

# STUDIES ON WINGS SYMMETRY AND HONEY BEE RACES DISCRIMINATION BY USING STANDARD AND GEOMETRIC MORPHOMETRICS

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**Abstract:** Morphometric is an essential tool for honey bee races discrimination and characterization. Such vital tool has been applied widely in honey bee researches. Unfortunately there is no available literature for confirming honey bee wings symmetry. Therefore, standard and geometric morphometric analyses were employed for investigating wings symmetry as well as for discriminating between Carniolan and Yemeni honey bees. Moreover, three angles of hind wings (H1, H2 and H3) were evaluated in the discrimination between the two races. Results of morphometric analyses strongly confirmed the symmetry of honey bee wings. Standard and geometric morphometric analyses successfully discriminate between the two races. Hind wing length and angle H3 could be incorporated in honey bee races discrimination.

**Key words:** honey bee, morphometry, symmetry, wings

## Introduction

The common method for the characterization and classification of honey bee subspecies is based mainly on measuring honey bee wing characters, which were considered as strong tool (*Rattanawanee et al., 2010*). Various honey bee colonies, races and species were discriminated by employing morphometric analysis (*Moradi and Kandemir, 2004; Raina and Kimbu, 2005; Farhoud and Kence, 2005; Shaibi et al., 2009; Rattanawanee et al., 2010; Nedić et al., 2011*). Standard morphometric was used in honey bee studies by measuring different wing angles, indices and distances (*Ruttner, 1988*) while geometric morphometric was used in honey bee studies by measuring the coordinates of fore wing points to calculate the centroid size (*s, Tofilski, 2008*). Not many studies were done on geometric morphometric of honey bees.

Concerning hind wings, only hind wing distances and number of hooks were used widely during morphometric studies (*Moradi and Kandemir, 2004 and*

*Abou-Shaara et al., 2012*) but no hind wing angles were incorporated in the morphometric analysis previously. Wing characters were found to be affected by different factors e.g. temperature (*Tan et al., 2005*), season (*Mattu and Verma, 1984*) and bee age (*Herbert et al., 1988*).

The right fore and hind wings were used by some authors during their morphometric analysis (*Nazzi, 1992; Andere et al., 2008; Uzunov et al., 2009; Mladenović et al., 2011 and Abou-Shaara et al., 2012*) while left wings were used by others (*Bouga and Hatjina, 2005 and Tofilski, 2008*). Fluctuating asymmetry and directional asymmetry were studied by some authors (*Smith et al., 1997 and Schneider et al., 2003*). *Smith et al. (1997)* found that honey bee drones were with higher directional asymmetry than females while *Schneider et al. (2003)* found higher fluctuation asymmetry of shape for European and hybrid workers than for African workers. The symmetry of left and right wings is the normal hypothesis as far there is no any malformations or abnormalities within wings. However, there are no available literatures that can confirm honey bee wings symmetry. Thus, standard and geometric morphometrics were employed in combination with various computer softwares for investigating wings symmetry and for discriminating between Carniolan and Yemeni honey bees. Moreover, three hind wing angles were measured and evaluated for the discrimination between the two races.

## Materials and Methods

The study was done at the Bee Research Unit, King Saud University. Thirty right and left fore wings and hind wings for two honey bee races, Yemeni honey bees (*Apis mellifera jementica*) and Carniolan honey bees (*Apis mellifera carnica*), were used in this study (total of 120 fore wings and 120 hind wings for the two races). Samples of honey bee workers were collected from brood combs and subsequently killed at -20°C and dissected by using forceps to separate fore wings and hind wings. Separated wings were scanned by using HP scanner at 1200 dpi to obtain wing images then the following investigations were performed.

### 1. Symmetry of fore wings

Cubital index and discoidal shift for left and right wings were measured by Beemorph program (<http://www.hockerley.plus.com/>). Inner wing length, inner wing width, distance C and distance D were measured by ScanPhoto method according to *Abou-Shaara et al. (2011)* by using Photoshop program. Fore wing angles (A1, A4, D7, J16, k19 and O26) were measured by ImageTool 3.0 program (<http://compdent.uthscsa.edu/dig/itdesc.html>). Analysis of Variance (ANOVA) was done for measured characters and means were compared by using Least Significant Difference ( $P < 0.05$ ),  $L.S.D_{.05}$ , by SAS 9.1.3 program (*SAS Institute, 2004*).

Coordinates of 18 points (Figure 1) were determined by using ImageJ 1.46 program then MorphoJ program (Klingenberg, 2011) was used for geometric morphometric analysis. Point sets of right and left fore wings were compared by measuring centroid size, is the square root of squared distance sums of points set from their centroid, and matrix correlation.

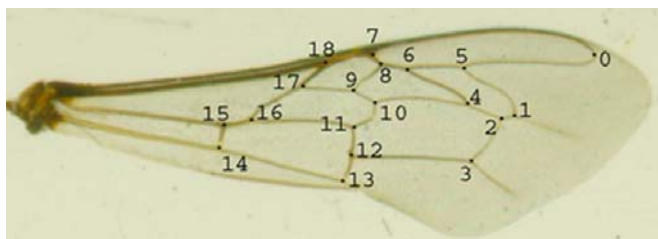


Figure 1. Eighteen fore wing points

## 2. Symmetry of hind wings

Hind wing length and width were measured by using ScanPhoto method for right and left wings. Three hind wing angles; H1, H2 and H3 (Figure 2) were measured by using ImageTool 3.0 program. These angles were used for confirming the symmetry of hind wings and for discriminating between the two races. ANOVA was performed for measured characters and means were compared by using L.S.D.<sub>0.05</sub>.

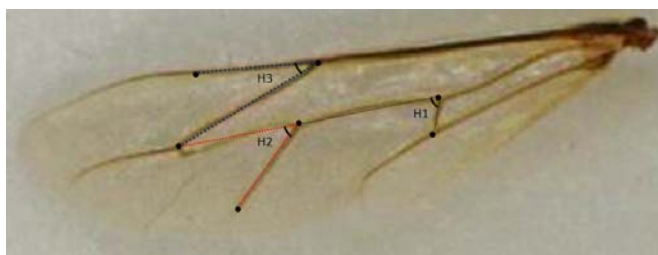


Figure 2. Three suggested angles for hind wings

## 3. The discrimination between Carniolan and Yemeni honey bees

Standard morphometric was used for the discrimination between the two races by using four lengths and six angles of right fore wings as well as by using right hind wing characters. Forward stepwise with tolerance = 0.01, F-to-Enter: 1.000 and F-to-Remove: 0.900 was used to identify canonical discriminant functions by using SYSTAT 13 program. Also, ANOVA was performed and means of fore and hind wing were compared by using L.S.D.<sub>0.05</sub> to identify significant differences between the two races. Moreover, geometric morphometric

of fore wing points was used for the discrimination between the two races based on their transformation grids and their centroid size.

## Results and Discussion

### 1. Symmetry of fore wings

Mean of right wings for Yemeni honey bee was  $2.54 \pm 0.12$  for cubital index and  $-2.25 \pm 0.51$  for discodial shift while for left wings mean was  $2.45 \pm 0.11$  for cubital index and  $-2.14 \pm 0.55$  for discodial shift. No significant differences were found between cubital index and discodial shift means (L.S.D.<sub>0.05</sub> values were 0.3259 and 1.5118 for cubital index and discodial shift, respectively). For Carniolan honey bees, means of cubital index and discodial shift were  $2.32 \pm 0.05$  and  $2.71 \pm 0.38$ , for right fore wings, respectively and means for left wings were  $2.34 \pm 0.07$  and  $2.60 \pm 0.39$ , respectively. No significant differences were found between cubital index and discodial shift (L.S.D.<sub>0.05</sub> values were 0.19 and 1.11 for cubital index and discodial shift, respectively). Also, no significant differences were found between studied sides in all measured lengths and angles (Table 1).

**Table 1. Means  $\pm$  SE of lengths and angles for Carniolan and Yemeni honey bee wings**

Carniolan honey bees:										
Side	IWL	IWW	DC	DD	A1	A4	D7	J16	K19	O26
Right	4.37 $\pm$ 0.02	1.95 $\pm$ 0.01	0.85 $\pm$ 0.01	1.87 $\pm$ 0.01	22.30 $\pm$ 0.46	31.39 $\pm$ 0.59	94.61 $\pm$ 0.63	104.20 $\pm$ 1.25	79.99 $\pm$ 0.96	40.57 $\pm$ 1.07
Left	4.37 $\pm$ 0.02	1.94 $\pm$ 0.01	0.85 $\pm$ 0.01	1.91 $\pm$ 0.01	24.06 $\pm$ 0.76	31.08 $\pm$ 0.39	94.96 $\pm$ 0.66	103.39 $\pm$ 1.08	80.59 $\pm$ 0.88	42.23 $\pm$ 1.18
L.S.D. <sub>0.05</sub>	0.05*	0.03	0.03	0.03	1.78	1.43	1.83	3.30	2.61	3.19
Yemeni honey bees:										
Side	IWL	IWW	DC	DD	A1	A4	D7	J16	K19	O26
Right	3.96 $\pm$ 0.01	1.80 $\pm$ 0.003	0.78 $\pm$ 0.001	1.73 $\pm$ 0.004	22.75 $\pm$ 0.80	33.23 $\pm$ 0.65	101.78 $\pm$ 0.84	95.14 $\pm$ 1.10	79.05 $\pm$ 0.85	44.49 $\pm$ 1.40
Left	3.96 $\pm$ 0.01	1.81 $\pm$ 0.005	0.78 $\pm$ 0.002	1.73 $\pm$ 0.004	21.23 $\pm$ 0.69	35.05 $\pm$ 0.64	101.26 $\pm$ 0.82	96.17 $\pm$ 0.92	78.44 $\pm$ 1.16	45.69 $\pm$ 1.59
L.S.D. <sub>0.05</sub>	0.06*	0.03	0.02	0.03	2.13	1.83	2.35	2.89	2.88	4.24

\* No significant differences were found between left and right means for all characters.

IWL: Inner wing length, IWW: Inner wing width, DC: Distance C and DD: Distance D.

Centroid size for Carniolan honey bees was 0.0229 for right wings and 0.0233 for left wings and matrix correlation between matrix 1 for right wings and Matrix 2 for left wings was 0.77. On the other side, centroid size for Yemeni honey bees was 0.0233 for right wings and 0.0258 for left wings and matrix correlation between matrix 1 and matrix 2 was 0.83.

## 2. Symmetry of hind wings

No significant differences were found for measured right and left hind wing characters for Yemeni and Carniolan honey bees (Table 2).

**Table 2. Means  $\pm$  SE of lengths and angles for Yemeni honey bee wings**

Side	Carniolan honey bees (Mean $\pm$ SE)					Yemeni honey bees (Mean $\pm$ SE)				
	HWL	HWW	H1	H2	H3	HWL	HWW	H1	H2	H3
Right	6.17 $\pm$ 0.02	1.88 $\pm$ 0.02	67.41 $\pm$ 1.16	47.45 $\pm$ 0.55	27.13 $\pm$ 0.18	5.63 $\pm$ 0.03	1.82 $\pm$ 0.02	64.61 $\pm$ 1.36	45.41 $\pm$ 0.92	27.22 $\pm$ 0.38
Left	6.15 $\pm$ 0.02	1.89 $\pm$ 0.03	68.61 $\pm$ 1.36	47.50 $\pm$ 0.50	26.69 $\pm$ 0.30	5.61 $\pm$ 0.04	1.81 $\pm$ 0.04	66.50 $\pm$ 0.65	45.77 $\pm$ 0.70	26.62 $\pm$ 0.25
L.S.D. <sub>0.05</sub>	0.07*	0.08	3.59	1.50	0.71	0.11	0.09	3.02	2.33	0.92

\* No significant differences were found between left and right means for all characters.

HWL: Hind wing length, HWW: Hind wing width, H1: Angle H1, H2: Angle H2 and H3: Angle H3.

In general, standard morphometric analysis strongly confirmed the symmetry of honey bee wings where no significant differences were found between all measured characters for fore and hind wings. The most identical characters were inner wing length, inner wing width, distance C and distance D. Based on geometric morphometric, high degree of similarity was found between centroid size of left and right wings for Carniolan and Yemeni honey bees. Relatively high correlation values were found between point sets of right and left wings for the two races. Thus the symmetry of honey bee wings was confirmed by standard and geometric morphometric analyses. Some factors were reported to impact on the normal wing venation pattern including pupal rearing temperature (*Tan et al., 2005*), season (*Mattu and Verma, 1984*) and deformed wing virus (DWV). Apart from these factors, right and left wings are identical.

## 3. The discrimination between the two races

Standard morphometric analysis by using fore wing characters showed that inner wing length and width were the most successful characters in discriminating between the two races without overlapping (Figure 3). Significant differences were found between the two races in all measured fore wing characters except angle A1 and angle K19 (Table 3). Concerning the discrimination between the two races by using hind wing characters, only hind wing length and angle H3 were used successfully for the discrimination between the two races without overlapping (Figure 4). Significant difference was found between means of hind wing length while no significant differences were found between means of all other hind wing characters (Table 3).

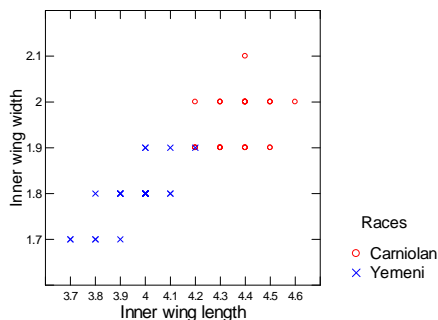


Figure 3. The discrimination between Yemeni and Carniolan honey bees based on inner wing length and width

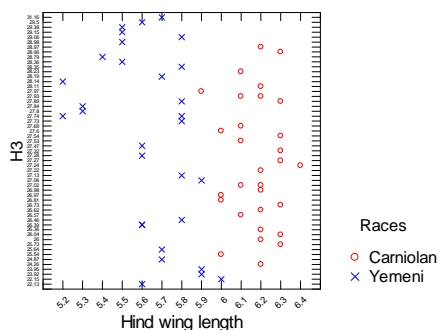


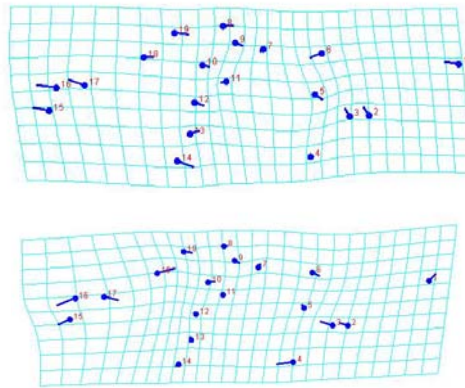
Figure 4. The discrimination between Yemeni and Carniolan honey bees based on angle H3 and hind wing length

Table 3. Lengths and angles means  $\pm$  SE for Carniolan and Yemeni honey bee wings

Race	Fore wing characters (Mean $\pm$ SE)									
	IWL	IWD	DC	DD	Angle A1	Angle A4	Angle D7	Angle J16	Angle K19	Angle O 26
Carniolan	4.37 $\pm$ 0.02	1.95 $\pm$ 0.01	0.85 $\pm$ 0.01	1.87 $\pm$ 0.01	22.30 $\pm$ 0.46	31.39 $\pm$ 0.59	94.61 $\pm$ 0.63	104.20 $\pm$ 1.25	79.99 $\pm$ 0.96	40.57 $\pm$ 1.07
Yemeni	3.96 $\pm$ 0.01	1.80 $\pm$ 0.003	0.78 $\pm$ 0.001	1.73 $\pm$ 0.004	22.75 $\pm$ 0.80	33.23 $\pm$ 0.65	101.78 $\pm$ 0.84	95.14 $\pm$ 1.10	79.05 $\pm$ 0.85	44.49 $\pm$ 1.40
L.S.D. <sub>0.05</sub>	0.05	0.029	0.02	0.03	1.85*	1.76	2.09	3.33	2.58*	3.53
Race	Hind wing characters (Mean $\pm$ SE)					* No significant differences were found between Yemeni and Carniolan character.				
	HWL	HWW	H1	H2	H3					
Carniolan	6.17 $\pm$ 0.02	1.88 $\pm$ 0.02	67.41 $\pm$ 1.16	47.45 $\pm$ 0.55	27.13 $\pm$ 0.18					
Yemeni	5.63 $\pm$ 0.03	1.82 $\pm$ 0.02	64.61 $\pm$ 1.36	45.41 $\pm$ 0.92	27.22 $\pm$ 0.38					
L.S.D. <sub>0.05</sub>	0.09	0.07*	3.59*	2.16*	0.85*					

IWL: Inner wing length, IWD: Inner wing width, DC: Distance C, DD: Distance D. HWL: Hind wing length, HWW: Hind wing width, H1: Angle H1, H2: Angle H2 and H3: Angle H3.

Geometric morphometric analysis was used for the discrimination between the two races by using centroid size and transformation grid shape. Right and left fore wings of Yemeni honey bees had higher centroid size than Carniolan honey bees. Also, differences in transformation grids were found between fore wings of the two races (Figure 5).



**Figure 5. Transformation grids of right fore wing for Carniolan honey bees (above) and Yemeni honey bees (below)**

The effectiveness of fore wing angles in separating between honey bee groups was identified by *Nedić et al. (2011)* during their work on collected honey bee samples from different locations of Serbia. The discrimination between the two races based on standard morphometric of fore wings showed that all measured morphological characters, including fore wing angles, were with significant differences between the two races except angle A1 and angle K19. Inner wing length and width were the most successful characters in discriminating between the two races accurately. The discrimination between the two races based on hind wing characters showed that no significant differences were found between character means except hind wing length. Canonical discriminant analysis showed that hind wing length and angle H3 were used correctly for discriminating between the two races. Thus, hind wing length and angle H3 could be incorporated in honey bee races discrimination.

Moreover, geometric morphometric was also succeeded in discriminating between the two races. In accordance with *Tofilski (2008)*, who classified successfully colonies of three honey bee races; *A. m. mellifera*, *A. m. carnica* and *A. m. caucasica* by using standard and geometric morphometric and correctly geometric morphometric identified African and European honey bees by about 99% (*Francoy et al., 2008*) as well as Asian honey bee species (*Rattanawanee et al., 2010*). In general, standard and geometric morphometric analyses were used successfully in the discrimination between the two races.

## Conclusion

Apart from the abnormal wing venations which occasionally occur within wings due to diseases or other factors, fore and hind wings of honey bees showed high symmetric degree based on standard and geometric morphometric and by employing various software programs. However, mixing right and left wings during the morphometric analysis is not recommended. The discrimination between the studied races was done successfully by standard and geometric morphometric analyses. Also, MorphoJ program can be used sufficiently for performing geometric morphometric analysis of honey bee wings as an integrated program. Finally, hind wing length and angle H3 could be incorporated in honey bee races discrimination.

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## Ispitivanje simetrije krila i razlikovanja rasa medonosnih pčela korišćenjem standardne i geometrijske morfometrije

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## Rezime

Morfometrija je neophodan instrument u razlikovanju rasa medonosnih pčela i njihovoj karakterizaciji i nalazi široku upotrebu u istraživanjima pčela. Na žalost, ne postoji literatura koja može da potvrdi studije simetrije krila pčela. Dakle, standardne i geometrijske morfometrijske analize su korišćene u analizi simetrije krila kao i za razlikovanje medonosnih pčela rase Carniolan i Yemeni. Štaviše, tri ugla zadnjih krila (H1, H2 i H3) su korišćena u razlikovanju rasa pčela. Rezultati morfometrijske analize potvrđuju simetriju krila pčela. Korišćenjem standardne i geometrijske morfometrijske analize mogu se uspešno razlikovati rase pčela. Dužina zadnjih krila i ugao H3 se uspešno mogu uključiti u analizu i određivanje rasa medonosnih pčela.



## References

- ABOU-SHAARA H.F., DRAZ K.A., AL-AW M., EID K. (2011): Simple method in measuring honey bee morphological characters. Proceedings of 42<sup>nd</sup> International Apicultural Congress– APIMONDIA in Buenos Aries (Argentina), 21<sup>th</sup>-25<sup>th</sup> September, p. 222.
- ABOU-SHAARA H.F., DRAZ K.A., AL-AW M., EID K. (2012): Stability of honey bee morphological characters within open populations. *Uludag Bee Journal*, 12,1, 31-37.
- ANDERE C., GARCIA C., MARINELLI C., CEPEDA R., RODRIGUEZ E.M., PALACIO A. (2008): Morphometric variables of honeybees *Apis mellifera* used in ecotypes characterization in Argentina. *Ecological Modelling*, 214,53–58.
- BOUGA M., HATJINA F. (2005): Genetic variability in Greek honey bee (*A. mellifera* L.) populations using geometric morphometrics analysis. Proceedings of the Balkan scientific conference of biology in Plovdiv (Bulgaria), 1<sup>st</sup> -19<sup>th</sup> May, p. 598–602.
- FARHOUD H.J., KENCE M. (2005): Morphometric and MtDNA analysis in honeybee populations (*Apis mellifera* L.) of north and northwest Iran. Proceedings of the Balkan scientific conference of biology in Plovdiv (Bulgaria), 1<sup>st</sup> -19<sup>th</sup> May, p. 594–597.
- FRANCOY T.M., WITTMANN D., DRAUSCHKE M., MULLER S., STEINHAGE V., BEZERRA-LAURE M.A.F., DE JONG D., GONCALVES L.S.(2008): Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. *Apidologie*, 39,5,488-494.
- HERBERT E.W., SYLVESTER H.A., VANDENBERG J.D., SHIMANUKI H. (1988): Influence of nutritional stress and the age of adults on the morphometrics of honey bees (*Apis mellifera* L.). *Apidologie*, 19,3,221-230.
- KLINGENBERG C.P. (2011): MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11,353-357.
- MLADENović M., RENATA R., STANISAVLJEVIĆ L.Ž., RAŠIĆ S. (2011): Morphometric traits of the yellow honeybee (*Apis mellifera carnica*) from Vojvodina (Northern Serbia). *Archives of Biological Sciences*. 63,1,251-257.
- MORADI M., KANDEMIR I. (2004): Morphometric and Allozyme Variability in Persian Bee Population from the Alburz Mountains, Iran. *Iranian International Journal of Science*, 5,2,151-166.
- MATTU V.K., VERMA L.R. (1984): Morphometric studies on the Indian honey bee, *Apis cerana indica* F. Effect of seasonal variations. *Apidologie*, 15,63-74.
- NAZZI F. (1992): Morphometric analysis of honey bees from an area of racial hybridization in northeastern Italy. *Apidologie*, 23,89-96.
- NEDIĆ N., JEVIĆ G., JEŽ G., ANĐELKOVIĆ B., MILOSAVLJEVIĆ S., KOSTIĆ M. (2011): Forewing differentiation of the honey bees from Serbia. *Biotechnology in Animal Husbandry*, 27,3,1387-1394.

- RAINA S.K., KIMBU D.M. (2005): Variations in races of the honeybee *Apis mellifera* (Hymenoptera: Apidae) in Kenya. *International Journal of Tropical Insect Science*, 25,4,281–291.
- RATTANAWANNEE A., CHANCHAO C., WONGSIRI S. (2010): Gender and Species Identification of Four Native Honey Bees (Apidae: *Apis*) in Thailand Based on Wing Morphometric Analysis. *Annals of the Entomological Society of America*. 103,6,965-970.
- RUTTNER F. (1988): *Biogeography and taxonomy of honeybees*, Springer-Verlag, Berlin.
- SAS INSTITUTE (2004): *The SAS System Version 9.1.3*. SAS Institute, Cary,NC.
- SCHNEIDER S.S., LEAMY L.J., LEWIS L.A., DEGRANDI-HOFFMAN G. (2003): The influence of hybridization between African and European honeybees, *Apis mellifera*, on asymmetries in wing size and shape. *Evolution*, 57,10,2350–2364.
- SMITH D.R., CRESPI B.J., BOOKSTEIN F.L. (1997): Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. *Journal of Evolutionary Biology*, 10,551-574.
- SHAIBI T., FUCHS S., MORITZ R.F.A. (2009): Morphological study of Honeybees (*Apis mellifera*) from Libya. *Apidologie*, 40,97–105.
- TAN K., BOCK F., FUCHS S., STREIT S., BROCKMANN A., TAUTZ J. (2005): Effects of brood temperature on honey bee *Apis mellifera* wing morphology. *Acta Zoologica Sinica*, 51,4,768-771.
- TOFILSKI A. (2008): Using geometric morphometrics and standard morphometry to discriminate three honeybee subspecies. *Apidologie*, 39,558–563.
- UZUNOV A., KIPRIJANOVSKA H., ANDONOV S., NAUMOVSKI M., GREGORC A. (2009): Morphological diversity and racial determination of the honey bee (*Apis mellifera* L.) population in the Republic of Macedonia. *Journal of Apiculture Research*, 48,3,196 – 203.