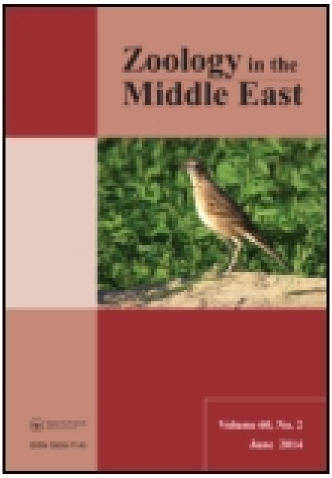

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Morphometric characterisation of the native Honeybee, *Apis mellifera* Linnaeus, 1758, of Saudi Arabia

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The morphometry of native honeybees from Saudi Arabia was analysed and compared with 7 *Apis mellifera* subspecies, based on 198 colony samples from 36 locations. Twenty five standard morphological characters were evaluated, and samples were compared with seven reference honeybee subspecies (*Apis mellifera carnica, A. m. ligustica, A. m. meda, A. m. syriaca, A. m. lamarckii, A. m. litorea* and *A. m. jemenitica*) obtained from the Oberursel Data Bank (Institut für Bienenkunde, Frankfurt University, Germany). Results confirmed that samples from Saudi Arabia are very similar to samples from the subspecies *A. m. jemenitica* (Ruttner, 1967), previously described from Oman, Yemen and Saudi Arabia. Samples were well-separated from the other subspecies, but the distinction was less in relation to *A. m. litorea*. While locally kept bees were well-separated, samples from migratory beekeeping showed broader variation and were less clearly separated, indicating the influence of ingression and hybridization with introduced honeybee subspecies.

Keywords: *Apis mellifera jemenitica*, *Apis mellifera litorea*, morphometry, variants, Saudi Arabia.

Introduction

Current beekeeping in Saudi Arabia is in a state of development. The estimated numbers of beekeepers is 4,000 with an estimated number of 700,000 bee hives (Alqarni, Hannan, Owayss, & Engel, 2011). About 70% of these colonies are kept traditionally in log hives inhabited by local bee varieties (Alqarni, Hannan, Owayss, & Engel, 2011). In a recent study, Al Ghamdi, Alsharhi, Alattal, and Adgaba (2012) undertook the first extended study to characterise the indigenous honeybee of Saudi Arabia based on 198 colony samples from 36 locations. Applying standard morphometry (Ruttner, 1988), this revealed significant NW-SE clinal variation through the main beekeeping areas of Saudi Arabia adjacent to the Red Sea coast, which was represented by three geographically widely overlapping morphometrical clusters. However, in that study they did not study the relationship of their samples to the currently recognised subspecies.

A first morphometric classification of the indigenous honey bee of Saudi Arabia by Ruttner in 1976 had placed it into the then newly recognized subspecies *Apis mellifera jemenitica* based on six samples from three regions (Jazan, Riyadh and Alhasa) (see also the classification of honeybees of the world by Ruttner, 1988). *A. m. jemenitica* represents an extreme end of the adaptive spectrum, being able to endure hot and dry climatic conditions. Also, it is one of the major subspecies extending over the enormous distance of 4500 km from Arabia to West Africa, and its homogeneity is under debate (Hepburn & Radloff, 1998; Al Ghamdi, Nuru, Khanbash, & Smith, 2013). We reinvestigate here the morphometric affiliation of the honey bees of Saudi Arabia based on the large

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Table 1. Mean characteristics (±SD) of 31 morphological traits and 4 indices of native Saudi honeybees colonies (n=198). For comparison, reference data for the Yemen honeybee (n=8) and the *litorea* honeybee (n=10) (Ruttner, 1988) were included. The table gives means and standard deviations. Asterisks mark calculated characters. Prob = length of proboscis; fem = length of femur; tib = length of tibia; ltar = length of tarsus; wtar = width of tarsus; pt2 = pigmentation of tergite2; pt3 = pigmentation of tergite3; pt4 = pigmentation of tergite 4; lt3 = length of tergite 3; lt4 = length of tergite 4; lst3 = length of sternite 3; wwm = width of wax mirror; lst6 = length of sternite 6; wst6 = width of sternite 6; lfw = length of forewing; wfw = width of forewing; a4, b4, d7, e9, j10, j16, l13, n23, o26 wing venation angles; lt3lt4= body size; leg=length of hind leg, lw_mtar = metatarsus index; lw_st6 = index of slenderness; bs_leg:= index of leg to body size; lwfw = index of wing width.

	Saudi Arabia	**Reference group**	
		jemenitica	***litorea***
prob	510.45 ±34.22	544.15±10.67	577.12±14.82
fem	230.89±6.63	234.99±4.59	242.13±2.07
tib	280.04±8.29	287.91±6.50	299.11±2.74
ltar	180.71±8.59	180.84±4.56	185.14±2.66
wtar	100.45±3.17	102.52±3.94	104.68±2.37
pt2	8.95±0.33	7.47±0.91	7.13±0.89
pt3	8.21±0.37	7.22±0.73	6.96±0.93
pt4	5.44±0.95	4.49±1.04	3.99±0.60
lt3	188.49±5.36	196.17±4.05	197.83±3.49
lt4	185.27±5.40	192.32±4.47	192.72±3.27
lst3	233.23±6.61	237.26±5.79	245.03±4.33
wwm	185.44±9.00	197.47±5.36	205.54±2.07
lst6	212.41±5.51	223.12±5.16	229.29±4.35
wst6	267.27±10.17	268.64±7.35	269.43±6.12
lfw	806.85±16.80	806.98±13.55	840.21±11.31
wfw	279.98±7.00	277.88±3.31	290.47±5.03
a4	33.5±1.28	33.89±1.00	33.65±1.43
b4	98.72±3.92	103.30±3.31	102.17±2.72
d7	104.86±2.00	105.01±1.20	102.53±2.65
e9	19.09±0.78	19.02±0.41	19.15±0.59
j10	54.78±2.14	55.29±2.50	54.18±1.36
j16	86.03±2.46	91.97±1.41	91.69±2.88
l13	14.50±0.77	14.21±0.89	15.51±0.76
n23	85.71±2.03	89.00±1.95	87.64±2.77
o26	43.35±2.03	39.86±1.91	39.55±2.83
It3It4*	371.98±10.26	388.49±8.49	390.55±6.69
leg*	691.54±19.45	703.74±15.05	726.37±5.64
lw_mtar*	55.73±3.66	56.70±1.98	56.55±1.37
lw_st6*	79.54±2.19	83.08±1.89	85.11±1.10
bsleg*	53.81±1.38	55.21±1.06	53.77±0.97
lwfw*	34.70±0.50	34.44±0.42	34.58±0.78

sample size of the Al Ghamdi et al. (2012) study, and relate them to reference samples of 7 subspecies in the Oberursel Data Bank.

Material and Methods

Data for Saudi Arabia were taken from Al Ghamdi et al. (2012). In that study, samples from 198 local colonies at 36 locations within 11 beekeeping areas in Saudi Arabia (n=32: Tabouk, Madinah, Al-Taif, Asir, Al-Baha, Jazan and Najran, Al-Jouf, Hail, Al-Qaseem and Riyadh) (Figure 1) had been analysed. 45.6% of the samples were from local hives which were not moved, the others were reported as migratory. 10 worker bees from each sample had been dissected, following Ruttner et al. (1978). Body parts were mounted on slides, which were scanned with 600 ppi connected to a desktop computer system with image tool software (Image Tool® 3.0). The morphological traits used in this analysis were associated with the honey bee size, cuticular pigmentation, and wing angles. We measured a total of 25 morphometric characteristics recommended by Ruttner (1988) to be highly discriminatory. Colony sample means were calculated for each character. To capture honeybee variation in Saudi Arabia samples due to clinal geographic variation (Al Ghamdi et al., 2012) and to differences in beekeeping, we subdivided the samples into five groups (Figure 1): a south-local and a south-migratory group (46.2% and 20.2% of the samples, respectively, >20°20'N and >40°40'E); a west-local and a west-migratory group (6.4% and 25.4% of the samples, respectively, >20°20'N and <40°40'E); and a north-migratory group (1.7% of the samples, >25°00'N and >34°00'E). Then we included reference bee data for the corresponding characteristics of seven other subspecies (*A . m. carnica, A. m. ligustica, A. m. meda, A. m. syriaca, A. m. lamarckii, A. m. litorea* and *A. m. jemenitica*) obtained from the Oberursel Data Bank (Institut für Bienenkunde, Oberursel, Goethe-Universität, Frankfurt, Germany). Subspecies were selected with regard to geographic vicinity, or to likelihood of importation. Data were analysed by creating factor analysis, hierarchical cluster analysis, and discriminant analysis using SPSS Statistics 20, release 20.0.0 (20011).

Results

Morphometric data indicate that the local honeybee of Saudi Arabia is the smallest honeybee and is lightly coloured compared with other reference honeybee samples including *A. m. jemenetica* and *A. m. litorea*. The means are presented in Table 1, in comparison with data from the Oberursel Data Bank (for *A. m. jemenitica* from Yemen, and for *A. m. litorea*).

Morphometric character measures were reduced to 3 principal components by factor analysis, and sample scores on these three axes are presented in Figure 2a-b. The three axes capture 73.1% of the sample variation. Factor 1 (61.7% of variation) is mainly positively correlated with characters of size and wing angle e9 but negatively with wing angles a4 and o26; factor 2 (6.2%) is mainly negatively correlated with colour (light colours produce low factor 2 values) and positively with wing angles j16 and n23; factor 3 (5.1%) predominantly but less clearly correlates with other wing venation angles.

Sample groups were marked by different colours or labels, and for each group (except local Saudi Arabian groups) 95% confidence ellipses were drawn. In Figure 2a-b, the Saudi local group and the Saudi migratory group mostly overlap, indicating a basic similarity. However, there is a shift towards higher factor 1 values and lower factor 2 values in the Saudi migratory group, indicating a tendency towards higher size values and lighter colours. This shift is also clearly expressed if southern and western regions are differentiated. While most of the 80 southern-local Saudi samples do mostly occupy a quite concentrated area not exceeding 0 on factor 1 axis, and only few samples below -1 on factor 2 axis, the 35 southern-migratory Saudi colonies cover a much wider range encompassing higher factor 1 and lower factor 2 regions. Similarly, all 11 western-local Saudi samples, though positioned towards the right-hand lower part of the southern-

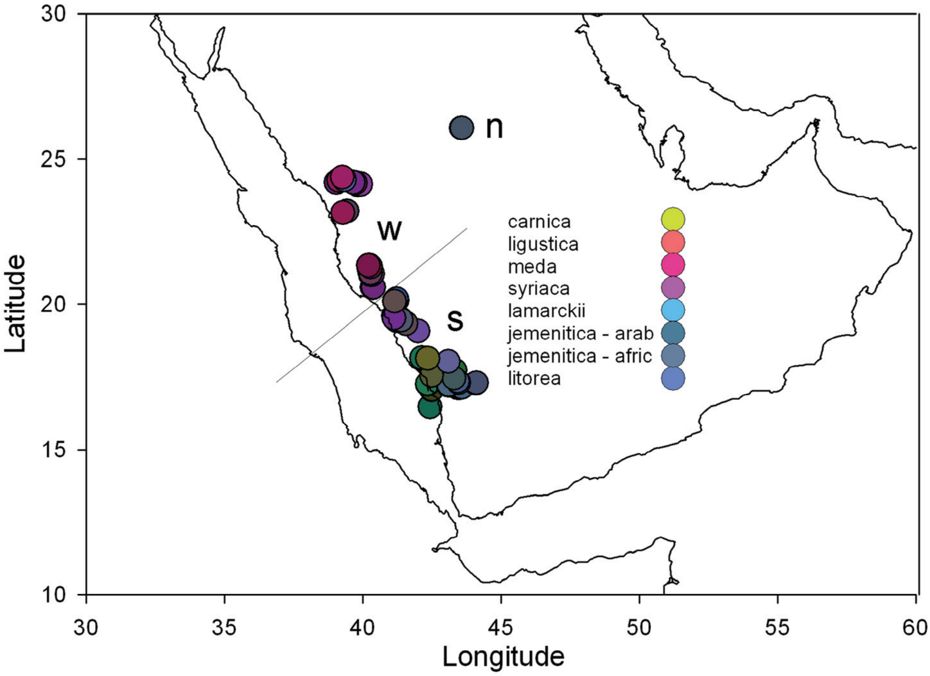

Figure 1. Sampling locations: The red, green and blue components of the sample colours were calculated from location means of sample scores on the first three PC axes of factor analysis. In the legend mean colour values are given for the reference samples. n = north group, w = west group, s = south group.

local distribution, likewise do not exceed 0 on factor 1 axis and fall below -1 on factor 2 axis. However, the 44 western-migratory samples clearly occupy the region above and below, respectively, of these limits together with part of the southern-migratory Saudi samples. Clusters of *A. m. carnica*, *A. m. ligustica*, *A. m. meda*, *A. m. syriaca* and *A. m lamarckii* are firmly distant from the Saudi Arabian samples (in that order), and there is no overlap of confidence ellipses. This basically holds also in Figure 2b for *A. m. carnica*, *A. m. ligustica and A. m. meda.* However, there is a slight overlap of *A. m. syriaca* with the Saudi migratory group, and a fairly substantial overlap with *A. m. lamarkii* in the area of higher factor 3 values. *A. m. litore*a is clearly separated from the Saudi local group in Figure 2a, but overlaps substantially with the Saudi migratory group in the area of large and light-coloured bees in Figure 2b, where it encompasses almost all samples of the Saudi migratory group, both southern and western, and about half of the Saudi local group. Together this still indicates that *A. m. litorea* is distinct from Saudi Arabian bees, but also points to closer morphometric similarities particularly with the migratory colonies. Last, the reference sample area of Arabian *A. m. jemenitica* includes the majority of Saudi samples, both local and migratory in Figure 2a and 2b, showing that Saudi Arabian samples closely relate to this group (mainly larger and lighter coloured Saudi migratory bee samples fall outside). Arabian *A. m. jemenitica* mostly overlap with Saudi samples in both plots, but African *A. m. jemenitica* do partly differentiate with an extension into the areal of larger and darker bees in Figure 2a, and stretches into high factor 3 value area in Figure 2b, which they share with *A. m. lamarckii* but which is not

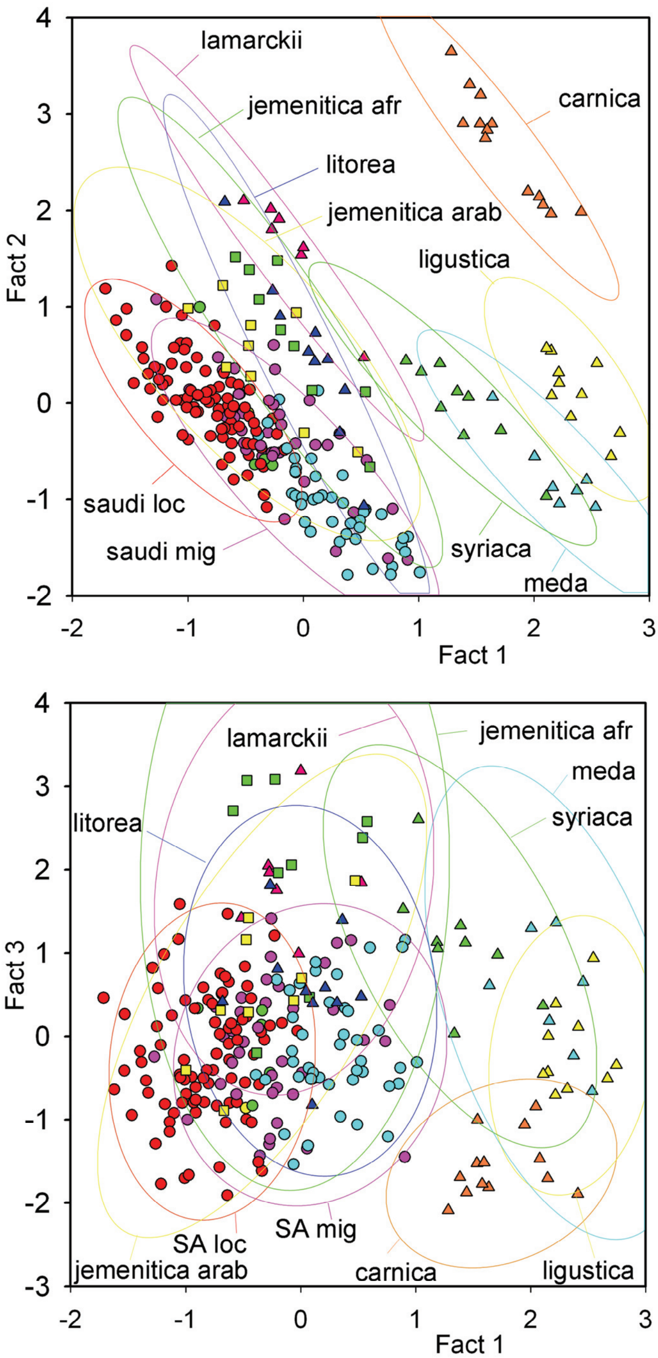

Figure 2a-b. Sample scores on the first three principal components of the factor analysis. A: Factor 2 versus factor 1. B: Factor 3 versus factor 1. Ellipses give 95% confidence areas for the groups. SA samples: Circles: Red: south group local. Pink: south group migratory. Green: west group local. Cyan: west group migratory. Squares: Yellow: Arabian *A. m. jemenitica*. Green: African *A. m. jemenitica*. Triangles: Blue: *A. m. litorea.* Bramble coloured: *A. m. lamarckii*. Green: *A. m. syriaca*. Light blue: *A. m. meda*. Yellow: *A. m. ligustica*. Brown: *A. m. carnica*.

Table 2. Squared Euclidian distances between z-standardised character group means for the Saudi Arabian (SA) groups and the Arabian *A. m. jemenitica* reference samples from the respective reference subspecies.

Group	**SA south-local**	**SA south-migratory**	**SA west-local**	**SA west-migratory**	**SA north-migratory**	***jemenitica*-arab**	***litorea***
carnica	218.89	185.02	207.21	180.72	167.38	156.55	131.29
ligustica	143.47	113.14	129.45	106.43	98.94	99.91	81.14
meda	100.79	73.18	90.53	69.14	62.75	68.14	44.78
syriaca	65.27	45.72	61.06	44.17	38.01	38.28	28.35
lamarckii	48.19	35.95	49.57	44.02	31.38	32.48	21.81
jemenitica-arab	10.41	7.60	9.60	9.76	4.78	.000	10.27
litorea	21.75	13.51	20.13	14.45	9.90	10.27	0.00
jemenitica-afric	25.63	18.53	20.95	20.81	13.27	13.36	8.88

occupied by Arabian *A. m. jemenitica*. On the other hand, both Arabian and African *A. m. jemenitica* cover greater areas not occupied by Saudi Arabian samples, indicating the wider variation in reference samples due to their more inclusive regional coverage.

In Figure. 1, which shows the sample locations to enable visualisation of the morphometric properties, symbol colours were created by calculating their RGB components from the sample scores on the three principal components axes as derived in the above factor analysis, averaged for colonies from the same location. Colours clearly demonstrate a clinal shift along the Red Sea coast. The average colour for reference samples is given in the legend, indicating closest similarity to both *A. m. jemenitica* groups.

The relations between the respective Saudi Arabian groups and the reference groups are further explored by calculating group centroid distances in the z-normalized factor space. Table 2 lists the squared Euclidian distances of the four SA groups and the reference subspecies, and additionally of the *jemenitica*-arab reference group to the other subspecies. Generally, distances decrease in the order *carnica* / *ligustica* / *meda* / *syriaca* / *lamarckii* / *jemenitica*-afric / *litorea* / *jemenitica*-arab for all Saudi Arabian groups. In particular, the distance to the *jemenitica*-arab group is fairly low for all Saudi Arabian groups, emphasizing the closeness to this reference group. However, the *A. m. litorea* reference group is clearly closer than the African *A. m. litorea* group, which is, however, much more marked in the Saudi Arabian migratory groups. This matches with the result that the *jemenitica*-arab group is by about 20% closer to *A. m. litorea* than to the *jemenitica*-afric group.

The Saudi Arabian south-local group is generally more distant from all other reference subspecies than all the other Saudi Arabian groups. The west-local group is slightly less separated (more than 10% less), except concerning *A. m. lamarckii*. More substantially, the Saudi Arabian migratory groups are distinctly less separated (by 13% to 38%) from the other subspecies than the respective local groups (except Saudi Arabia west in relation to *A. m. jemenitica* where distances are similar).

Distance relations between all groups are shown in Figure 3, showing an average linkage dendrogram constructed on the above squared distance matrix. It clearly underlines the close relationship between all Saudi Arabian subgroups, the close relation to *A. m. litorea* and the *jemenitica*-afric subgroup, the more distant link to *A. m. lamarckii* and the clear separation from the other reference subspecies *A. m. meda*, *A. m. syriaca*, *A. m. ligustica*, and *A. m. carnica*.

For further confirmation, the relations of the Saudi Arabian samples to reference groups were investigated by allocating them with discriminant analysis. In a first approach all *jemenitica* reference samples were joined in one group. All reference samples were correctly reallocated into their groups, except one *A. m. jemenitica* sample which was reallocated as *A. m. litorea*. Of the ungrouped Saudi Arabian samples, 163 (94.2%) were allocated into the *jemenitica* group. The remaining 10 samples (5.8%) were allocated into the *litorea* group. All of these originated from the Saudi Arabian migratory groups, one from the south-migratory group, and 9 from the west-migratory group.

If the two *jemenitica* reference groups were kept separate, two samples of the *jemenitica*-arab group were reallocated into the *jemenitica*-afric group, and one sample from the *jemenitica*-afric group was reallocated into the *litorea* group. 158 (91.3%) of the ungrouped Saudi Arabian samples were allocated to the *jemenitica*-arab group, while 11 (6.4%) were allocated into the *jemenitica*-afric group, and 4 (2.3%) were allocated into the *litorea* group. Most samples placed into the *jemenitica*-afric group were from the west-migratory group (7), whilst the others were from the south-local (2) or south-migratory (2) groups. Again, all samples allocated to *A. m. litorea* were from the west-

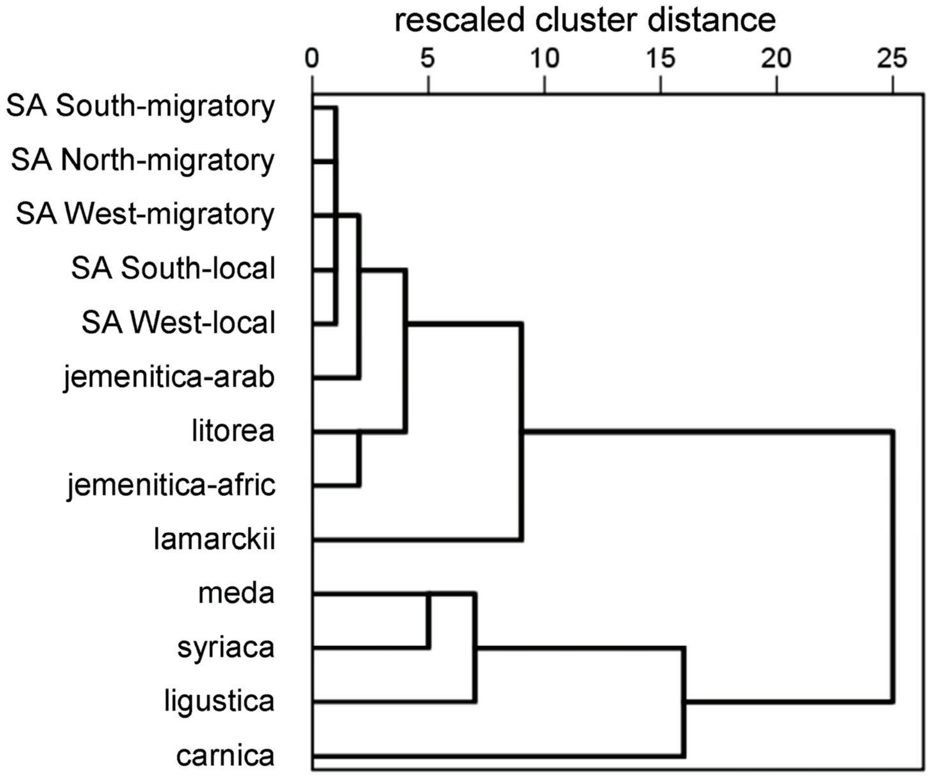

Figure 3. Cluster analysis dendrogram (average clustering) of the group means for Saudi Arabian subgroups and for reference groups.

migratory group. If the *jemenitica*-afric reference group was excluded, 160 (92.5%) of the Saudi Arabian samples were allocated into the *jemenitica*-afric group, and 13 (7.5%) into the *litorea* group.

Discussion

Our morphometric study basically confirmed that the Saudi Arabian samples do closely relate to *A. m. jemenitica* as defined by reference samples stored in the Oberursel data bank, described in Ruttner's (1988) textbook on honeybee biogeography. Samples showed an unequivocal separation from *A. m. carnica, A. m. ligustica, A. m. meda, A. m. syriaca* and *A. m. lamarckii* in factor analysis principal component plots, in squared Euclidian group distances and in discriminant analysis allocations. However, relations to *A. m. litorea* are not as clearly distinct. Though Euclidian group distance is still about 3 times longer to the *litorea* reference group than to the *jemenitica* reference group, there is some overlap in PC plots and allocation of up to 13 (7.5%) of the Saudi Arabian samples into the *litorea* group. This reflects the close morphometric relation between *A. m. jemenitica* and *A. m. litorea*, but also a high degree of variation within *A. m. jemen-*

itica over its extensive range of distribution. Though the *jemenitica* reference group is distinct from all other groups (except one sample reallocated into the *litorea* group in discriminant analysis), reference samples of the Arabian and the African *A. m. jemenitica* can be separated by discriminant analysis (with 2 of 9 samples reallocated from Arabian *jemenitica* group to African *jemenitica* group). It is beyond the scope and data base of this study to clarify general questions about variation within *A. m. jemenitica* or proximity to and differentiation from *A. m. litorea* (Al Ghamdi et al., 2013), and molecular tools might be needed for this. However, for the current study, it seemed feasible to consider Arabian and African *A. m. jemenitica* separately. This reveals a somewhat closer relation of the African *A. m. jemenitica* to *A. m. litorea* than that of the Arabian *A. m. jemenitica* in group distances, which is also apparent from the reallocation of that one sample from *jemenitica*-afric group to the *litorea* group, and results in a minor fraction of the Saudi Arabian samples affiliating morphometrically with either African *A. m. jemenitica* (6.4%), or *A. m. litorea* (2.3%). Overall, the analysis confirmed that Saudi Arabian samples were morphometrically identical or very close to *A. m. jemenitica* reference samples of Arabian origin (Ruttner, 1988), and clearly separated from all other subspecies with the exception of *A. m. litorea* where the distinction is less marked.

Variation within Saudi Arabian honeybees did affect their morphometric position. In the factorial plots, local colonies occupied a denser cluster than migratory samples. In particular, the region of smaller and somewhat darker bees is exclusively occupied by migratory samples. Local samples were more clearly separated from all other subspecies (except *A. m. jemenitica*) with no overlap with any of them. Likewise, south samples were distributed differently from west samples, mostly occupying the region of smaller and darker bees, compared to west samples. There were only very few north samples, positioning within the migratory cluster, which are not further interpreted. Though sample frequencies are unbalanced between the categories, sample positions indicate that both the mode of beekeeping (local or migratory) as well as the geographic region did affect the morphological position, with the highest separation of the southern-local group from all other subspecies, and the least separation of the west-migratory group. These relations are also confirmed in Euclidian distance matrix, and by the fact that in discriminant analysis all samples associating with *A. m. litorea* were, with one exception, from the west-migratory group.

The higher variability of the migratory group compared to the local group, together with its lesser separation from other subspecies, may indicate an influence of ingression from imported bees. However, if there is hybridization, the increased allocation and lower distances to *A. m. litorea* in the migratory group does not necessarily imply ingression of this subspecies. Influences of hybridization on morphometric positions are difficult to interpret, as hybrids might just get "pulled" into an area occupied by another, intermediate group. To clarify this, molecular tools need to be applied.

This study confirmed that the Saudi Arabian samples should be considered *A. m. jemenitica*. Although they differ from African *A. m. jemenitica* by degrees, as do the reference samples, there is no particular reason to regard them as separate subspecies but rather as local variants or ecotypes, similar to what is found in the other subspecies covering a more extended area. In spite of the geographic variation, the presumably more pure local samples appear fairly well defined and uniform in comparison to the reference groups, and there is no indication that the geographic pattern of variation is influenced by proximity of adjacent subspecies, such as *A. m. syriaca* in the north.

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