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Comparison of Morpho-skeletal Characteristics (Using Standardised Criteria Sets) of the Great Tit *Parus major* (Linnaeus 1758) in Three Iranian Populations (Mashhad, Noor and Hamedan)

TAYEBEH ARBABI[1*], MANSOUR ALIABADIAN[2] & ABOLGHASEM KHALEGHIZADEH[3]

1. Institut für Pharmazie und Molekulare Biotechnologie (IPMB), Abt. Biologie, Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany.

2. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, IRAN.

3. Ornithology Lab., Iranian Research Institute of Plant Protection, Tehran, IRAN.

**Correspondence Author. Email: tayebeh_arbabi@hotmail.com*

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Abstract: In order to study morphometrical and skeletal differences between three populations of the Iranian subspecies of the Great Tits *Parus major*, 40 specimens were collected during autumn 2001 to spring 2002. The specimens were obtained from three localities: Vakilabad forest park (Mashhad), Noor forest park (Noor) and Abbasabad gardens on the southern slopes of Alvand Mountain (Hamedan), representing *P.m. intermedius, P.m. karelini* and *P.m. blanfordi*, respectively. We utilised a large set (29) of morpho-skeletal characteristics for comparison in univariate and multivariate analyses using SPSS and Past programs. The Bonferonni univariate test revealed that 21 characteristics were significantly different among the three populations ($p<0.05$). The Tukey multivariate test showed that in a pairwise comparison of the measured factors among three paired populations, only nares to tip, humerus and mid-toe length were different in all the three pairwise populations, while wing breadth, tail length, tail index, carpo-metacarpus, tarso-metatarsus bone, brain-case height, brain-case breadth, lacrymal breadth and mandible length differed in only one of the three paired populations, and body length, shield width, wingspan, wing length, proportion of white wedge of the outer pair tail feathers to tail length, femur, tibiotarsus, profile length and height of the ramus mandible differed in two paired populations ($p<0.05$). There was no significant difference between any of the three pairwise populations for the remaining factors, *i.e.* bill length, bill depth, tarso-metatarsus length, ulna, radius, coracoid, interorbital constriction and bill length by side ($p>0.05$). While PCA analysis showed that the populations of Mashhad and Noor are of different ecotypes, cluster analysis (which considered all morphometric measurements) suggested the Hamedan birds as the most differentiated population of the three. Mitochondrial sequences by other authors showed that the three geographically close populations are distinct from each other.

Keywords: Great Tit, *cinereus* group, *major* group, morpho-skeletal factors, *Parus major, P.m. blanfordi, P.m. intermedius, P.m. karelini*, population, skull, subspecies, Iran.

INTRODUCTION

The family Paridae has 51 species, six of which are distributed in Iran – Great Tit *Parus major*, Coal Tit *P. ater*, Blue Tit *P. caeruleus*, Sombre Tit *P. lugubris*, Turkestan Tit *P. bokharensis* and Yellow-breasted Tit *P. flavipectus* (Scott & Adhami 2006, Mansoori 2008). The Great Tit varies geographically throughout its enormous range and, as a result, has been split into no fewer than 33 subspecies (Dickinson 2003).

The various subspecies belong to three groups of subspecies (*P. m. minor, P. m. cinereus, P. m. major*) differing in coloration (Vaurie 1950). Different plumage between three groups of subspecies can be distinguished as follows: *major* group: green back and yellow underparts; *cinereus* group: blue-grey back and white under parts; *minor* group: green back and whitish underparts (Vaurie & Snow 1957). Hybridisation has been reported in some regions, for instance the *major* and the *minor* groups hybridise in the Amur valley (Vaurie 1950, Kvist & Rytkönen 2006).

In Iran the range of the Great Tit is a narrow band; in the north from the east to the west and southwest, and in the south from the Zagros Mountains eastward to Kerman (Mansoori 2008). Three subspecies of the Great Tit have been described in Iran, namely *P. m. karelini, P. m. blanfordi* and *P. m. intermedius*. Zarudny (1890) reported a new race of Great Tit in Afghanistan and Baluchistan mountains and named it *P. m. intermedius* and Prazák (1894) reported a new race of Great Tit in Tehran, west and southwest Iran and named it *P. m. blanfordi* (Vaurie 1950). Witherby (1903) stated that he considered it inadvisable to separate the southwest Persian Great Tit from specimens of Europe, but based on an examination of a larger series in 1907, he found that the paleness of the colouration of the breast is constant and marked when compared with typical examples from Europe and suggested separation of the subspecies (Witherby 1907). Zarudny (1910) reported a new race of Great Tit in Talesh (northwest Iran) and named it *P. m. karelini.* He stated this form averages a little smaller than typical nominate *major* from Europe and western Iran (Vaurie 1950). Considering the controversy about subspecies of the Great Tit Kvist *et al.* (2003) analysed the subspecies groups of the species using mitochondrial sequences and suggested that they have not yet diverged into different species as they have interbreeding in some regions.

In 1957, Vaurie & Snow (1957) reviewed the Palearctic subspecies of Great Tit and proposed a few changes in their previous classification. Other researchers such as Delacour & Vaurie (1950), Vaurie (1950, 1959) and Kvist *et al.* (2007) have studied the populations of Great Tit in Iran. However, so far there are no inclusive studies of Iranian populations involving multiple morpho-skeletal factors to evaluate whether or not mitochondrial and molecular studies are supported by morpho-skeletal measurements. We therefore aimed to study the morphological factors of the subspecies occurring in Iran. However, we are aware that recently-published molecular studies have found apparent incongruencies with non-molecular taxonomic conclusions (*e.g.* Olsson *et al*. 2009). Previous studies on morphometrical comparisons of the Great Tits had been focused only on a few factors such as wingspan, wing breadth, wing length, tail length, tail index, yellow lipochrome and white wedge on tail feathers mentioned in the important work of Vaurie (1950) and Vaurie & Snow (1957). Nevertheless, our results were comparable with the earlier studies for these factors. However, it is vital that future studies, like ours, whether concerned with the morphometric and skeletal characteristics of *Parus major* or any other bird species, endeavour to employ a large set of standardised criteria.

MATERIALS AND METHODS

The three study sites (Fig. 1) represented populations from all the three subspecies; *intermedius, karelini* and *blanfordi*, respectively; Vakilabad forest park in Mashhad, northeastern Iran, elevation 970 m a.s.l. (36°20'N, 59°30'E), Noor Forest Park in Noor, south Caspian lowlands, –22 m a.s.l. (36°34'N, 51°50'E) and Abbasabad gardens on the southern slopes of Alvand mountain around Hamedan, western Iran, 1850 m a.s.l. (34°40'N, 48°30'E). A sample of 40 Great Tits was obtained during autumn 2001 to spring 2002. The number of specimens from Mashhad, Noor and Hamedan was 12 (7 males and 5 females), 21 (8 males, 11 females and 2 unsexed specimens) and 7 (4 males and 3 females), respectively. Specimens were transferred to the laboratory after freezing. After boiling skulls and bones in water, they were cleaned and separated. Twenty-nine factors were measured using a 0.05 mm precision callipers (Table 1). These factors were bill depth, nares to tip, shield width (Eason *et al.* 2001), body length, bill length, wingspan, wing length, wing breadth, tail length, tarso-metatarsus length, mid-toe length (Dementev & Gladkov 1970), humerus, ulna, radius, carpo-metacarpus, coracoid, femur, tibiotarsus, tarso-metatarsus bone (Gilbert 1990), profile length (PL), brain-case height (BcH), brain-case breadth (BcB), bill length by side (BL), interorbital constriction (IC), lacrymal breadth (LB), mandible length (MdL) and height of ramus mandible (HRM) (Ruprecht 1984) (Fig. 2). Additionally, we calculated the tail index based on the proportion of tail length to wing length. The length of white wedge on the inner web of the outer pair tail feathers (t_6) were measured, and then calculated as the proportion to the tail length (Vaurie 1959, Vaurie & Snow 1957). We also considered the existence of yellow lipochrome on underparts and nape but had not measured this factor. Body weights were omitted from this study because crop contents varied between individuals. All measured variables followed a normal distribution verified by the Kolmogorov-Smirnov test. The *t*-test, Bonferonni univariate test and Tukey multivariate test (one-way ANOVA), PCA Factor Analysis and Cluster Analysis were performed using SPSS and Past programs.

Figure1. Map of the study areas in Iran (extracted from MapSource software).

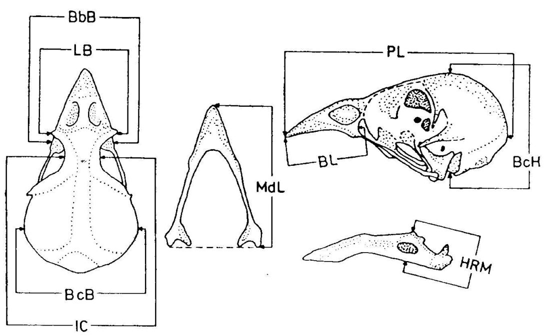


Figure 2. A scheme for skull measurements: lacrymal breadth (LB), brain-case breadth (BcB), interorbital constriction (IC), mandible length (MdL), profile length (PL), bill length by side (BL), brain-case height (BcH), and height of ramus mandible (HRM) (Ruprecht 1984).

RESULTS AND DISCUSSION

The results of comparison of biometric data of external, bone and skull factors revealed that 21 out of 29 characteristics showed significant differences among the three populations of Mashhad, Noor and Hamedan ($p<0.05$; Table 2). However, we could not find any significant difference between the three populations for the following factors: bill length, bill depth, tarso-metatarsus length, ulna, radius, coracoid, bill length by side and interorbital constriction ($p>0.05$; Table 2)

The differences between the sexes of the 29 measured factors were also studied within the populations. In general nares to tip, tarso-metatarsus length, humerus, tibiotarsus and brain-case breadth were significantly different between the sexes ($p<0.05$; Table 1). The only difference found in Noor was the brain-case height. In Mashhad the sexes differed significantly in the following factors: bill length and depth, nares to tip, wingspan, tail index, and carpo-metacarpus. In Hamedan, bill depth, tarso-metatarsus bone, mid-toe length, carpo-metacarpus, ulna, coracoids and lachrymal length showed significant differences ($p<0.05$; Table 1). Despite these findings, the small number of specimens remains a constraint on reaching any strong conclusions. Therefore, we suggest future studies are needed in at least one of the populations where these factors were significant dimorphically.

The body length was longer in Mashhad (160.55 mm, N=12) than in Hamedan (152.76 mm, N=7) and Noor (149.21, N=21; Table 1), but there was no significant difference between the Noor and Hamedan populations (Table 2).

The wingspan and wing length and breadth showed significant differences between the three populations ($p<0.05$), all measures were smallest in the Noor birds and largest in the Mashhad birds (Table 2). Four males of *P. m karelini* measured by Stresemann (1928) had a wing length of 70–72 mm, being smaller than those of nominate *major*. A larger series of *P. m. karelini* measured by Zarudny & Bilkevitch (1913) had wing lengths of 68.4–76.3 mm (N=32 males). These measures overlap those of nominate *major* in which the wing length of males ranges from 73 to 83 mm (Vaurie & Snow 1957). The wing length does not appear to be correlated with migration. In supposedly sedentary populations in Iran the mean wing length in the Zagros is 76.8 mm, 77.4 mm, and in *P. m. intermedius* in Khorasan averaging 77.0 mm (N=28) (Vaurie 1950). This measure is shorter in *P. m. karelini* in Gilan (Mean=71.4 mm, N=4) and South Caspian (Mean=72.8, N=32), being also shorter than in other subspecies in the region. In Hamedan, the wing length was 76.2 mm (N=5), very close to that of *P. m. intermedius* in Khorasan. The wing length of the southern Caspian region corresponds very well to the measurements of European birds (Vaurie & Snow 1957).

Our results showed that the tail length showed significant differences between the three pairwise populations (p<0.05). The longest tails were in Mashhad (64.23 mm) and shortest in Noor (59.67 mm). In previous studies, the mean tail length in *P. m. intermedius,* and in *P. m. blanfordi* in Hamedan were 65.0 mm (N=26) and 62.0 mm (N=5), respectively (Vaurie & Snow 1957).

In this study the tail index was found to be different only between the Noor and Mashhad populations. Vaurie & Snow (1957) reported values of 84.5 (N=26), 88.0 (N=27) and 81.5 (N=5) for this index for *P. m. intermedius* in Khorasan, *P. m. karelini* in Gilan, and *P. m. blanfordi* in Hamedan, respectively. In our results this index was measured more for Mashhad (87.41) and Hamedan (86.96) but less for Noor (84.89) (Table 1). In the populations from Turkestan the tail index is 100.5 to 103.6 (Vaurie 1950) contrary to the index for the mean of all three populations from Iran (86.01±2.75; Table 1).

The proportion of the white wedge (on the inner web of the tail feather t_6) to the tail length was different between Hamedan birds and those from Noor and Mashhad. In Noor the highest proportion of birds were in the 0–9% category, while in Mashad most birds were in the 40–49% category and in Hamedan, in the 50–59% category (Table 3). The length of the white wedge in Hamedan specimens was distinctly more than in the specimens of Mashhad and Noor (p<0.01), 71.4% of the birds occupied the upper the two categories of white wedge length (50%–69%) ,whereas only 16.7% of birds from Mashhad and 0% of Noor fell into this category (Table 3). Figure 3 shows the frequency of the categories of white wedge length among Iranian clines (from the north to the south) (based on data presented in Vaurie & Snow 1957). The differences in white wedge in Iran strongly suggest that the south Caspian birds are genetically rather well isolated from *blanfordi* from west-central Iran (Vaurie & Snow 1957).

Regarding bone measurements, the measurements of humerus and mid-toe length showed significant differences between the populations (p<0.05). Although femur and tibiotarsus were different among the three populations, the difference between Noor and Hamedan birds was not significant (p>0.05; Table 2). Skull measurements, were significantly different for profile length, brain-case height, brain-case breadth, lacrymal breadth, mandible length and height of ramus mandible (p<0.05; Table 2). In detailed analysis, brain-case height, brain-case breadth and lacrymal breadth were significantly different between the populations of Noor and Hamedan, while mandible length was different between the Mashhad and Noor populations. Profile length was not significant between Mashhad and Noor populations nor was the height of ramus mandible between Mashhad and Hamedan (p>0.05; Table 2).

The yellow lipochrome is completely absent in Mashhad specimens (Fig. 4). Regarding the paleness, specimens from the regions of Noor and Hamedan are similar, but different from Mashhad birds, whereas specimens from Noor were slightly darker than those from Hamedan. This was mentioned by Slagsvold & Lifjeld (1985) in their description of pale plumage in southern subspecies. Subspecies discrimination is based very largely on the presence or absence of the yellow lipochrome, the greater or lesser degree of saturation and the slight differences in the distribution of pigment (Fig. 4, Cramp & Perrins 1993). In our study region, the yellow lipochrome in the western populations (in which the plumage of the adult is pigmented with yellow above and below) is different from the eastern populations (in which the yellow lipochrome is completely absent in the adult form and is present or absent in the immature). The subspecies *intermedius* that intervenes geographically between the yellow and grey subspecies has been suggested as a hybrid population (Vaurie 1950), the adult showing a varying amount of yellow on the back but none on the underparts. In the yellow western subspecies, there is variation on the amount of yellow lipochrome on the lower surface of the body. In the grey eastern subspecies, the plumage above and below is saturated to a greater or lesser degree and the greater coverts and inner tail feathers have a varying degree of melanin. The variation also affects the presence, absence or amount of pigment on the second outer pair of tail feathers and sometimes on the tips of the third. It has been suggested that this degree of saturation correlates with humidity (Vaurie 1950).

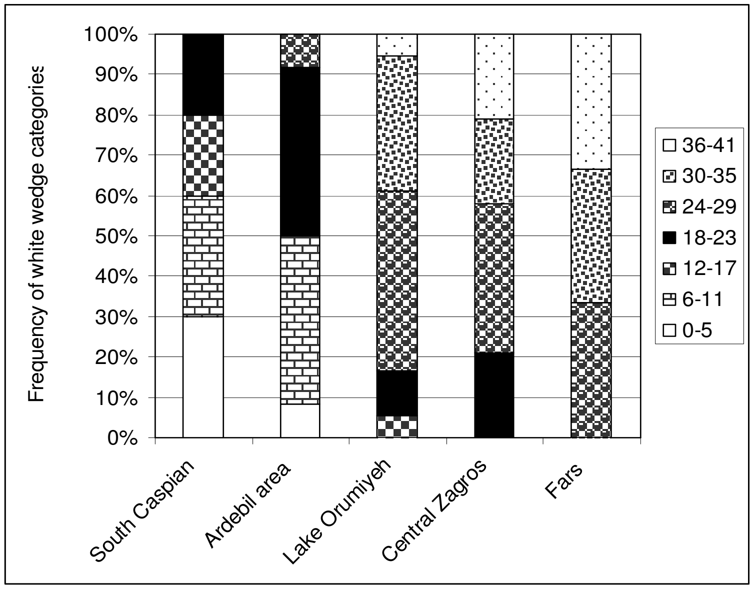


Figure 3. The length of white wedge on outer tail feather in different clines in Iran (based on data presented in Vaurie & Snow 1957).

Mashad

Noor

Hamedan

Figure 4. Different plumage of specimens from Mashhad, Noor and Hamedan.

Table 1. Averages (mm)±SD of measurements of morpho-skeletal factors in Great Tit populations from Mashhad, Noor and Hamedan. Results of *t*-tests between the sexes are shown: *= *p*<0.05, **= *p*<0.01, ns= *p*>0.05.

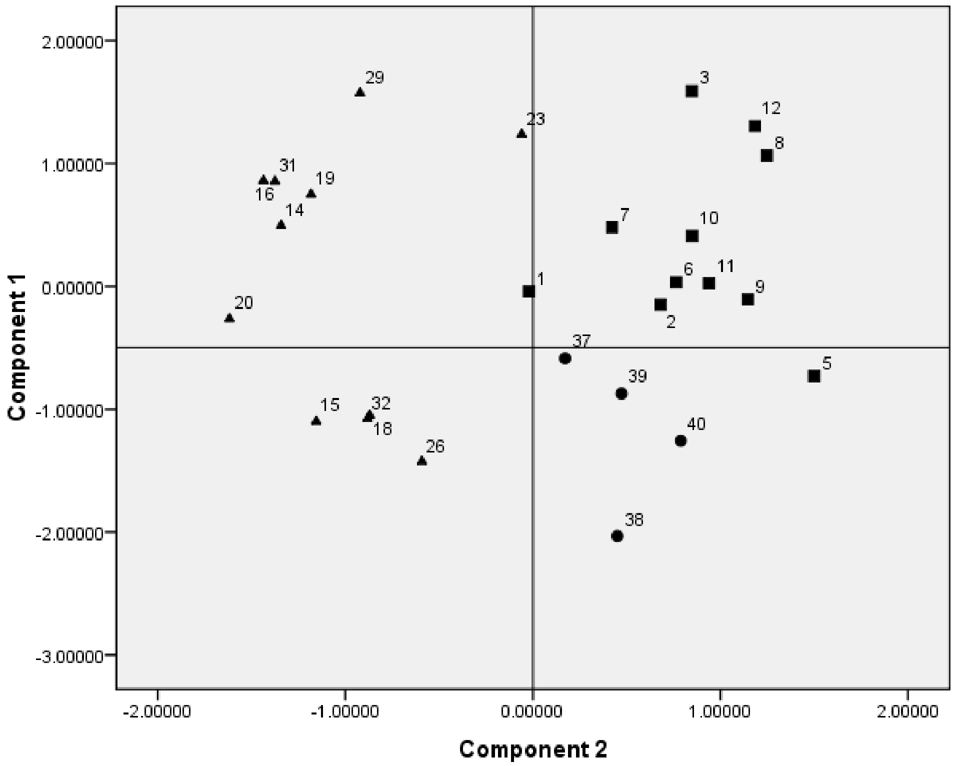
Factors		**N**	**Mashhad (*t*-test *p*-value)**	**Noor (*t*-test *p*-value)**	**Hamedan (*t*-test *p*-value)**	**TOTAL (*t*-test p-value)**
	Sample size (N)		**12 (7M, 5F)**	**21 (8M, 11F)**	**7 (4M,3F)**	**19 M, 19 F**
Body	Body length	39	160.55±3.22ns	149.21±4.76ns	152.76±0.65ns	153.25±6.39ns
Bill	Bill length	40	11.44±0.45*	11.53±0.41ns	11.20±0.10ns	11.45±0.40ns
	Bill depth	40	5.02±0.01**	5.10±0.22ns	5.20±0.28*	5.09±0.20ns
	Shield width	39	5.36±0.23ns	5.62±0.20ns	5.30±0.09ns	5.49±0.24ns
	Nares to tip	40	9.46±0.36**	9.15±0.43ns	8.66±0.26ns	9.18±0.48*
Wing	Wingspan	38	227.61±2.79**	214.55±8.97ns	216.84±0.27ns	219.04±8.90ns
	Wing length	40	73.47±1.49ns	70.31±2.28ns	70.60±0.69ns	71.31±2.33ns
	Wing breadth	39	61.07±1.24ns	59.04±2.32ns	60.08±1.00ns	59.80±2.05ns
Tail	Tail length	40	64.23±8.89ns	59.67±2.39ns	61.39±0.93ns	62.09±5.90ns
	Tail index	40	87.41±2.75*	84.89±2.79ns	86.96±0.47ns	86.01±2.75ns
	Proportion of the white wedge on the inner web of t_6 to tail length (%)	40	45.17±6.28ns	13.62±10.49ns	54.14±12.56ns	30.17±20.29ns
Foot	Tarso-metatarsus length	40	19.72±0.56ns	19.81±0.50ns	19.23±0.40*	19.55±0.51*
	Mid-toe length	40	13.87±0.12ns	14.24±0.41ns	13.46±0.42*	13.99±0.45ns
Bones (wing)	Humerus	38	17.83±0.30ns	17.39±0.30ns	17.00±0.32ns	17.46±0.42*
	Ulna	40	18.32±0.34ns	18.24±0.49ns	17.96±0.11**	18.22±0.42ns
	Radius	39	19.90±0.29ns	20.03±0.64ns	19.84±0.31ns	19.96±0.50ns
	Carpo-metacarpus	40	11.01±0.15*	11.11±0.31ns	10.77±0.22**	11.02±0.28ns
Bones (body)	Coracoid	37	15.85±0.22ns	15.84±0.40ns	15.73±0.10**	15.82±0.31ns
Bones (foot)	Femur	38	15.94±0.38ns	15.46±0.29ns	15.35±0.26ns	15.59±0.39ns
	Tibiotarsus	36	28.32±0.56ns	27.60±0.71ns	27.08±0.68ns	27.74±0.80*
	Tarso-metatarsus bone	40	19.57±0.47ns	19.63±0.48ns	19.31±0.45ns	19.68±0.51ns
Skull	Profile length (PL)	40	27.65±0.41ns	27.34±0.53ns	26.82±0.44ns	27.34±0.55ns
	Brain-case height (BcH)	40	12.05±0.36ns	12.30±0.27*	11.89±0.19ns	12.15±0.33ns
	Brain-case breadth (BcB)	38	15.03±0.51ns	15.18±0.21ns	14.69±0.35ns	15.04±0.39*
	Interorbital constriction (IC)	40	2.71±0.30ns	2.64±0.22ns	2.61±0.15ns	2.65±0.23ns
	Bill length by side (BL)	40	9.43±0.29ns	9.42±0.30ns	9.35±0.02ns	9.41±0.27ns
	Lacrymal breadth (LB)	40	5.44±0.24ns	5.57±0.18ns	5.36±0.16*	5.49±0.21ns
	Mandible length (MdL)	39	19.32±1.10ns	18.64±0.39ns	18.57±0.30ns	18.84±0.74ns
	Height of ramus mandible (HRM)	40	2.35±0.25ns	2.53±0.17ns	2.26±0.09ns	2.43±0.21ns

Table 2. Results of Tukey tests with 95% confidence for morphometrical factors from the three populations of Great Tits from Mashhad, Noor and Hamedan and *p*-values from Bonferonni univariate test between Great Tit populations from Mashhad, Noor and Hamedan. *= $p<0.05$, **= $p<0.01$, ns= $p>0.05$.

Factors		Mashhad-Noor	Mashhad-Hamedan	Noor-Hamedan	Bonferonni *p*-value
	Sample size (n)	12 (7M, 5F)	21 (8M, 11F)	7 (4M,3F)	
Body	Body length	0.000**	0.001**	0.146 ns	0.000**
Bill	Bill length	0.779ns	0.424 ns	0.142 ns	0.167 ns
	Bill depth	0.498 ns	0.135 ns	0.462 ns	0.156 ns
	Shield width	0.003**	0.803 ns	0.004**	0.000**
	Nares to tip	0.020*	0.000**	0.018*	0.000**
Wing	Wingspan	0.000**	0.009**	0.751 ns	0.000**
	Wing length	0.000**	0.007**	0.936 ns	0.000**
	Wing breadth	0.018*	0.534 ns	0.430 ns	0.023*
Tail	Tail length	0.002**	0.088 ns	0.726 ns	0.002**
	Tail index	0.025*	0.926 ns	0.165 ns	0.021 *
	Proportion of the white wedge on the inner web of t_6 to tail length (%)	0.000**	0.148 ns	0.000**	0.000**
Foot	Tarso-metatarsus length	0.953ns	0.425 ns	0.250 ns	0.276 ns
	Mid-toe length	0.019*	0.046*	0.000**	0.000**
Bones (wing)	Humerus	0.001**	0.000**	0.016*	0.000**
	Ulna	0.872 ns	0.180 ns	0.275 ns	0.190 ns
	Radius	0.775 ns	0.957 ns	0.661 ns	0.627 ns
	Carpo-metacarpus	0.550 ns	0.154 ns	0.015*	0.020*
Bones (body)	Coracoid	0.997 ns	0.765 ns	0.774 ns	0.758 ns
Bones (foot)	Femur	0.001**	0.001**	0.716 ns	0.000**
	Tibiotarsus	0.018*	0.001**	0.203 ns	0.001**
	Tarso-metatarsus bone	0.847 ns	0.089 ns	0.020*	0.026*
Skull	Profile length (PL)	0.201 ns	0.002**	0.042*	0.003**
	Brain-case height (BcH)	0.067 ns	0.487 ns	0.008**	0.005**
	Brain-case breadth (BcB)	0.476 ns	0.128 ns	0.010*	0.013*
	Interorbital constriction (IC)	0.705 ns	0.656 ns	0.953 ns	0.623 ns
	Bill length by side (BL)	0.987 ns	0.767 ns	0.804 ns	0.769 ns
	Lacrymal breadth (LB)	0.175 ns	0.643 ns	0.044*	0.033*
	Mandible length (MdL)	0.028*	0.071 ns	0.971 ns	0.021*
	Height of ramus mandible (HRM)	0.037*	0.536 ns	0.006**	0.003**

Table 3. Proportions of categories of the white wedge on the inner web of the outer pair tail feathers in three populations of the Great Tit in Iran.

Proportion of the white wedge $_6$ to tail length (%)	Mashhad (%)	Noor (%)	Hamedan (%)
00–09	-	47.6	-
10–19	-	28.5	-
20–29	-	14.3	-
30–39	-	4.8	28.6
40–49	83.3	4.8	-
50–59	16.7	-	42.8
60–69	-	-	28.6



	Value	Variance
Eigenvalue 1:	8.29	28.60%
Eigenvalue 2:	5.64	19.45%

Figure 5. PCA graph of biometrical factor analysis (and Eigenvalue of two most important factors) for three populations: Mashhad (solid square), Noor (solid triangle) and Hamedan (solid circle).

The PCA analyses showed a clear separation of the Mashhad and Noor populations, while the Hamedan population was located between them, closer to Mashhad than Noor (Fig. 5). When all the morphometric measurements were included, the cluster analysis suggested the populations of Mashhad and Noor to be more similar to each other than to the population of Hamedan and that bill depth was the most important factor for classifying the other factors. The specimens from the east of Iran are larger than specimens from the west (Table 1). Our results showed that the three studied populations vary clearly in morphology and plumage coloration, showing a clinal pattern.

The birds from Khorasan are morphologically quite different from pure *major*, having an almost white belly and a greyish mantle. The birds from Mazandaran Province are yellower, and the birds from the westernmost sampling site in Hamedan Province appear phenotypically as pure *major* (Vaurie & Snow 1957).

However, of the three sampling sites, the easternmost population in Khorasan Province is located on a region where *P. m. blanfordi* of the *major* group is assumed to hybridise with the *cinereus* group giving rise to *P. m. intermedius* (Formozov *et al*. 1993, Martens 1996). It has also been suggested that *P. m. bokharensis* may hybridise there with Great Tits from other adjacent subspecies groups (Harrap & Quinn 1996, Martens 1996). It is possible that specimens from the southern Caspian average somewhat paler than nominate *major*, and thus show a tendency towards the paler *blanfordi*, which replaces the nominate *major* at the eastern corner of the Caspian, on the Iranian Plateau and in the Zagros. Nonetheless, Vaurie & Snow (1957) stated that the population in the north of Iran is identical in coloration and size with the nominate *major* from Europe and doubted that the difference is sufficient to warrant the recognition of the subspecies *karelini*. If we consider the overlap of wing length and the fact that the difference in coloration can be very slight at the best, it seems that *karelini* is much too poorly differentiated to deserve subspecies status and should be synonymised with the nominate *major* (Vaurie & Snow 1957).

The mitochondrial sequences revealed that in Iran, the three geographically close populations are distinct (Kvist *et al.* 2003) but the Hamedan population did not differ from the Central *major* population. This differentiation was not observed in the nuclear microsatellite loci. In their sample of 31 birds, Kvist *et al.* (2007) did not find any *cinereus* or *bokharensis* haplotypes in Iranian populations. In a phylogenetic tree, the Iranian haplotypes were placed closer to the root (*bokharensis*) than the other haplotypes of the *major* group (Kvist *et al.* 2007). However, because present or recent admixture was detected by Kvist *et al.* (2003, 2007), more studies are necessary to test for genetic diversification in relation to morphological diversification in the Great Tit.

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