199.

Mathai, S. 1972. Studies on the effect of host nutrition on *Bracon brevicornis* Wesmael. *Agricultural Research Journal of Kerala*, 9(1): 1–3.

Sarup, P., Singh, D.S., Srivastava, M.L. and Singh, R.N. 1971. Laboratory evaluation of pesticides against the adults of an important parasite *Bracon brevicornis* (Braconidae: Hymenoptera) of some crop pests. *Indian Journal of Entomology*, 33(3): 346–349.

Subba Rao, B.R. and Kumar, S.S. 1960. Effect of temperature and host density on the rate of increase of *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae). *Beitrage zur Entomologie*, 10(7-8): 872–885

Lal, K.B. 1947. The identification of *Bracon hebetor* (Say) and *B. brevicornis* (Wesmael). *Indian Journal of Entomology*, 8(1): 85–88.

Mathew, K.P., Abraham, C.C., Visalakshi, A. and Nair, M.R.G.K. 1980. On the improvement of female production in *Bracon brevicornis* Wesmael. *Entomon*, 5(3): 173–174.

Mohapatra, L.N. and Mohapatra, R. 2003. Incidence of cashew leaf and blossom webber, *Lamida moncusalis* Walker (Lepidoptera: Pyralidae) in relation to its natural enemies and weather factors. *Indian Journal of Plant Protection*, 31(1): 119–121.

Muesebeck, C.F.W. 1925. A revision of the parasitic wasps of the genus *Microbracon* occurring in America north of Mexico. *Proceedings of the United States National Museum,* 67: 1–85.

Naik, C.B., Kranthi, S., Gharade, S., Kumbhare, S., Nagrare, V.S. and Singh, L.R.K. 2018 Endoparasitoid: *Bracon lefroyi* (Dudgeon & Gough) of pink bollworm *Pectinophora gossypiella* (Saunders) on cotton. *Indian Journal of Entomology*, 80(2): 361-366.

Narayanan, E.S., Subba Rao, B.R. and Sharma, A.K. 1958. Studies on the *Bracon brevicornis-hebetor* complex (Hymenoptera: Braconidae). *Proceedings of the Indian Academy of Sciences*, *Section B*, 48(1): 1–13.

Narendran, T.C., Mohamed, U.V.K., Abdurahiman, U.C. and Sudheendra Kumar, V.V. 1981, Oviposition behaviour of *Braeon brevicornis* Wesmael (Hymenoptera: Braconidae). *Agricultural Research Journal of Kerala*, 18(2): 239–241.

Nasser, M. and Abdurahiman, U.C. 1998. Efficacy of *Cardiastethus exiguus* Poppius (Hemiptera: Anthocoridae), as a predator of the coconut caterpillar, *Opisina arenosella* Walker (Lepidoptera: Xylorictidae). *Journal of Entomological Research* (New Delhi), 22(4): 361–2368.

Papp, J. 2008. A revision of the *Bracon* (subgenera *Bracon* s. str.; *Cyanopterobracon*, *Glabrobracon*, *Lucobracon*, *Osculobracon* subgen. n., *Pigeria*) species described by Szépligeti from the Palaearctic Region (Hymenoptera: Braconidae: Braconinae). *Linzer Biologische Beiträge*, 40(1): 1741–1837.

Papp, J. 2012. A revision of the *Bracon* Fabricius species in Wesmael's collection deposited in Brussels (Hymenoptera: Braconidae: Braconinae). *European Journal of Taxonomy*, 21: 1–154.

Puttarudriah, M. and Channa Basavanna, G.P. 1956. A study on the identity of *Bracon hebetor* Say and *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae). *Bulletin of Entomological Research*, 47(1): 183–191.

Quicke, D.L.J. 1982. The genus *Shelfordia* Cameron (Hymenoptera: Braconidae): Discovery of type specimen, reclassification of species, new synonymy and notes on related genera. *Oriental Insects*. 15: 227–233.

Quicke, D.L.J. 1987. The Old World genera of braconine wasps (Hymenoptera: Braconidae). *Journal of Natural History*, 21: 43–157.

Ramakrishna Ayyar, T.V. 1928. A contribution to our knowledge of south Indian Braconidae. Part I. Vipioninae. *Memoirs of the Department of Agriculture in India* (*Entomological Series*), 10(3): 29–60, 60a–f.

Rukhsana, K., and Sebastian, C.D. 2015. Genetic structure and molecular phylogeny analysis of *Bracon brevicornis* Wesmael, a larval parasitoid of coconut black headed caterpillar, *Opisina arenosella* Walker. *Research in Biotechnology*, 6: 17–23.

Sharma, K.K., Jaiswal, A.K. and Kumar, K.K. 2000. A new record of *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae) as a parasitoid of *Eublemma amabilis* Moore – a major predator of lac insect, *Kerria lacca* Kerr. *Journal of Applied Zoological Researches*, 11(2–3): 156–156.

Sheeba, M. and Narendran, T.C. 2007. On the identity of two confusing species of Braconidae (Hymenoptera) parasitic on *Opisina arenosella* (Walker). *Journal of Plantation Crops*, 35(3): 195-197.

Sheeba, M. and Narendran, T.C. 2013. A review of *Bracon* species of India with a key to Indian species. *Records of the Zoological Survey of India*, *Occational paper no.* 348: 1–52.

Sudheendrakumar, V.V., Narendran, T.C., Mohamed, U.V.K. and Abdurahiman, U.C. 1982, Biology and morphology of immature stages of *Bracon brevicornis* (Hymenoptera: Braconidae) an important biological control agent of the black headed caterpillar pest of coconut. *Journal of the Bombay Natural History Society*, 79(2): 254–2260.

van Achterberg, C. 1979. A revision of the subfamily Zelinae auct (Hymenotpera: Braconidae). *Tijdschrift voor Entomologie*, 122: 1–479.

van Achterberg, C. 1992. Four new genera of the subfamily Braconinae (Hymenoptea: Braconidae) from the Indo-Australian region. *Zoologische Mededelingen* (*Leiden*), 66(27): 381–397.

van Achterberg, C. 1993. Illustrated key to the subfamilies of the Braconidae. (Hymenoptera: Ichneumonoidea). *Zoologische Verhandelingen* (*Leiden*), 283(1): 1–189.

van Achterberg, C. and Polaszek, A. 1996. The parasites of cereal stem borers (Lepidoptera: Cossidae, Crambidae, Noctuidae, Pyralidae) in Africa, belonging to the family Braconidae (Hymenoptera: Ichneumonoidea). *Zoologische Verhandelingen* (*Leiden*), 304: 1–123.

van Achterberg, C. and Quicke, D.L.J. 1992. Phylogeny of the subfamilies of the family Braconidae: a reassessment assessed. *Cladistics*, 8: 237–264.

van Achterberg, C. and Walker, A.K. 1998. 17. Braconidae, pp. 137–185. *In*: Polaszek, A. (ed.). African cereal stem borers. Economic importance, taxonomy, natural enemies and control. CAB International, Wallingford, UK. 530 pp.

Venkatesan, T., Jalali, S.K. and Srinivasamurthy, K. 2009. Competitive interactions between *Goniozus nephantidis* and *Bracon brevicornis*, parasitoids of the coconut pest *Opisina arenosella. International Journal of Pest Management*, 55(3): 257–263.

Yu, D.S., van Achterberg, C. and Horstmann, K. 2012. Taxapad 2012, Ichneumonoidea 2011. Ottawa, Canada. Database on CDROM. Available from: http://www.taxapad.com (accessed 16 June 2018).