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The Morphological and Behavioral Analysis of Geographically Separated *Rammeihippus turcicus* (Orthoptera: Acrididae: Gomphocerinae) Populations: Data Result in Taxonomical Conflict

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ABSTRACT. *Rammeihippus* Woznessenskij, 1996 (Orthoptera: Acrididae: Gomphocerinae) is a genus represented by two species. *Rammeihippus turcicus* (Ramme, 1939) is the only known species of the genus from Anatolia. As for most of the Gomphocerinae species in Anatolia, all populations of the species are intermittently distributed at high altitudes. In this study, three populations of *R. turcicus* were studied for the first time to determine the song and mating behavior. Males of the species produce typical calling song for Gomphocerinae and complex courtship songs and mating behavior. Thus, an accurate taxonomy requires extensive material and different character sources. In this study, the Anatolian *Rammeihippus* was re-examined on the basis of qualitative and morphometric morphology, male songs, and behavioral characteristics. There was no agreement between the results of the song and morphology. Acoustic analysis suggested one species and patchy distribution in the area, whereas morphology pointed out that each population was a different taxonomical unit. The results of the study show that the aberrant morphology does not necessarily indicate a new species in the Gomphocerinae genus.

Key Words: song complexity, courtship behavior, Anatolia

Gomphocerinae is one of a species-rich subfamily, and these groups involve closely related species with low-level differences in morphological characters (e.g., Harz 1975). The species of the subfamily are well known for their elaborate acoustic signals, and they have a complex bidirectional (both partners stridulate and respond to each other) acoustic communication system that plays the most important role in reproductive isolation between species (e.g., Elsner 1974, Helversen and Elsner 1977, Helversen and Helversen 1983, Mayer et al. 2010, Vedenina and Mugue 2011). Therefore, communication signals are a useful tool to clarify the taxonomic situation of morphologically similar taxa (e.g., *Chorthippus parallelus* group, Butlin and Hewitt 1985; *Chorthippus biguttulus* group, Ingrisch 1995, Willemse et al. 2009; *Chorthippus demokidovi* group, Çıplak et al. 2005; *Chorthippus dorsatus* group, Stumpner and Helversen 1994; *Chorthippus albamarginatus* group, Helversen 1986; in different numbers of groups of genus *Stenobothrus*, Berger 2008, Berger et al. 2010; *Ramburiella*, Savitsky 2002; *Omocestus*, Savitsky 2005; *Dociostaurus*, Blondheim 1990, Savitsky 2000, 2007; and *Myrmeleotettix*, Şirin et al. 2011).

Although most of the species in the subfamily were described based on the morphological characters (Bei-Bienko and Mistshenko 1951, Harz 1975, etc.) in the past, reliable identification of morphologically similar grasshoppers was recently achieved by analyzing their songs (Helversen 1989; Çıplak et al. 2005; Vedenina and Helversen 2009; Şirin et al. 2010b, 2011, etc.). This may have caused misidentifications of subfamily in previous articles that deal with topographically and climatically rich areas like Anatolia. Consequently, it is not even certain whether previous records of morphologically similar species represent the respective nominate species or new ones. Two recent studies about *Chorthippus brunneus* and *Myrmeleotettix maculatus* are good examples to elucidate this situation. The distribution of *C. brunneus* species was given as from nearly all parts of Anatolia; conversely, *Chorthippus bornhalmi* was indicated in only one locality by several authors (Karabağ 1958, Weidner 1969, Demirsoy 1977, Çıplak et al. 2002, Yalim and Çıplak 2002) who used qualitative morphology for identification of the species. Using song analysis of the relevant species, Şirin et al. (2010b) determined that *C. brunneus* is not found in Anatolia and that *C. bornhalmi* is the most widespread member of lineage in Anatolia. *M. maculatus* is another example showing that morphological similarity causes a taxonomical conflict in the subfamily. According to the available literature, based on morphological characters for identification, the species is found commonly in highlands along the Black Sea basin of Anatolia and one additional locality in the south (Bei-Bienko and Mistshenko 1951, Ramme 1951, Karabağ 1958, Weidner 1969, Harz 1975, Demirsoy 1977, Çıplak et al. 2002). Şirin et al. (2011) revealed the northern populations have clear song differentiations compared with the southern population, which therefore represents a distinct new species.

Rammeihippus turcicus (Ramme, 1939) is present in the Ankara and Kastamonu districts of Anatolia (Ramme 1939, Karabağ 1958, Weidner 1969, Harz 1975, Demirsoy 1977). This species has been described using quantitative morphological characters by Ramme (1939), and there is no song record or behavioral analysis of it. The distribution of *R. turcicus* populations is geographically split from the north to the south. *R. turcicus* with these specifications is a good candidate to study differentiation rank in morphological and behavioral characters in allopatrically distributed populations.

In this study, all geographic *R. turcicus* (Ramme 1939) populations in Anatolia were studied in detail, using behavioral and morphological characters to answer the following questions: what is the distribution range of *R. turcicus* populations in Anatolia? Is there a phenotypic differentiation between the various geographically separated populations? If phenotypic differences are found between populations, could these differences be accepted to lie within the variation limits of one taxon?

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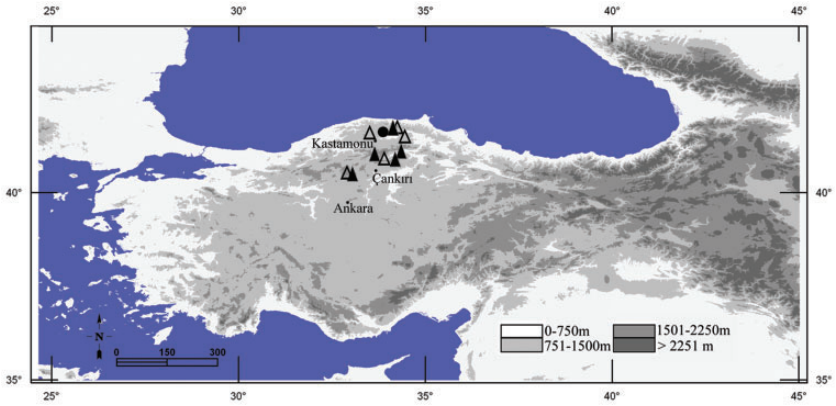

Fig. 1. The localities of *R. turcicus* members in Anatolia: 1) filled triangles represent the record of *R. turcicus*; 2) nonfilled triangles represent the location of studied samples from different museum and collection (Table 1); 3) large filled circle represents type locality of *R. turcicus* (Table 1).

Table 1. List of records of *R. turcicus* in Turkey

Locality	References	Reported as
Type locality: Anatolien, 60 km südl. Ineboli, 1,300 m, 15.X.1933, leg: Dr. Kummerlüve, Dr. Niethammer	Ramme (1939)	*Microhippus turcicus* n. sp.
Ankara-Aydos, Uluagaç yaylası, 1,800 m, 9.IX.1947, 4 ♂♂, 6 ♀♀, leg.: T. Karabağ	Karabağ (1958)	*M. turcicus*
Ankara-Aydos, Uluagaç yaylası, 1,800 m, 9.IX.1947, 1 ♂♂, 4 ♀♀, leg: T. Karabağ	HUZOM	*M. turcicus*
Rammeihippus nom. n. for *Microhippus* Ramme, 1939	Woznessenskij (1996)	*Rammeihippus*
Kastamonu, Devrekani-Catalzeytin yolu, Bozarmut koyu, Yaralıgöz geçidi, 26.VII.2004, 1,450 m, 31 ♂♂, 52♀♀	Mol (2007) (A. Mol collection)	*M. turcicus*
Kastamonu, Yaralıgöz geçidi, 1,450 m, 21.VIII.2004, 12 ♂♂, 9 ♀♀; 17.VII.2005, 11 ♂♂, 2 ♀♀; leg.: A. Mol	Mol (2007) (A. Mol collection)	*M. turcicus*
Kastamonu-Kure yolu, Oyrak geçidi, 1,210 m, 26.VII.2004, 5 ♂♂, 2 ♀♀; 21.VIII.2004, 4 ♂♂, 1 ♀; 17.VII.2005, 7 ♂♂, 3 ♀♀; leg.: A. Mol	Mol (2007) (A. Mol collection)	*M. turcicus*
Kastamonu, Tosya-İskilip yolu, Turbe geçidi, 1,625 m, 20.VII.2004, 5 ♂♂, 5 ♀♀; 20.VIII.2004, 6 ♂♂, 3 ♀♀; 13.VII.2005, 10 ♂♂, 4 ♀♀; leg.: A. Mol	Mol (2007) (A. Mol collection)	*M. turcicus*
Kastamonu, Turbe geçidi altı, 1,100 m, 20.VII.2004, 2 ♂♂, 4 ♀♀; 20.VIII.2004, 4 ♂♂, 3 ♀♀; 13.VII.2005, 1 ♂; leg.: A. Mol	Mol (2007) (A. Mol collection)	*M. turcicus*
Kastamonu, Devrakani- Çatalzeytin yolu, Yaralıgöz geçidine 6 km. kala, 1,308 m, N: 41°44.411 E: 34°02.020, 18.VIII.2011, 14 ♂♂, 12 ♀♀, leg.: D. Şirin, A. Mol, and G. Akyıldız	This study	*R. turcicus*
Kastamonu, Tosya-İskilip yolu, Türbe geçidi civarı, 1,622 m, N: 40°56.250 E: 34°12.702, 17.VIII.2011, 12 ♂♂, 12 ♀♀, leg.: D. Şirin, A. Mol, and G. Akyıldız	This study	*R. turcicus*
Kastamonu, Ilgaz Ulusal parkı, TRT vericisi yanı, N 41°03.246, E 033°43.053, 2,045 m N. N., 18.VIII.2011, 2 ♂♂, 1 ♀♀, leg.: D. Şirin and G. Akyıldız	This study	*R. turcicus*
Çankırı, Orta, Dodurga, Tutmaçbayındır köyü, Köyden 4.4 km sonra, Uluagaç yaylası, 1,589 m, N: 40° 31.141 E: 32°58.734, 19.VIII.2011, 12 ♂♂, 11 ♀♀, leg.: D. Şirin and G. Akyıldız	This study	*R. turcicus*

Materials and Methods

All cited areas in the literature and the possible habitats throughout the middle Black Sea region of Anatolia were visited (Fig. 1) to collect *R. turcicus* samples (Ramme 1939, Bei-Bienko and Mistshenko 1951, Harz 1975, Demirsoy 1977, Mol 2007). During these field studies, four populations of *R. turcicus* were encountered in unconnected areas (Fig. 1; Table 1). However, enough adult and nymphs samples could not be collected from Ilgaz populations; therefore, Ilgaz populations were not used in the analysis. Animals were collected as adults or nymphs from localities, and nymphs were raised in the laboratory. Collected animals were kept in wood cages (35 by 35 by 35 cm) and fed with *Dactylis glomerata* or *Poa densa.* Light and additional heat were provided for 12 h each day with a 50 -W bulb inside the cage in the laboratory. Cages were monitored daily. At the day of imaginal molt, sexes were separated and housed in different cages. Adult grasshoppers were marked individually with paint markers by a color code on their pronotum (Edding 751).

Song recordings of collected animals were made in the laboratory of the Biology Department at Namik Kemal University, Tekirdag, Turkey. All song recordings were carried out using a TASCAM DR-100 (TEAC Corporation, USA) recorder with a Philips-SBC ME 570 (Koninklijke Philips Electronics N.V., Netherland) condenser microphone (frequency response from 50 to 18,000 Hz with a sensitivity of −45 ± 3 dB). The microphone was kept about 5–15 cm away from the calling male. The male songs were digitalized at 48,000 Hz and analyzed with custom-designed software (Wolfram Schulze, Tierphysiologie an der Universität Bayreuth, Germany) developed in LabVIEW 7 (National Instruments, Austin, TX) and Turbolab 4.0 (Stemmer AG). The traditional Gomphocerinae song terminology (Ragge and Reynolds 1998; Berger and Gottsberger 2010; Şirin et al. 2010b, 2011) was slightly modified to describe the songs of *R. turcicus* more accurately. The following terms were used (Fig. 2): calling song—a song produced by an isolated male or a male distant to a female; courtship song—the special song produced by a male when close to a female; phrase—series of syllable groups exhibiting a unit characteristic; phase—each different part of a courtship song that includes a series of syllables or elements; syllable—the main unit of the song regularly repeated throughout a phrase and produced by one up and one downstroke of hind femurs; syllable period—the interval from the beginning of one syllable to that of the next; and

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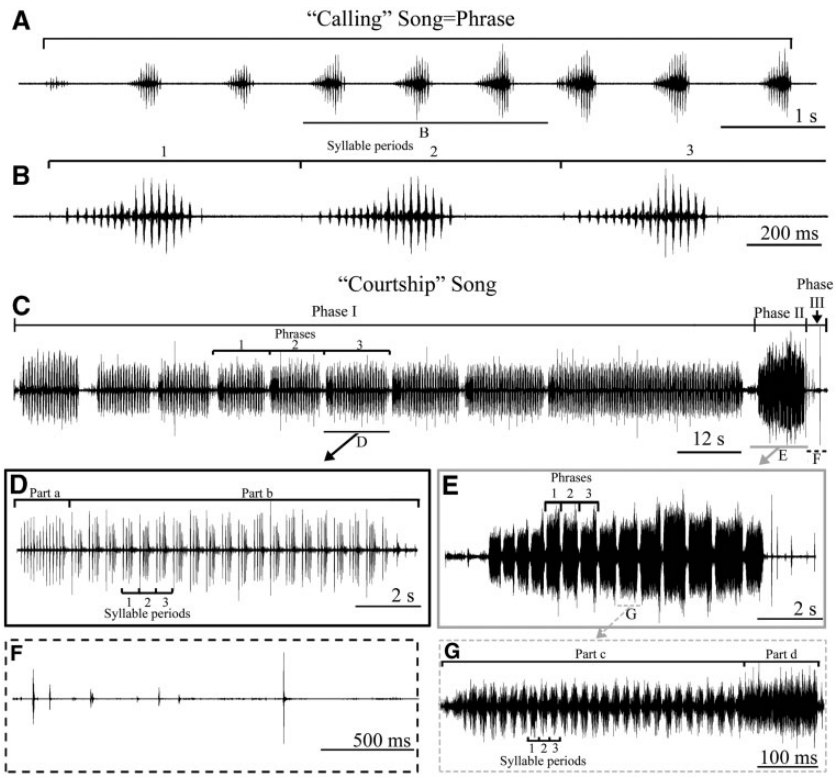

Fig. 2. Terminology for two general song types of *R. turcicus* calling song (A, total song and B, song part, showing a syllable group in detail) and courtship song (C, total song; D, song part of Phase I; E, song part of Phase II; F, song part of Phase III; and G, a complete phrase of Phase II, showing the fine structure of the sound).

pulse—a simple undivided transient train of sound waves. In song descriptions, seconds (s) or milliseconds (ms) were used for duration or intervals.

In addition, video recordings were obtained to observe the male body movements during song production in detail. These videos were recorded (30 frames/s) using Nikon P50 and Canon Powershot SX30 IS cameras. Analyses of video recordings were made with still frames using the programs Kinovea 0.8.15 (www.kinovea.org) and Wax2.0 (www.debugmode.com). Drawings were made in Adobe Photoshop CS4 (Adobe 1990-2008) and Gimp 2.8.2 (www.Gimpshop.com) using single frames of the video records.

Specimens collected during field studies were prepared as museum material by standard methods and preserved in 70% alcohol. Specimens examined during this study are at Namik Kemal University, Department of Biology, Entomological Museum, Tekirdag, Turkey (NKUEM) and the Zoology Museum at Hacettepe University, Department of Biology, Ankara, Turkey (HUZOM).

Statistical Analysis. In previous studies, species of the *Rammeihippus* genus have been distinguished based on qualitative morphology (Ramme 1939, Bei-Bienko and Mistshenko 1951, Harz 1975, Demirsoy 1977). The strength of morphological characters needs to be tested further using appropriate statistical methods in addition to qualitative examination. In this study, courtship songs and behaviors recorded for *R. turcicus* populations were used first, and then 11 morphological characters were measured using a digital camera attached to a stereo microscope (Optica SZM-SMD [Ponteranica, Italy] and a Micros stereomicroscope [Veit/Glan, Austria] attached to a Canon Powershot G-11 [Tokyo, Japan]). Two characters were measured from the head (LH: total length of head, LA: length of antenna). Five characters were measured from the pronotum (LMC: length of medial carina, LPBTS: length of pronotum before typical sulcus, LPATS: length of pronotum after typical sulcus, MADLC: maximum distance between lateral carinae, MIDLC: minimum distance between lateral carinae). Two characters were measured from the tegmina (TL: tegmina length, MWT: maximum width of tegmina). Two characters were measured from the femur (LHF: length of hind femur, MWHF: maximum width of hind femur). In addition, a number of stridulatory pegs were counted. All metric characteristics were analyzed by the appropriate statistical methods separately for male and female.

The parametric and nonparametric nature of morphometric data was determined by applying the Anderson-Darling (for normality) and Levene (homogeneity of variance) tests. As the data were nonparametric, log transformation was carried out to approximate normal distribution prior to factor analysis or canonical discriminate function (CDF) analysis. Factor analysis was used to interpret structural associations among 11 morphometric characters, as well as to reduce the large amount of data. Factors >1 in eigenvalue were selected and scored. These factors were characterized with respect to morphological characters, which had high absolute values for factor loading. Factor loadings point to the overall importance of each character, whereas eigenvalues indicate the proportion of total variance for each factor. The characters with ≥ 0.6 factor loading were included in the analysis because it is suggested as a threshold value for this kind of analyses (Kalaycı 2005). The final result of factor analysis was rotated to a component matrix, which was generated by principal component analysis and varimax with the Kaiser normalization method (Kalaycı 2005).

The CDF method is an ordination technique for displaying and describing the differences between group centroids by extracting the eigenvectors from the pooled variance–covariance matrix of within groups (Mclachlan 2004). CDF analyses were performed separately with factor results generated from male and female metric data. The most frequently used criterion to evaluate the discrimination is Wilks' lambda (Hanzelova et al. 2005, Kalaycı 2005, Radloff et al. 2005), which is the likelihood-ratio statistic for measuring the degree of between-class separation (Beharav and Nevo 2003). In stepwise discriminate function analysis, a model of discrimination is built up step by step

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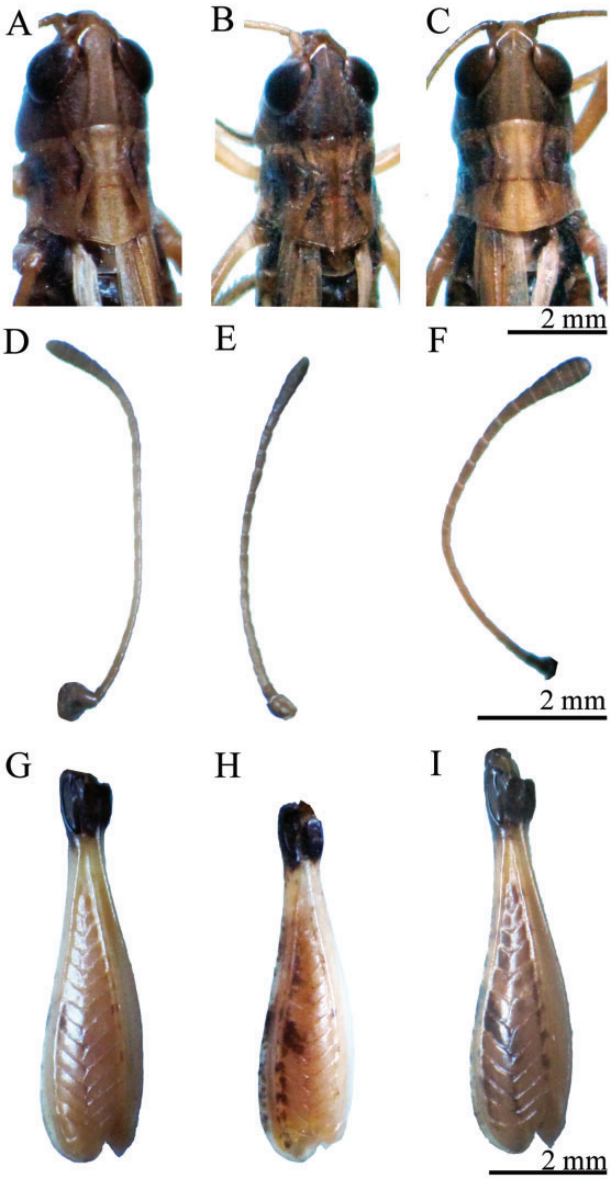

Fig. 3. Pronotum head, antennae, and hind femur of males of *R. turcicus* populations. (A, D, G) Türbe. (B, E, H) Uluağaç. (C, F, I) Yaralıgöz.

(Hill and Lewicki 2005). Specifically, at each step, all variables are reviewed and evaluated to determine which one will contribute mostly to the discrimination between groups. That variable is included in the model, and the process starts again (Hill and Lewicki 2005). For the stepwise computation, the maximum significance of the *F* value for the inclusion of a variable was set to 0.05; the minimal significance for the exclusion was set to 0.1 (Kalaycı 2005).

A factor and CDF, descriptive statistics (minimum and maximum value, means, and standard deviation), plots, and graphs were calculated and prepared using SPSS version 18 (SPSS Inc., New York, NY).

Mating Trial Experiment. In this study, females of three populations of the *R. turcicus* were tested with the males of the other two populations. The 5 females and 10 males were used from each population. During the experiment, females stayed in the cage with males, which belong to one of the other two populations. If the male was not motivated for courtship behavior, it was changed with another male from the same population. The attempt in this experiment is counted positively when the male starts and completes the courtship behavior and is accepted by the female for copulation. Egg pods were stored in moist sand in Petri dishes at 4–6°C over winter; unfortunately, none of them were raised in the successive year.

Results

Morphology. The antenna of both sexes in all *R. turcicus* populations exhibits a distinct club-like widening close to apex (Figs. 3D–F and 4D–F). The head is slightly wider and shorter than the pronotum (Figs. 3A–C and 4A–C). The lateral carinae of the pronotum are distinct and angularly incurved in one-third of the pronotum (Figs. 3D–F and 4D–F). The distal end of the hind femur is always black (Figs. 3G–I and 4G–I). The cerci are short with a distinct cavity before the pointed apex. The tegmina are greatly abbreviated in both sexes. The general features of antennae, pronotum, cerci, and wings are matched in the males and females of all populations of *R. turcicus*. However, some basic Gomphocerinae characteristics, which are used widely for taxon identification, differ from each other: 1) the male tegmina are 6.1–6.4 mm in the Uluağaç, 5.6–5.9 mm in the Yaralıgöz, and 6.9–7.1 mm in the Türbe populations; 2) the maximum length or maximum width of the tegmina is 4.2–4.6 mm in males of the Uluağaç and Türbe populations and 4.6–4.9 mm in the Yaralıgöz population; 3) antennae are 5.8–6.9 mm in the male (5.1–5.5 mm in the Yaralıgöz population) and 3.9–4.2 mm in the female (4.3–4.5 mm in the Türbe population); 4) the maximum length or maximum width of the male hind femur is 4.0–4.5 mm in the Uluağaç, 4.5–4.7 mm in the Yaralıgöz, and 4.8–5.0 mm in the Türbe populations; and 5) the number of stridulatory pegs in males is 103–122 in both the Uluağaç and Yaralıgöz populations and 136–147 in the Türbe population.

Measurements of 11 morphological characters obtained from 28 males from three different populations are listed in Table 2. After a factor analysis from the metric data, the three factors that had eigenvalues that summed to >1 were extracted (Table 3). The total accumulation of the extracted factors explained 81.8% of variation in the original data. The characters, their factor loadings, and eigenvalue of the factors are listed in Table 3. To study the relationship between variables and cumulative values of variation generated, a CDF was performed with the data selected by the factor analysis (Fig. 5A). The results of CDF suggested three distinctive groups with nearly no overlapping. Although the axis of Function 1 separates each population, that of Function 2 clearly separates the southern (Uluagaç) population from the other two northern (Yaralıgöz and Türbe) populations.

The same 11 morphological characters were measured from 35 females belonging to the same populations (Table 2). After a factor analysis, the four factors that had eigenvalues that summed to >1 were extracted (Table 3). The total accumulation of the extracted factors explained 66.36% of the variation in the original data. The characters, their factor loadings, and the eigenvalues of the factors were given in Table 3. The results of female CDF show that all populations were not separated clearly, and they overlapped with each other (Fig. 5B).

Song and Behavior. Calling and courtship songs recorded from three populations of *R. turcicus* were analyzed. This analysis shows that each of the calling and courtship songs shows a pattern that is similar in all populations. Paternal and temporal characteristics of the calling (Fig. 6) and courtship songs (Fig. 7) were very similar in the three populations.

Calling Song Characteristics. The calling song of males in all the populations is a typical phrase (Fig. 6) consisting of 6–10 syllables (7–9 in Yaralıgöz, 6–10 in Türbe, and 6–9 in Uluağaç) and a carrier frequency band of between 8 and 18 kHz (maximum intensity of frequency band 13–14 kHz). Phrase duration varies from 4.37 to 7.66 s (5.47–7.66 s in Yaralıgöz, 4.37–6.97 s in Türbe, and 5.24–7.12 s in Uluağaç). The phrase generally is a more rapid crescendo type and begins with maximum intensity (Fig. 6A and C); however, in some of the songs, the phrase begins quietly, and the maximum intensity is usually reached around 2/5–3/5 of the phrase (Fig. 6E). The syllable period

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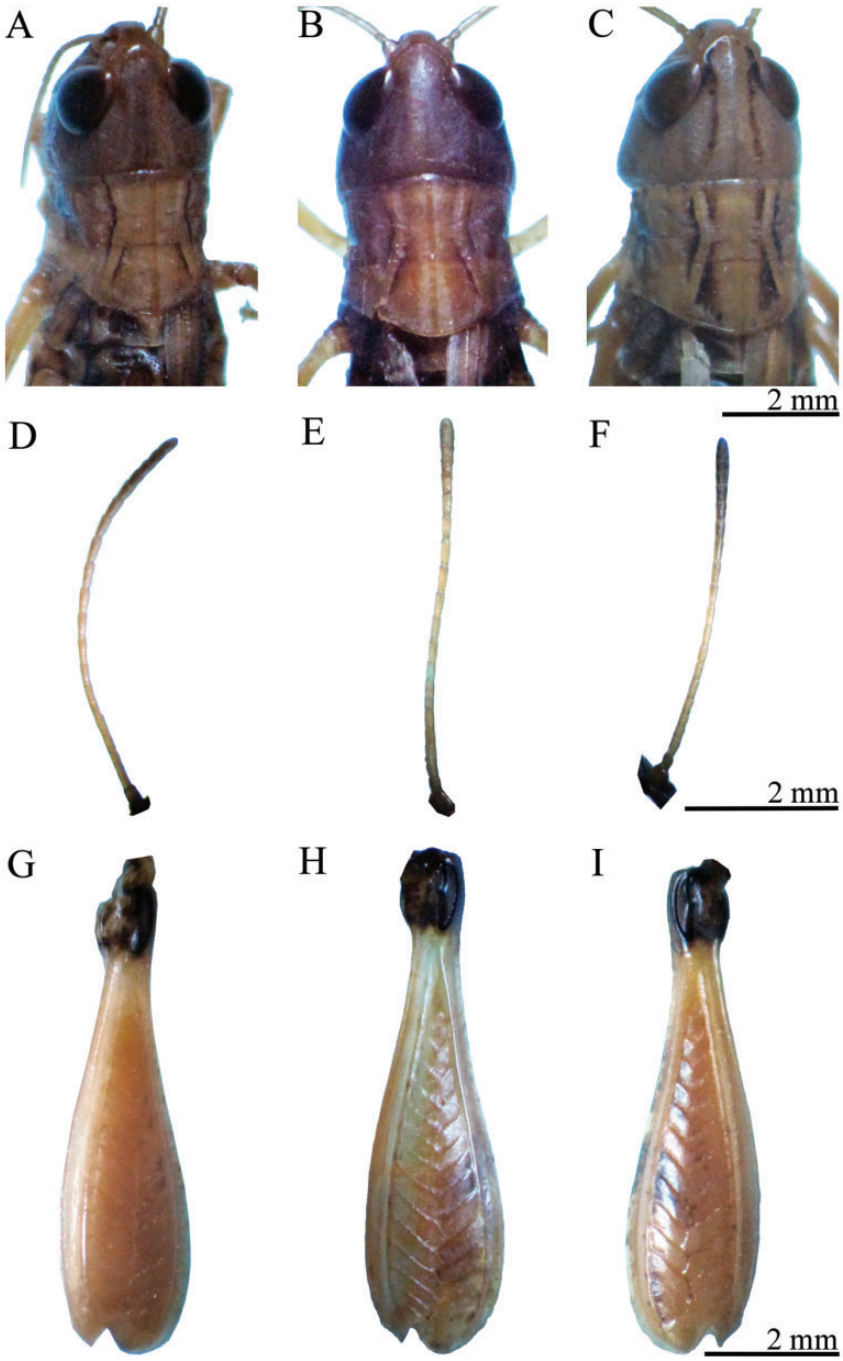

Fig. 4. Pronotum head, antennae, and hind femur of females of *R. turcicus* populations. (A, D, G) Türbe. (B, E, H) Uluağaç. (C, F, I) Yaralıgöz.

lasts about 0.530–1.01 s (0.530–0.997 s in Yaralıgöz, 0.574–0.857 s in Türbe, and 0.705–1.01 s in Uluağaç; Table 4).

Courtship Song and Body Movement Characteristics. R. turcicus has very long courtship song, and its duration ranges between 106 and 147 s (115–142 s in Yaralıgöz, 111–147 s in Türbe, and 106–146 s in Uluağaç). The song is composed of three complex phases and body movements that generate respective phases.

Phase I was always the longest: 96.2–136.5 (Table 4) in the main part of the song (Fig. 2C and D). The courtship begins with this phase, and males show high activity in this phase. Phase I starts without any preparation part and involves a series of phrases (Figs. 2C and 7A–C). Duration of each phrase ranges between 6 and 37 s (Table 4). All phrases in Phase I consist of two different parts (Fig. 2D). The first part of the phrase is Part a, which involves a series of very clear pulses being produced by only one leg (M1a; Fig. 8B). While one of the hind legs produces Part a of the phrase, the other leg stays in the higher position during the first half of Part a (Fig. 8B). The inactive leg goes down in two steps to the same position with the song-producing leg in the remaining part of Part a (Fig. 8B). Part a usually lasts about 0.47–2.82 s (Table 4).

The second part of the phrase is Part b, involving a series of very clear syllables that are produced by the alternation of the two legs (M1b1; Fig. 8B). Duration of Phase I, Part b ranges between 4.5 and 34.6 s and is composed of about 9–72 syllables, each usually lasting about 375–808 ms (Table 4; Fig. 7). Each syllable in the phrase is produced alternatively by the two legs. Each leg has a different role in each syllable production. When the active leg starts to move (up and down steps) to produce song elements, the passive leg always slightly

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Table 2. Means and standard deviations for 11 morphometric characters measured from three Anatolian populations belonging to *R. turcicus*

Characters mm	Male			Female		
	Uluağaç, Ankara ($n = 12$)	Yaralıgöz, Kastamonu ($n = 14$)	Türbe pass, Kastamonu ($n = 12$)	Uluağaç, Ankara ($n = 11$)	Yaralıgöz, Kastamonu ($n = 12$)	Türbe pass, Kastamonu ($n = 12$)
LH	2.05–2.22	2.04–2.22	2.11–2.36	2.51–2.73	2.29–2.80	2.47–2.73
	2.11 ± 0.056	2.15 ± 0.054	2.25 ± 0.084	2.63 ± 0.072	2.56 ± 0.124	2.58 ± 0.078
LA	5.82–6.20	5.09–5.49	6.18–6.91	3.96–4.19	3.85–4.13	4.26–4.45
	6.04 ± 0.110	5.30 ± 0.125	6.52 ± 0.232	4.14 ± 0.152	4.09 ± 0.105	4.32 ± 0.053
LMC	2.29–2.44	2.18–2.40	2.29–2.58	2.80–3.02	2.62–2.95	2.76–2.95
	2.36 ± 0.050	2.26 ± 0.075	2.43 ± 0.095	2.91 ± 0.065	2.82 ± 0.102	2.86 ± 0.057
LPBTS	1.20–1.27	1.16–1.27	1.20–1.35	1.45–1.56	1.49–1.56	1.45–1.60
	1.24 ± 0.033	1.20 ± 0.039	1.27 ± 0.052	1.49 ± 0.041	1.53 ± 0.026	1.52 ± 0.058
LPATS	1.09–1.16	1.02–1.13	1.09–1.24	1.35–1.45	1.13–1.38	1.20–1.45
	1.12 ± 0.026	1.06 ± 0.040	1.16 ± 0.047	1.41 ± 0.034	1.28 ± 0.081	1.34 ± 0.081
MADLC	1.31–1.38	1.20–1.31	1.13–1.27	1.38–1.60	1.42–1.60	1.38–1.53
	1.33 ± 0.029	1.26 ± 0.027	1.20 ± 0.044	1.49 ± 0.064	1.49 ± 0.060	1.46 ± 0.039
MIDLC	0.65–0.80	0.69–0.80	0.65–0.69	0.87–0.95	0.95–1.09	0.87–1.02
	0.70 ± 0.047	0.74 ± 0.026	0.67 ± 0.019	0.91 ± 0.030	1.00 ± 0.040	0.95 ± 0.049
TL	6.11–6.44	5.60–5.94	6.87–7.05	4.00–4.36	4.00–4.25	4.11–4.40
	6.25 ± 0.119	5.76 ± 0.132	6.97 ± 0.063	4.20 ± 0.126	4.15 ± 0.077	4.24 ± 0.105
MWT	1.42–1.45	1.20–1.27	1.49–1.67	0.98–1.09	0.91–1.05	0.95–1.05
	1.45 ± 0.014	1.22 ± 0.031	1.57 ± 0.062	1.02 ± 0.032	0.97 ± 0.047	1.01 ± 0.043
LHF	6.55–7.60	7.20–7.78	7.60–7.93	8.98–9.56	8.91–9.31	9.05–9.60
	6.98 ± 0.379	7.56 ± 0.160	7.75 ± 0.127	9.31 ± 0.193	9.14 ± 0.114	9.33 ± 0.180
MWHF	1.60–1.71	1.64–1.71	1.56–1.64	1.89–1.96	1.78–1.96	1.78–1.96
	1.65 ± 0.036	1.66 ± 0.030	1.59 ± 0.027	1.92 ± 0.034	1.85 ± 0.050	1.87 ± 0.059

n, number of individuals. Data are shown as range and mean ± SD.

Table 3. Factors, eigenvalues, and cumulative percentages obtained by factor analysis of 11 morphological characters from the males and females of *R. turcicus* (Uluağaç, Yaralıgöz, and Türbe populations)

Characters	Factor 1	Factor 2	Factor 3	Factor 4
Males				
LH	0.050	0.526	0.567	
LA	0.603[a]	0.717[a]	0.011	
LMC	0.946[a]	0.255	0.130	
LPBTS	0.947[a]	0.111	0.132	
LPATS	0.875[a]	0.363	0.118	
MADLC	−0.132	−0.280	−0.840[a]	
MIDLC	−0.415	−0.603[a]	−0.045	
TL	0.498	0.770[a]	0.220	
MWT	0.542	0.774[a]	−0.029	
LHF	0.104	−0.085	0.875[a]	
MWHF	−0.008	−0.856[a]	−0.258	
Eigenvalue	6,066	1,729	1,203	
Cumulative %	55,146	70,867	81,803	
Females				
LH	0.468	−0.041	−0.020	0.265
LA	−0.044	−0.707[a]	0.238	0.397
LMC	0.944[a]	0.123	−0.168	0.010
LPBTS	0.058	0.008	−0.820[a]	−0.310
LPATS	0.888[a]	0.115	0.268	0.173
MADLC	−0.012	0.828[a]	0.215	0.164
MIDLC	−0.556	0.514	−0.319	−0.005
TL	0.100	−0.174	0.058	0.654[a]
MWT	0.591	−0.326	0.043	0.119
LHF	0.291	0.188	−0.070	0.697[a]
MWHF	0.139	−0.003	0.770[a]	−0.288
Eigenvalue	3,037	1,683	1,415	1,164
Cumulative %	27,614	42,909	55,772	66,358

[a]Factor loadings are considered to be important to each factor.

changes the position to the starting point, and the roles of these two legs change in the next syllable (Fig. 8B). Thus, each syllable involves several pulses with high amplitude, produced by the active leg, and a noisy part with low amplitude, produced by the passive leg (Fig. 8B). The male body tilts through the active leg, which is responsible for the high-amplitude pulses in the syllable. This movement continues from right to left during Part b (Fig. 8B). Antennal movements of the males during Part a and Part b song production are such that they open the antenna side to side nearly 180° during M1a and M1b1 (Fig. 8B).

The males produce a visual display of M1b2 when Part b is finished (Fig. 8B). After completing Part b in the phrase, male raised the tibia upward (M1b2-II); then the tibia was jerked into the air (M1b2-III), and the antennae (Fig. 8M1b2) were pushed backward (Fig. 8B). The tibia returned to the same position very fast after the jerk movements, and during this period, the antennae also returned to the initial position (Fig. 8M1b2-IV). Because the jerk needs fast and strong body movements, the body could be stabilized after two or three times move the M1b2-V and M1b2-VI. During this circulation, the antennae first moved back (Fig. 8M1b2-V) and returned to the initial position (Fig. 8M1b2-VI) like the first direction of the antennae, but this time the distance was shorter than M1b2-III and M1b2-IV (Fig. 8B).

The males start the courtship behavior with Phase I and mostly stop somewhere else in the phrase series, which stems from the environmental effect or female movements. If courting is interrupted, courtship behavior is adopted again from the beginning of the ritual.

Phase II was the second regular part of the song, and it was always shorter, 5.9–10.3s (Table 4), than Phase I (Fig. 7). Phase II starts after Phase I and involves a series of short phrases (Fig. 2E). The duration of each phrase ranged between 0.42 and 0.98 s (Table 4). All phrases in Phase II consist of two parts (Fig. 2G). The first part is Part c and involves a series of isolated and repeated similar syllables (Fig. 2G), which are produced with nearly synchronous leg movements (Fig. 8M2c). Part c usually lasts about 0.25–0.72 s and is composed of approximately 6–37 syllables, each of which usually lasts about 10–45 ms (Table 4). The last syllable in the Part c is always longer, 54–377 ms (Table 4), than previous syllables in this part (Fig. 2G). The second part of the phrase is Part d, which is faster and represents a shorter version of Part c (Fig. 2G). Syllables in this part are not clearly differentiated, as a result of the unsynchronized leg movements (Fig. 8M2d). Part d usually lasts about 112–638 ms (Table 4).

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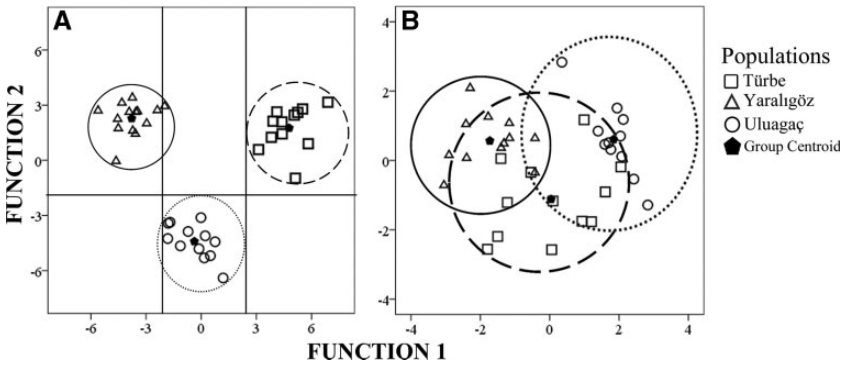

Fig. 5. First two axes of canonical discriminant function analyses for the 11 morphometric characters measured from *R. turcicus* (Türbe, Uluağaç, and Yaralıgöz populations); (A) males and (B) females.

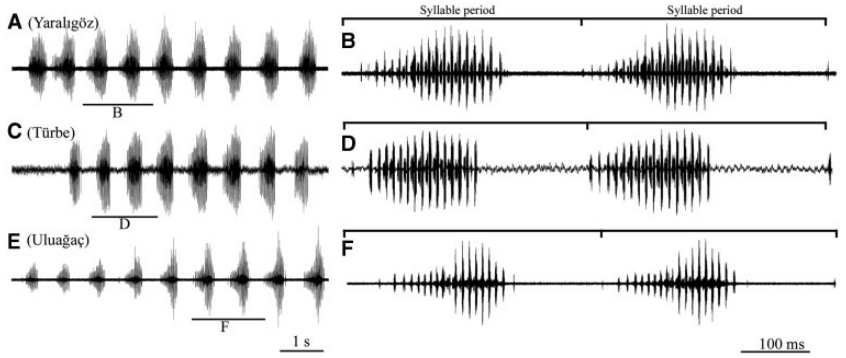

Fig. 6. Complete phrase and faster oscillograms of the indicated part of male calling songs in *R. turcicus* populations; (A–B) Yaralıgöz, (C–D) Türbe, and (E–F) Uluağaç.

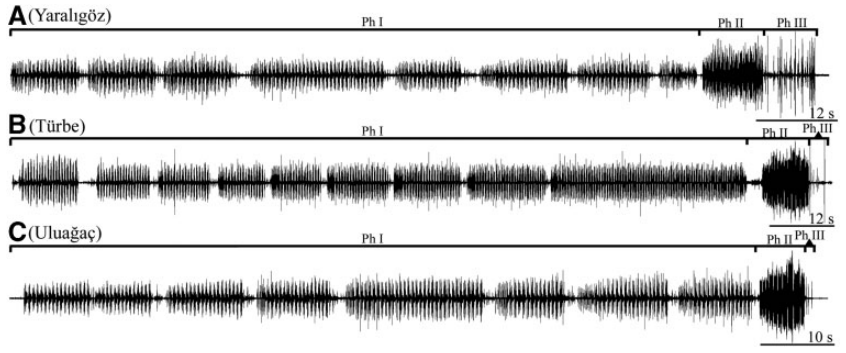

Fig. 7. Complete phrase of male courtship songs in *R. turcicus* populations; (A) Yaralıgöz, (B) Türbe, and (C) Uluağaç. Ph, phase of the song.

The regular part of the courtship song ends with Phase II. After this phase, males always move toward the female. In most cases, the female jumps away. If the female keeps near the male after Phase II, the male starts to produce the last phase of the courtship behavior.

Phase III was the last and irregular part of the song with a duration ranging between 1.07 and 7.44 s (Table 4). Although males do not produce a complex and extensive song pattern in Phase III, they show visual complexity and different behavioral activities in the last period of the song (Fig. 8D). In this part, males reveal high activity to convince the female to mate. Male succession on mating period directly link to change the female directions by the male (Fig. 8M3-I–IV). Throughout the complete courtship ritual until this phase, males generally stay in opposite directions of the females (Fig. 8M3-I). When the Phase II part of the song finishes, the male moves toward the female head to touch it and then turns back to the initial position (Fig. 8M3-I). This cycle is completed with making M1b2 movements after M3-I by the males (Fig. 8D). The number of cycles in Phase III shows differences from song to song and from male to male.

Measurements of the six song parameters (durations of Phase I, Part a; Phase I, Part b; Phase II, Part c; and Phase III, Part d; and syllable period of Phase I, Part a and Phase II, Part c) obtained from 14 males from three different populations are listed in Table 4. A CDF analysis was performed with the use of these six parameters of the courtship song, and the results of the song CDF show that all populations were not separated, and they exhibit unique patterns (Fig. 9).

Mating Trial Experiment. The experiment was performed to understand the basic female choice of the males belonging to other populations. In this experiment, we concentrated on what is the number of attempts made by the male that is accepted by a female for copulation.

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Table 4. Temporal parameters measured from male calling and courtship songs of *R. turcicus* populations

Characters			Taxon (place), individual		
			Yaralıgöz pass (Kastamonu), $N = 2$	Türbe pass (Kastamonu), $N = 2$	Uluağac plateau (Ankara), $N = 2$
Calling song					
Song = phrase		Duration (s)	5.47–7.66 6.23 ± 0.87 $n = 5$	4.37–6.97 5.67 ± 0.95 $n = 5$	5.24–7.12 6.29 ± 0.75 $n = 5$
		Syllable period (s)	0.530–0.997 0.772 ± 0.11 $n = 37$	0.574–0.857 0.699 ± 0.07 $n = 41$	0.705–1.01 0.839 ± 0.09 $n = 39$
		Syllable number	7–9 8.2 ± 0.84 $n = 5$	6–10 7.8 ± 1.48 $n = 5$	6–9 7.4 ± 1.14 $n = 5$
			Yaralıgöz pass (Kastamonu), $N = 4$	Türbe pass, (Kastamonu), $N = 4$	Uluağac plateau (Ankara), $N = 6$
Courtship song					
Song		Duration (s)	114.61–142.36 126.6 ± 11.4 $n = 6$	111.1–146.7 130.1 ± 13.2 $n = 6$	105.7–145.9 125.8 ± 16.5 $n = 9$
Phase 1		Duration (s)	98.0–126.1 112.0 ± 11.9 $n = 6$	100.2–136.5 118.8 ± 12.8 $n = 6$	96.2–134.7 116.3 ± 15.2 $n = 9$
	Phrases	Duration (s)	6.1–21.2 12.4 ± 3.3 $n = 57$	9.8–37.0 13.6 ± 5.4 $n = 39$	9.2–22.1 13.5 ± 3.8 $n = 64$
	Part a	Duration (s)	1.40–2.59 1.91 ± 0.3 $n = 57$	0.47–2.63 1.68 ± 0.5 $n = 39$	0.55–2.82 1.56 ± 0.7 $n = 64$
	Part b	Duration (s)	4.48–19.66 10.47 ± 3.3 $n = 57$	7.58–34.57 11.88 ± 5.4 $n = 39$	6.74–20.89 11.77 ± 3.8 $n = 64$
		Syllable period (s)	0.407–0.701 0.549 ± 0.04 $n = 1,038$	0.375–0.640 0.502 ± 0.04 $n = 583$	0.443–0.808 0.562 ± 0.05 $n = 1,190$
		Syllable number	9–54 19.3 ± 7.9 $n = 57$	11–47 17.1 ± 6.5 $n = 39$	10–72 19.9 ± 11.7 $n = 64$
Phase 2		Duration (s)	6.18–10.30 8.93 ± 1.5 $n = 6$	7.01–9.48 8.32 ± 0.8 $n = 6$	5.91–8.52 6.97 ± 0.8 $n = 9$
	Phrases	Duration (s)	0.44–0.73 0.59 ± 0.08 $n = 82$	0.42–0.93 0.61 ± 0.1 $n = 67$	0.43–0.98 0.66 ± 0.2 $n = 78$
	Part c	Duration (s)	0.25–0.36 0.30 ± 0.03 $n = 82$	0.34–0.72 0.48 ± 0.1 $n = 67$	0.29–0.68 0.43 ± 0.09 $n = 78$
		Syllable period (ms)	11–45 20 ± 0.004 $n = 711$	10–29 16 ± 0.002 $n = 1,165$	13–21 17 ± 0.001 $n = 1,251$
		Last syllable period (ms)	189–365 254 ± 0.04 $n = 53$	54–174 111 ± 0.03 $n = 60$	108–377 213 ± 0.06 $n = 68$
		Syllable number	7–37 21.1 ± 5.6 $n = 82$	6–28 17.6 ± 4.9 $n = 67$	8–34 22.3 ± 5.2 $n = 78$
	Part d	Duration (ms)	112–434 289 ± 0.1 $n = 82$	213–252 125 ± 0.05 $n = 67$	102–638 226 ± 0.1 $n = 78$
Phase 3		Duration (s)	3.82–7.44 5.65 ± 1.4 $n = 6$	2.63–4.69 4.03 ± 0.7 $n = 6$	1.07–5.45 2.51 ± 1.6 $n = 9$

N, number of individuals; *n,* number of the analyzed characters. Data are shown as range and mean ± SD.

When the females of the Türbe population were confronted with males of other populations, the males from the Uluağaç population were accepted on the fifth attempt and males from the Yaralıgöz population were accepted in the sixth attempt by females. The females of the Uluağaç population were accepted the male on the sixth attempt for the Türbe population and on the fourth attempt for the Yaralıgöz population. The last stage was made using Yaralıgöz females, and the result of the experiment revealed acceptance on the seventh attempt for the Türbe population and on the third attempt for the Uluağaç population.

Discussion

Acoustic characteristics and courtship behavior of *R. turcicus* were studied for the first time in this research. The results of this study show that the calling song of the males involves basic and common Gomphocerinae song characteristics. In addition, the male courtship song shows a complexity that involves different song elements. Courtship behavior of the male consists not only in song but also in different optical elements, such as antenna hind leg attraction and substrate vibration, which show similarity at different levels for the *C. albomarginatus* group (Vedenina and Helversen 2003, 2009), *Stenobothrus*

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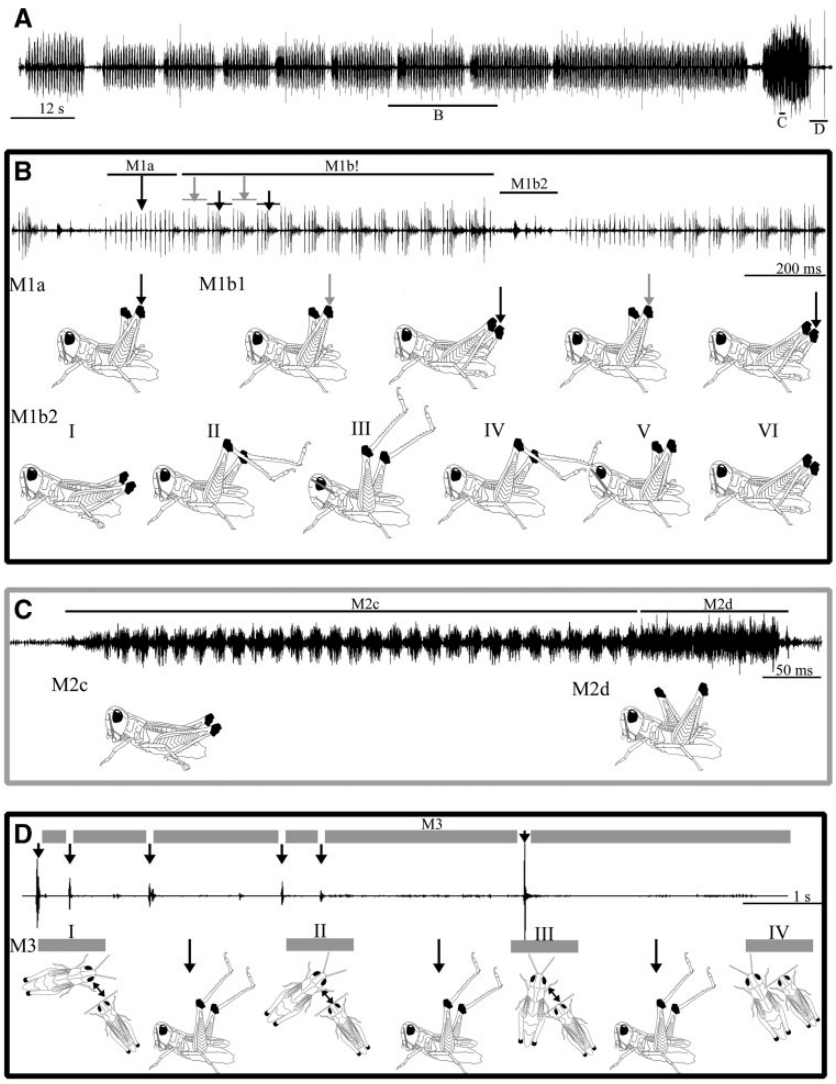

Fig. 8. Phases and parts of complete male courtship songs and responsible behavioral characters in *R. turcicus*. (B) Song part showing the fine structure of each song parts and their associated leg movements in Phase I (Part a = M1a movement and Part b = M1b1-M1b2 movements). (C) Song part showing the fine structure of each song parts and their associated leg movements in Phase II (Part c = M2c movement and Part d = M2d movement). (D) Song part showing the fine structure of Phase III and mechanism of physical contact between male and female (M3).

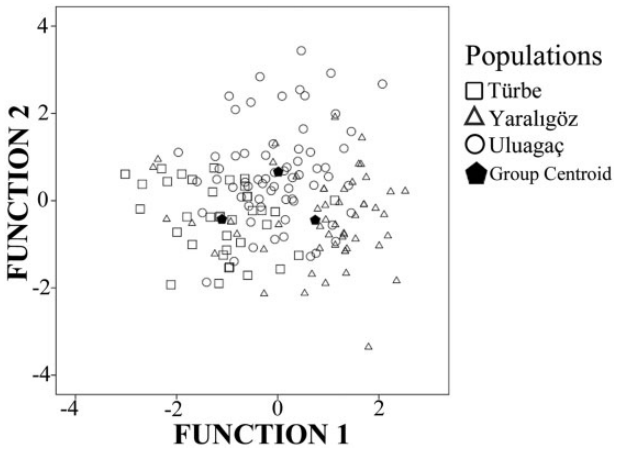

Fig. 9. Results of canonical discriminant function analysis using six song parameters.

eurasius group (Berger 2008), and *Myrmeleotettix antennatus* (Berger and Gottsberger 2010) species in Gomphocerinae.

The courtship song in Gomphocerinae species generally displays similarity to its calling song; however, it represents mostly different temporal parameters, like most species of the genus *Chorthippus* (Stumpner and Helversen 1994, Ingrisch 1995, Ragge and Reynolds 1998), *Dociostaurus* (Savitsky 2000, D.Ş. and A.M., unpublished data), *Ramburiella* (Savitsky 2002), etc. On the other hand, the courtship song shows differences with derived elements and pattern from the calling song of *Stenobothrus* (Berger 2008, Berger et al. 2010), *Myrmeleotettix* (Berger and Gottsberger 2010, Şirin et al. 2011), some of the *Chorthippus* (Vedenina and Helversen 2003, 2009), *Gomphocerripus rufus* (Ragge and Reynolds 1998), and others. *R. turcicus* belongs to the second group of the Gomphocerinae in which both song elements and courtship behavior are considered. Three geographically isolated populations (Fig. 1) of *R. turcicus* produce completely similar songs and courtship behavior (Figs. 6–8). In addition, they mate with each other in laboratory conditions without any outer stimulation.

The results from CDF analysis of the 11 morphometric characters, both male and female, suggested a different pattern of relationships

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(Fig. 5). Three isolated units were easily recognizable in the CDF results of males (Fig. 5*A*), in contrast to females, which were almost completely overlapped in CDF (Fig. 5*B*). The males of all populations are separated from each other by the axis of Function 1. On the other hand, males of the Uluağaç population are separated by the axis of Function 2 from the Yaralıgöz and Türbe populations. This result shows that the two northern populations, Yaralıgöz and Türbe, are closer to each other than the Uluağaç population, which is the most southern region of *R. turcicus*. In addition, males of the three populations are morphologically changed, and their characters are stabilized more than those of the females (Table 2; Fig. 5).

An important outcome of this study is that the courtship song and the behavioral characteristics are not concordant with the morphological data. The courtship song and behavioral characters suggest that *R. turcicus* is one valid species from north to south, and all compatible individuals of three populations (Fig. 9) can possibly copulate when they meet according to the female mate choice results. In contrast, both morphometric and qualitative morphology reveal that geographically isolated populations of the *R. turcicus* have been differentiated and some characteristics were more affected, and variation limits have been specified for each population (Table 2). Characteristic inconsistency in *R. turcicus* populations can be assumed to be a discrepancy between the rates of divergence in morphology and song, rapid in the first and slow in the second. A similar conflict is very common in allopatric populations of the Ensiferan species (Jang and Gerhardt 2006, Heller 2006, and references therein); however, it is rare in the Acrididae species (Heller 2006). The results of this study highlight the importance of the song characteristics in the definition and description of the Gomphocerinae species, which use the song as a premating isolation barrier.

So far, 43 species assigned to the subfamily Gomphocerinae have been described from Anatolia (Çıplak et al. 2002, Ünal 2007, Orthoptera Species File [OSF] 2012). However, only six species—*Chorthippus (Glyptobothrus) helverseni* Mol, 2003, *Chorthippus (Glyptobothrus) kazdaghensis* Mol and Çıplak, 2005, *Chorthippus (Glyptobothrus) taurensis* Şirin and Çıplak, 2005, *Chorthippus (Glyptobothrus) antecessor* Şirin and Çiplak, 2010, *Chorthippus (Glyptobothrus) relicticus* Şirin, Helversen, and Çiplak, 2010, and *Myrmeleotettix ethicus* Şirin and Çıplak, 2011—had been described using a combination of song and morphological characteristics. The remaining 35 species were described by classic morphology because the importance of song character was possibly discovered much more recently. The situation of *R. turcicus* populations indicated that misidentifications and descriptions are most likely to be seen (D.Ş. and A.M., unpublished data) in 35 species of Gomphocerinae in Anatolia. However, sibling species situation is also within the bounds of possibility, such as *C. demokidovi* group (Çıplak et al. 2005) and *Myrmeleotettix* genus (Şirin et al. 2011) situations in Anatolia. All these genera and most others in Gomphocerinae prefer colder climatic conditions (Weidner 1969, Demirsoy 1977, Şirin et al. 2010a). The climate in Anatolia shows rapid change in short distances because of the topographical diversity. Therefore, both climate and topography could generate refugial areas such as plains during glacial periods and uplands during interglacial periods for species preferring colder climatic conditions (Çıplak et al. 2005; Şirin et al. 2010a, 2011; Albayrak et al. 2011, 2012, etc.). All these results indicated that Gomphocerinae species from Anatolia should be revised to understand the natural taxonomic situation in respect to subfamily.

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