

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

THE SOUTH-EASTERN DISTRIBUTION LIMIT OF *Apis mellifera*, A MORPHOMETRIC STUDY OF THE HONEY BEES (*Apis mellifera* L.) OF SISTAN-BELUTSCHISTAN

Apis mellifera'nın Güney-Doğu Yayılış Sınırları, Sistan-Belutschistan Balarılarında (*Apis mellifera* L.) Morfometrik Çalışma

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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ABSTRACT

The western honeybee, *Apis mellifera*, covers an immense range of natural distribution. In contrast to the limits in the North, West and South defined by the sea, the continental limits in the East are far less clear. Here we present detailed morphometric data on the Bees of Sistan-Belutschistan in the far South-Eastern End of the *A. mellifera* distribution. These are of particular interest because they inhabit a comparatively narrow desert climate land strip separating the distribution range from the Eastern honey bee, *Apis cerana*. Samples from the area differed quite substantially from the next-neighbouring Iran bee population, particularly by small body size, short hair, coloration patterns, and comparatively broad wings, but were clearly allocated to *A. m. meda* rather than to other representative *A. mellifera* subspecies. The study expands the South-Eastern range of *A. m. meda* by a particularly heat-adapted subpopulation, considerably narrowing the geographic gap to *A. cerana* in Pakistan.

Keywords: *Apis mellifera meda*, distribution range, morphometry

ÖZ

Batı bal arısı *Apis mellifera* çok geniş doğal bir yayılışa sahiptir. Batı ve Güney'deki sınır denizle tanımlanırken Doğu'daki kıtasal sınır Kuzey sınırına göre çok daha belirsizdir. Burada *Apis mellifera*'nın Güneydoğu sınırının son kısmında Sistan-Belutschistan arıları hakkında detaylı morfometrik verileri sunuyoruz. Bu bölge özel ilgi alanıdır çünkü bu arılar oldukça dar bir çöl iklim bölgesinde Doğu arısı *Apis cerana* yayılış bölgesini ayıran bir çizgide bulunmaktadır. Bölgeden alınan numuneler hemen aynıdaki İran arı popülasyonundan oldukça farklı olup özellikle küçük vücut büyüklüğü, kısa kıllar, renk durumları, karşılaştırmalı geniş kanatları ile başka bir *Apis mellifera* alt türünden çok net bir şekilde *A. m. meda*'ya dahil olmaktadır. Bu çalışma *A. m. meda*'nın güneydoğu bölgesine uzanan sıcaklığa adapte olmuş alt popülasyonu olup *A. cerana* ile aradaki coğrafik boşluğu ciddi şekilde daraltmaktadır.

Anahtar Kelimeler: *Apis mellifera meda*, yayılış alanı, morfometri

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INTRODUCTION

In this study we reinvestigate the south-eastern distribution of *Apis mellifera*, in an area of specific interest. The South-Eastmost region of Iran, Sistan-Belutschistan, forms a land strip of about 1100 km, which stretches from Zabol bordering Afghanistan in the North, along the border of Pakistan to Chabahar in the South, at the Gulf of Oman (Fig. 1). It covers an area of about 181578 km² inhabited by about 2.4 m people. Little is known about honey bees and beekeeping in this region dividing the

distribution ranges of *A. mellifera* and *A. cerana* without any apparent overlap. According to Ruttner et al (1985), the most eastern *A. mellifera* were found close to Dalfarh (28°58N 57°38E) in the district of Kerman, located west of Sistan-Belutschistan. They reported that there were no *A. mellifera* bees in Sistan-Belutschistan due to its extremely dry desert climate, but reported the presence of the dwarf honey bee, *A. florea*. More to the East, from Pakistan and Afghanistan onwards, the Eastern honey bee, *Apis cerana*, is found.

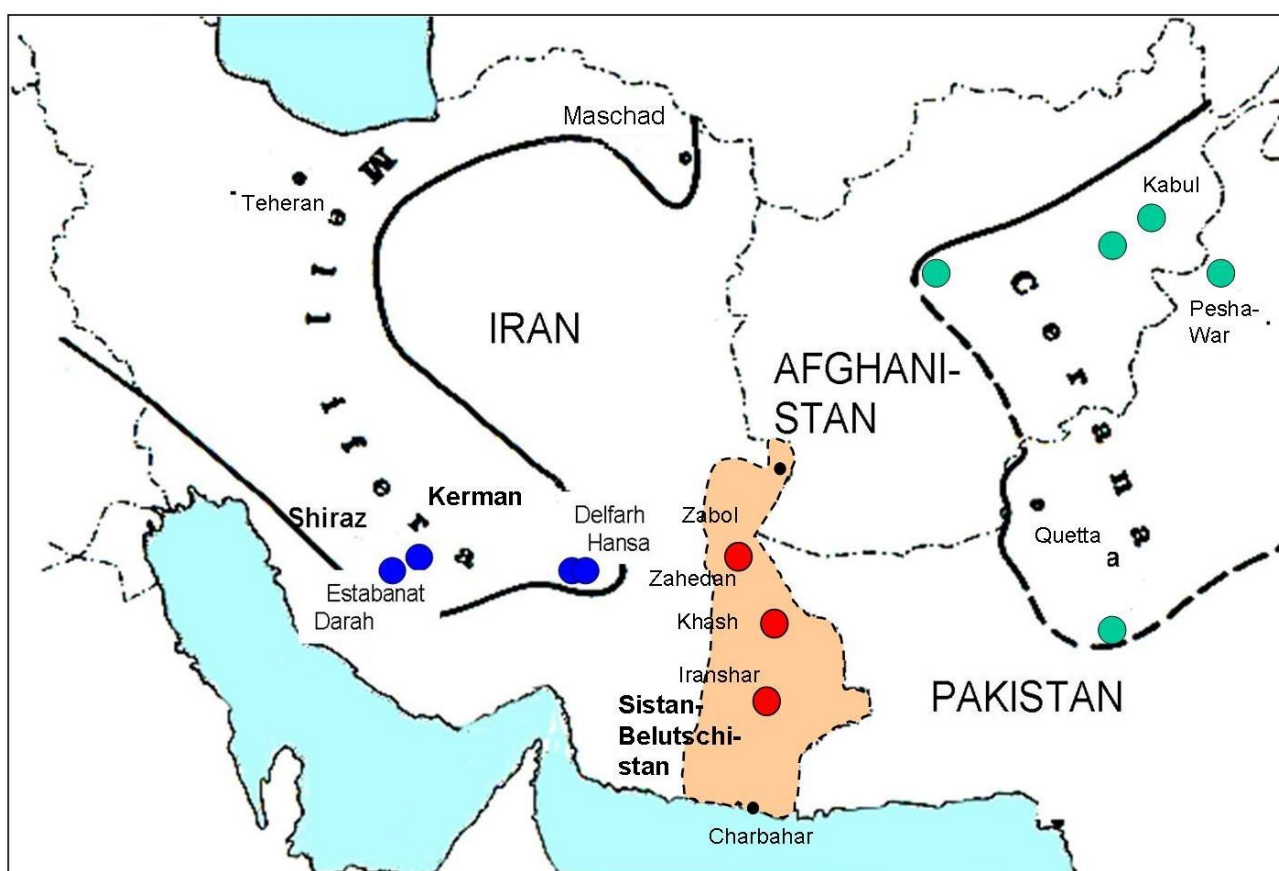


Fig. 1 The south-eastern boundaries of the *A. mellifera* distribution area, as well as the west-eastern boundaries of *A. cerana*, modified from Ruttner et al. (1985). The blue symbols give the hitherto eastmost sampling locations of *A. mellifera*, green symbols the westmost of *A. cerana*. Red symbols show the sampling locations in this study. No *A. mellifera* bees were found at Charbahar.

In this study, a reinvestigation of that Eastern limit of *A. mellifera* distribution range was undertaken. In a sampling excursion in 2005, we found that quite some beekeeping takes place in the area.

According to local estimates this comprised of 1299 beekeepers in the year 1998, which had decreased to about 200 in 2005 due to long-lasting periods of extreme dearth (>22mm rainfall/year). Traditional

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beekeeping had been performed using common clay pot hives, but at that time the about 3.000 colonies were kept modern Langstroth hives. A wide variety of plants are visited by the bees for honey and pollen. Bees are prone to swarming, and are fairly defensive. Common natural enemies of the bees were Beewolves (*Philantus spec.*), bee eaters, and ants. In 2015, Rahini undertook a mtDNA study on *A. meda* including 3 sample locations of bees in Sistan-Belutschistan. In the current study we present a detailed morphometric study of these bees, and explore their morphological relation to other bee subspecies.

MATERIALS AND METHOD

One of us (Pour Elmi) collected *Apis mellifera* honey bees from 7 colonies in the region. In 2005, four locations were visited. From South to North: no *A. mellifera* bees were found at Chabahar (Bandar Beheshti, (25° 17'N 60°36'E, altitude 0 to 100 m), 3 samples were taken from Iranshahr (27°10'N, 60°38'E, altitude 660 m). 1 from Khash (28°13'N, 61°11'E, altitude 14900 m, and another 3 from Zahedan (29°30'N, 60°50'E, altitude 1384 m). Mean annual temperatures were high in all locations (20-28°C) with average July temperatures between 27-34°C). Average rainfall is generally low (<110 mm/year), humidity is high at the coast (85%, Chabahar), but low within the inland (25 to 50%). *A.florea* was noted in all locations except Zahedan. Sampling beeyards were selected where beekeepers were not engaged in breeding, and did not move their colonies. Each sampled colony was at least 30 km distant from any other to avoid close relatedness. At least ten worker bees were taken from each sampled colony, and were stored in 95% Ethanol for later analysis.

10 worker bees per colony were analyzed according to the methods described in Ruttner (1988), using 37 morphometric characters (16 sizes, 7 colors, 3 pilosity, and 11 wing angles). Measurements of size and wing venation were performed using a stereo microscope and a computer-aided measuring system based on a video system and measuring program (Meixner 1994). For measurements of pilosity and color scaling a stereo microscope was used. To determine the morphometric position within the species, data from 67 samples of *A. m. meda* and 128 samples of other adjacent subspecies were obtained from the Oberursel data bank. Results

were analysed by discriminant analysis and UPGMA Cluster Analysis using SPSS 23 (2015).

RESULTS

A selection of morphometric data are listed in Table 1, together with Oberursel data bank data for the most relevant subspecies and *A. m. meda* subgroups. Due to low numbers of colonies sampled in Sistan-Belutschistan, sampling locations were not differentiated. The honey bees of Sistan-Belutschistan were small in relation to most of the other bee groups, and differed highly significantly in almost all length measurements from the data bank measurement of *A. m. mellifera*, *A. m. carnica*, *A. m. caucasica* and *A. m. anatolica* (univariate comparisons with LSD post-hoc comparisons), but were similar in size to *A. m. syriaca*. However, they were markedly bigger than *A. m. jemenitica*, where all size measurements were significantly smaller. They were also slightly smaller in relation to other *meda* groups (Iran central, Iran North-East (NE), Iran South-East (SE) Iran Mazandaran, and Irak, but only about half of the measurements were statistically different. In particular, they were very similar to the other *meda* groups in the waxmirror dimensions and the metatarsal length and width. An outstanding character was wing dimensions. While wing length ranged similar as other length measurements, wing width measures were significantly higher in comparison to all other groups except *A. m. carnica* and Iran NE. Consequently, forewing index (length/width) was significantly higher in all comparisons. Sistan-Belutschistan bees did not show an exceptional position in relation to the other groups in slenderness (length/width of sternum 6) nor in the relation of body size to leg length.

Hairlength was significantly shorter than in all the other bee groups, with the exception of *A. m. jemenitica* where it was significantly shorter. Tomentum width differed significantly only from the higher value of *A. m. caucasica*, while the dark stripe differed significantly only from the higher values in *A. m. mellifera* and *A. m. Iran NE*.

Sistan-Belutschistan bees were fairly light colored. In particular, pigmentation of tergite 4 was significantly lighter than in any of the other groups. Tergite 2 and 3 pigmentations were considerably and significantly lighter in comparison to *A. m. mellifera*, *A. m. carnica*, *A. m. caucasica* and *A. m.*

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anatoliaca. While tergite 2 pigmentation did not differ throughout the other groups, tergite 3 pigmentation was significantly darker in comparison to *A. m. syriaca*, *A. m. jemenitica* and the *A. m. meda* groups from Central Iran and Irak. Pigmentation of the scutellum was generally lighter than in all other groups, the differences were significant except for scutellum 1 in comparison to *A. m. jemenitica*, Iran SE and Irak, and for scutellum 2 in comparison to *A. m. caucasica*, *A. m. syriaca* and *A. m. jemenitica*. In contrast,

pigmentation of labrum 1 was significantly darker than all other groups (except *A. m. jemenitica*, Iran SE and Irak where the difference was not significant). Pigmentation of labrum 2 was darker than in the *A. m. meda* groups and *A. m. jemenitica*, which difference was significant for Iran SE and Irak, but was lighter in comparison to the other groups which was significant only for *A.m.caucasica*.

Table 1. Morphometric characteristics of Sistan-Belutschistan honey bees (*A. mellifera*), and data on other subspecies or *A. meda* subgroups obtained from the Oberursel Data Bank. Significance of difference to Sistan-Belutschistan: * $P>0.05$, ** $P<0.005$ *** $P<0.0005$

					<i>A. m. meda</i>			
	<i>A. m. caucasica</i>	<i>A. m. anatoliaca</i>	<i>A. m. syriaca</i>	<i>A. m. jemenitica</i>	Iran central	Iran SE	Irak	Sistan-Belutschistan
Body size (LT3+LT4). (mm)	4.54 0.075 ***	4.48 0.059 ***	4.19 0.132 *	3.88 0.115 ***	4.35 0.089 ns	4.48 0.111 ***	4.29 0.113ns	4.28 0.057
Complete leg fem+tib+mtar. (mm)	8.27 0.144 ***	8.14 0.104 ***	7.87 0.144 *	7.02 0.201 ns	7.81 0.163 ns	7.92 0.153 *	7.76 0.127 ns	7.75 0.138
Forewing index wfw/lfw	0.34 0.003 ***	0.34 0.004 ***	0.33 0.003 ***	0.34 0.004 ***	0.34 0.004 ***	0.34 0.005 ***	0.34 0.005 ***	0.35 0.005
Hair length. (mm)	0.34 0.034 ***	0.29 0.025 **	0.22 0.037 *	0.18 0.019 ***	0.28 0.033 **	0.28 0.016**	0.28 0.018 *	0.25 0.029
Pigment tergite 4	1.53 0.86 ***	2.81 0.70 ***	3.83 0.25 ***	4.38 0.80 ***	4.33 0.68 **	4.66 0.89 ***	5.82 0.27 ***	6.41 0.49
Pigment tergite 2	3.60 0.86 ***	4.77 0.89 ***	8.56 0.43 ns	8.06 0.85 ns	8.56 0.43 ns	8.34 0.55 ns	8.92 0.25 ns	8.07 0.59
Pigment scutellum 1	1.57 0.82 ***	3.91 1.42 ***	5.91 0.95 *	6.30 0.69 ns	5.93 0.99 *	6.11 0.44	6.69 0.53 *	6.78 0.56
Angle A4 (degree)	34.6 1.54 ***	33.0 1.12 ***	32.6 1.29 ***	34.3 1.21 ***	30.6 1.37 ***	29.9 0.85 *	30.20 0.98	28.4 *1.07
Angle K19 (degree)	75.0 2.32 ***	77.8 2.00 ***	79.9 2.16**	81.1 1.53 ns	78.7 1.58 ***	80.0 1.46 ns	80.5 1.65 ns	82.4 1.89

In the 11 wing venation angles, Sistan-Belutschistan bees differed significantly from the other groups in 72 of 121 possible comparisons. They differed in most angles (9 out of 11) from *A. m. mellifera*, *A. m. carnica*, *A. m. anatoliaca* and *A. m. anatoliaca*, in somewhat more than half from *A. m. syriaca* and *A. m. jemenitica*, and in somewhat less than half from the *A. m. meda* groups (about 5 out of 11). Some angles took extreme positions. Angle A4 and Angle O26 were significantly smaller than in all other groups (except A4 in comparison to *A. m. carnica* and Iran NE, and O26 in comparison

to Iran NE Iran Mazandaran, and Irak, where differences were not significant. Angle b4, G18 and K19 were significantly larger than in all other groups (except b4 in comparison to *A. m. carnica* and G18 as well as K19 in comparison to *A. m. jemenitica* where differences were not significant). Cubital vein 1 was significantly longer than in *A. m. jemenitica*, but shorter than in *A. m. carnica*, IranNE and IranSE, vein 2 was also significantly larger than in *A. m. jemenitica* but smaller than *A. m. mellifera*, *A. m. caucasica* and *A. m. anatoliaca*. Cubital index was significantly lower in comparison to *A. m.*

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carnica and IranSE, but higher in comparison to *A. m. mellifera*, *A. m. caucasica*, *A. m. anatoliaca* and *A. m. jemenitica*. Differences were not significant in the other comparisons.

In discriminant analysis, when entered as an own group the bees from Belutschistan were positioned adjacent to the *A. m. meda* groups (Fig. 2 a), with some affinity to *A. m. syriaca* or *A. m. anatoliaca*. They were distinct enough from all other groups that each sample was reallocated to their own group with very high probability ($P > 0.9995$). When entered as unclassified samples, all were assigned with very high post-hoc probability to *A. m. meda*

($P > 0.9995$ in all but the sample from Khash, where P was 0.996).

A separate discriminant analysis of the Sistan-Belutschistan samples together with the *A. m. meda* groups alone again emphasized the comparatively high distance of these bees from the other groups, predominantly because of their smaller size reflected on discriminant function 1 (Fig 1b). As ungrouped samples, all Sistan-Belutschistan samples were allocated to the Iran SE group with very high probability ($P > 0.9995$), except one sample from Iranshar which was allocated to the Irak meda group with lower probability of $P = 0.965$).

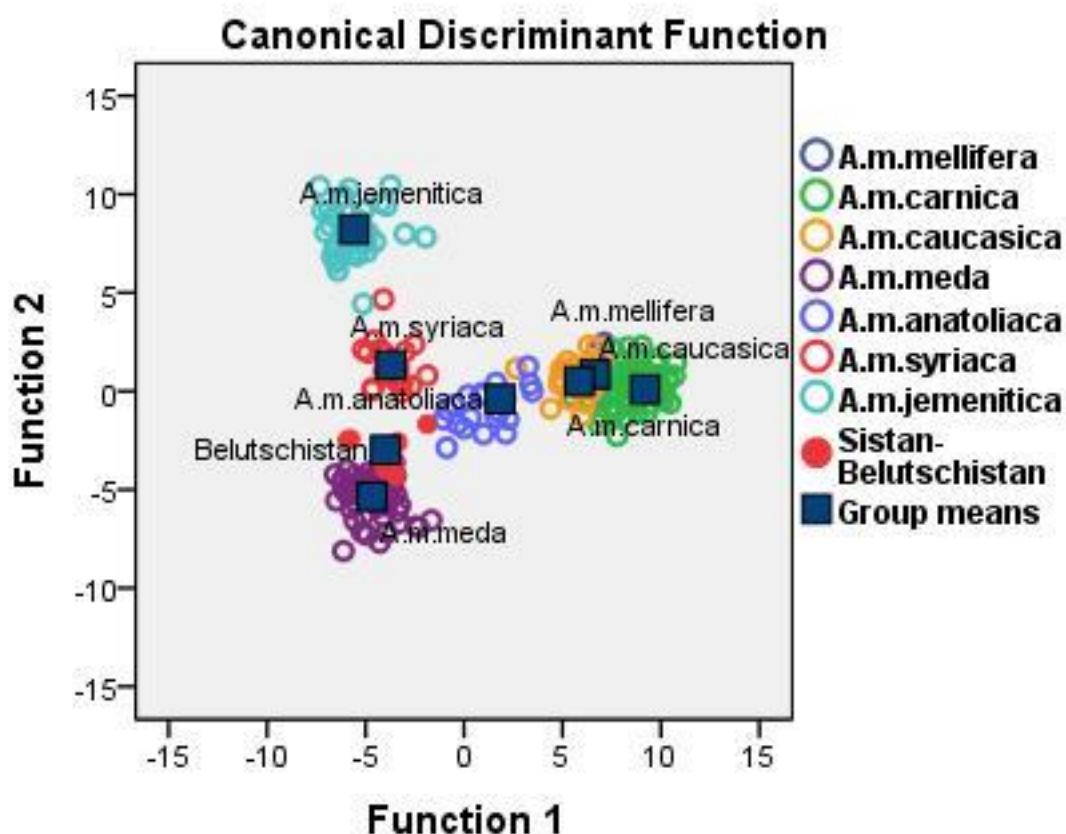


Fig.2a Discriminant analysis function plots. a for the grouped Sistan-Belutschistan samples together with all other subspecies, b for the ungrouped Sistan-Belutschistan samples together with the *A. m. meda* subgroups.

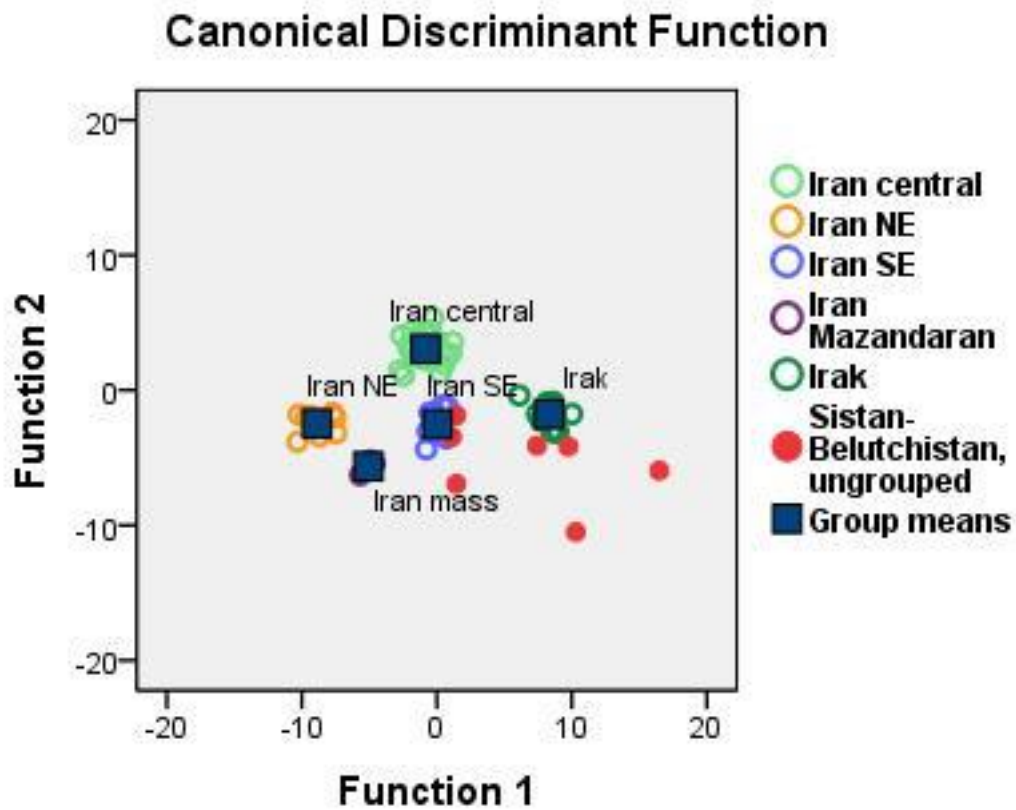


Fig. 2 b. Discriminant analysis function plots. a for the grouped Sistan-Belutschistan samples together with all other subspecies, b for the ungrouped Sistan-Belutschistan samples together with the *A. m. meda* subgroups.

A cluster analysis based on of group means of z-normalized character data also showed the Sistan-Belutschistan bees to be closely linked to the *A. m. meda* cluster (Fig. 3). Again they had closer affinity to the Iran SE group, and from the other subspecies they were next linked to *A. m. syriaca*, then to the *A. m. anatoliaca* / *A. m. caucasica* cluster, and then to *A. m. carnica*, to *A. m. mellifera* followed at some

distance by *A. m. jemenitica*. Table 2 gives an excerpt from the distance matrix reflecting the Euclidian distances from Belutschistan bees, similarly showing the relative closeness to *A. m. meda* groups, to *A. m. syriaca*, followed by the other subspecies with *A. m. jemenitica* placed furthest away.

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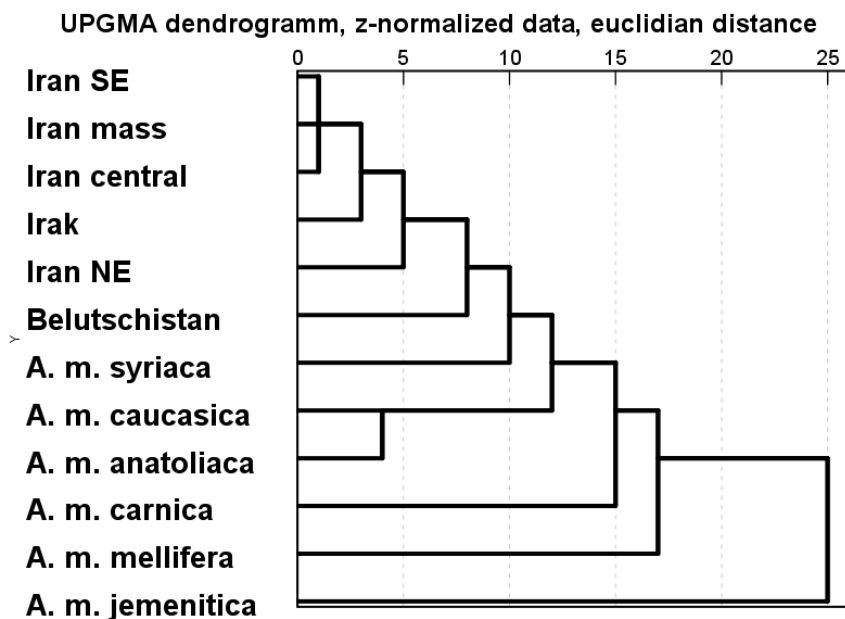


Fig. 3 Dendrogram showing the position of the Belutschistan bees in relation to other subspecies, and to subgroups of *A. m. meda*, based on z-normalized morphometric data

Table 2. Euclidian distance from the Belutschistan group based on z-normalized character data.

Euclidian distance	
Group	Belutschistan
<i>A. m. mellifera</i>	10,487
<i>A. m. carnica</i>	9,465
<i>A. m. caucasica</i>	9,721
<i>A. m. anatoliaca</i>	7,628
<i>A. m. syriaca</i>	6,741
<i>A. m. jemenitica</i>	11,378
Iran central	5,436
Iran NE	6,550
Iran SE	5,558
Iran mass	6,091
Irak	5,516
Belutschistan	0,000

DISCUSSION

In this study we investigate the honey bees (*A. mellifera*) at the south-eastern distribution boundary of *Apis mellifera*, in an area of specific interest due to its vicinity to the distribution area of the Eastern honey bee *A. cerana*. These sampling positions

were approximately 300 km east of the currently most eastward sample of *A. mellifera*, at Delfarh (28°58N 57°38E) in the district of Kerman, which had been morphometrically analyzed by Ruttner *et al.* (1985). No bees had been suspected by these authors because of the extremely hot and dry desert climate generally adverse to beekeeping. This hot desert strip constitutes the separation line to the beginning of *A. cerana* territory in Pakistan, the width of which has thus been reduced by about one third until the next record of *A. cerana* at Thari (Pakistan, 34°30' N 64°51' E), but possibly the distance of separation might be even less. It might be not impossible but unlikely that there is any overlap between the species as the separating region is extremely uninhabitable. The geographic situation is sketched out in Fig 1, based on Ruttner *et al.* (1985).

At the same time, the bees of Sistan-Belutschistan enrich the spectrum of *A. m. meda* by a subpopulation of clearly deviating properties. *Apis mellifera meda*, distributed throughout Iran, was first described by Skorikov in 1929 (Ruttner 1988), for a small location in its northern range. The first more detailed investigation by Ruttner *et al.* (1985) still remains the main reference in which, based on

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63 samples from Iran, they roughly outlined the approximate distribution limits. They further identified 5 discernible subpopulations in Iran (West and central Iran, Mazandaran, Northeast Iran and South-east Iran) but also extensions into Iraq and Southeast Anatolia. This work was later supplemented by Ftayeh *et al.* (1994), who clarified the distribution limit into northern Syria, and by Ruttner *et al.* (2000) showing altitude-related clinal variation pattern along the 36°N latitude up and down the Elbrus Mountains to the Caspian sea. Later, Adl *et al.* (2007) confirmed the clear separation between west Iranian *A. m. meda*, east Anatolian *A. m. anatoliaca* and *A. m. caucasica*. Dolati *et al.* (2013) further differentiated Iranian *A. m. meda* into 9 subpopulation by geometric wing analysis. All these investigations were based on morphometry, but were recently supplemented by a few molecular studies, mostly restricted to partial areas (Kandemir *et al.* 2004, Rahini 2015). However, the complete amount of variation within *A. m. meda* and its geographical the pattern still needs to be sorted out, which is beyond the scope of this paper.

Discriminant analysis, as well as cluster analysis, clearly confirmed that Sistan-Belutschistan bees belong to *A. m. meda*, in spite of quite some deviations which in part may be interpreted as adaptations to the extremely dry-hot climate. Some particular features set them apart, which are small size (but bigger than *A. m. jemenitica*), light coloration particularly of tergite 4 which was lighter-colored than in any of the other groups, and extremely short hairs. A distinctive trait is the relatively broad wings, and some extreme wing angles (A4, O26, b4, G18 and K19 which might be resulting from general wing shape. While distinctly different from all other *A. m. meda* populations, Sistan-Belutschistan bees were closest related to the most adjacent South-Eastern *A. m. meda* population. Next to the *A. meda* population Sistan-Belutschistan bees were most similar to *A. m. syriaca* with resemblances in size and pigmentation. An mtDNA analysis including bees from Sistan-Beluschistan (Rahini, 2015) had shown their membership to the evolutionary C line, thus supporting their status within the *A. m. meda* subspecies.

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GENİŞLETİLMİŞ ÖZET

Giriş

Bu çalışmada özel bir ilgi alanına sahip olan coğrafyada *Apis mellifera*'nın güney-doğu dağılım sınırları yeniden araştırılmıştır. Daha öncesinde Sistan-Belutchistan bölgesinin çok fazla kuru çöl iklimine sahip olmasından dolayı balarısı *Apis mellifera*'nın bu bölgede olmadığı ancak *A. florea*'nın varlığı rapor edilmiştir. 2005 yılında yapılan bir arazi çalışmasında bu bölgede hem modern hem de geleneksel yöntemlerle bayağı bir arıcılık faaliyetleri yapıldığı bulunmuştur. Bu çalışmada bu bölge balarılarının detaylı morfometrik çalışması yapılmış ve diğer balarılar ile morfolojik ilişkileri araştırılmıştır.

Materyal ve Metot

Yazarlar biri olan Pour Elmi 2005 yılında bölgeyi güneyden kuzeye doğru ziyaret ziyaret etmiş ve 7 balarısı kolonisinden alkol içerisine örnekler toplamıştır. Bu örneklerden 3 tanesi Iranshahr (27°10'N, 60°38'E, rakım 660 m). 1 tanesi Khash (28°13'N, 61°11'E, rakım 14900m, ve diğer 3 tanesi ise Zahedan (29°30'N, 60°50'E, rakım 1384 m) şehrinden toplanmıştır (Şekil 1). Kovanlardan toplanan örneklerden her kovandan 10 birey olacak şekilde Ruttner (1988)'de tanımlanan metoda göre 37 morfometrik karakter (16 vücut büyüklüğü, 7 renk, ve 11 kanat açısı) ölçülmüştür. Bu bölge arılarının morfometrik pozisyonunu belirlemek amacıyla 67 *A. m. meda* ve 128 diğer yakın balarısı alttürlerinin Oberursel veri tabanında bulunan kovan verileri ile karşılaştırılmıştır. Elde edilen veriler SPSS 23 kullanılarak diskriminat fonksiyon analizi ve UPGMA kümeleme analizleri ile çok değişkenli istatistiksel analizlere tabi tutulmuştur.

Sonuç ve Tartışma

Morfometrik karakterlerden bazılarının karşılaştırmalı sonuçları ve farklılıkları Tablo 1'de verilmiştir. Sistan-Beluchistan'dan elde edilen arıların diğer örnekler göre az sayıda olması nedeniyle bu bölge arılarının farklı lokasyonları gruplanamamıştır. Ancak tüm morfometrik ölçümlerde bu bölge arıları veri tabanında bulunan *A. m. mellifera*, *A. m. carnica*, *A. m. caucasica* ve *A. m. anatoliaca*'dan istatistiksel olarak anlamlı bir şekilde farklı bulunmuştur. *A. m. syriaca*'ya benzer büyüklükte fakat *A. m. jemenitica*'dan çok daha büyük bulunmuştur. Aynı zamanda diğer İran ve Irak bölgesindeki arılardan da çok az küçüktür. Kullanılan 37 karaktere göre diğer balarısı grupları ile benzerlik ve farklılıklara sahiptir. Bu karakterlerin tamamı kullanılarak yapılan çok değişkenli istatistiksel analizlerde Beluchistan bölgesinden toplanan arıların *A. m. meda* grubuna daha yakın çıktığı fakat *A. m. syriaca* ve *A. m. anatoliaca*'ya da benzerlik gösterdiği görülmüştür (Şekil 2). Morfometrik karakter ortalamalarının UPGMA kümeleme analizinde de *A. m. meda* kümesi ile bağlanmıştır. Diğer alttürlerden ise *A. m. syriaca*'ya ve daha sonra *A. m. anatoliaca* / *A. m. caucasica* grubuna daha sonra ise *A. m. carnica*, *A. m. mellifera* ve sonrasında ise uzaktan *A. m. jemenitica*'ya bağlanmaktadır (Şekil 3). Tablo 2'de Beluchistan balarılarının diğer balarısı alttürlerine olan öklid uzaklıkları gösterilmektedir. Bu uzaklıklar incelendiğinde UPGMA kümeleme analizindeki benzer ilişkiler görülmektedir. Bu bölge balarılarının yapılan detaylı çalışması ile *Apis mellifera* dağılımı diğer balarısı türü olan *A. cerana*'ya kadar yakınlaşmıştır. Hem diskriminant hem de Kümeleme analizi Beluchistan arılarının biraz farklılıklarla birlikte *A. m. meda*'ya bağlı olduğunu göstermiştir. Ayrıca yapılan mtDNA analizi (Rahini, 2015) ile bu bölge arılarının C evrimsel koluna ait olduğu ve *A. m. meda* alttürünün de pozisyonunu belirlenmektedir.