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Integrated systematics of the *Poecilimon luschani* species group (Orthoptera, Tettigoniidae): radiation as a chain of populations in a small heterogeneous area

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The *Poecilimon luschani* species group (Orthoptera, Tettigonioidea, Phaneropterinae), ranging along a narrow line in West Anatolia and the Balkan mountains, has been revised using integrated data from morphology, bioacoustics, and DNA sequences of two matrilineal markers. A taxonomic approach neutral with respect to existing species/subspecies was applied to the sampled populations. The male calling song showed poor variation amongst the populations. Of the morphological structures, only the male cerci and male subgenital plate offer diagnostic characters but they are secondary gains or losses in most cases. Phylogenetic analyses applied to a data matrix consisting of concatenated 1401 bp long sequences of 16S rDNA and cytochrome oxidase subunit I produced from specimens of the group resulted in trees with similar topologies. Both genetic and phenotypic data confirmed the species status of the geographically discrete Izmir, Balkan, Aydın, Kütahya, and Balıkesir populations. However, 13 populations located in the southern part of the group's range in Antalya and Muğla Provinces of Turkey present a complex phylogenetic and morphological pattern of three morphological units, but two phylogroups. By integrating song, morphology, and phylogenetic data the following conclusions were made. The species status of *Poecilimon ledereri* and *Poecilimon orbelicus* was confirmed. The Aydın population was re-established as a valid species, *P. tuncayi* stat. rev. The Kütahya population was elevated to species level as ***Poecilimon egrigozi*** Ünal, 2005, **stat. nov.** The Balıkesir population is here described as a new species ***Poecilimon helleri* sp. nov.** The southern lineage of the species group exhibits characteristics of a chain species with three subspecies. *Poecilimon luschani* is retained as the species name by priority and the *Poecilimon birandi* is considered its subspecies as ***Poecilimon luschani birandi* stat. nov.** A new subspecies ***Poecilimon luschani chobanovi* ssp. nov.** is described. The phylogenetic relationships amongst the proposed taxa are as follows: *P. ledereri* + (*P. orbelicus* + (*P. tuncayi* + (*P. egrigozi* + *P. helleri*) + (*P. luschani luschani* + *P. luschani birandi* + *P. luschani chobanovi*))).

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ADDITIONAL KEYWORDS: Anatolia – chain species – integrated taxonomy – *Poecilimon luschani* group.

INTRODUCTION

Poecilimon Fischer (Orthoptera, Tettigoniidae) is the most specious genus of Phaneropterinae. Ramme (1933) listed 68 species in eight groups in the first revision of the genus. Currently, the number stands at 150 because of new species described since then (Eades *et al*., 2012). The range of *Poecilimon* covers the Black Sea and the Eastern Mediterranean Basins. However, the main bulk of species is concentrated in Anatolia and Greece, the lands surrounding the Aegean Sea (Willemse & Heller, 1992; Heller *et al*., 1998; Çıplak *et al*., 2002), and the genus has been suggested to have an Aegeid origin (Çıplak, 2004).

Taxonomy of the genus was an active research area throughout the 20th century for several reasons. First, the high species number of the genus causes constraints in diagnosing species. Second, in many cases

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the descriptions and diagnoses of species are based solely on qualitative morphology. However, morphology may be misleading because of commonly occurring homoplasies. Third, as the radiation at the species-group level is assumed to have occurred recently, *Poecilimon* possibly includes young species that have not sufficiently diverged (Kaya *et al*., 2012a). A recent trend is to study groups of related species using character sources other than morphology, such as male calling song (Heller *et al*., 1998, 2006, 2011; Heller, 2004; Heller & Lehmann, 2004; Heller, Willemse & Sevgili, 2004; Heller & Sevgili, 2005; Chobanov & Heller, 2010), because communication signals are assumed to be useful tools in defining reproductive units or biological species (Heller, 1988, 1990, 2006; Ragge & Reynolds, 1998). Other approaches to studying *Poecilimon* include DNA-based phylogenetic studies (Ullrich *et al*., 2010). These studies have considerably improved taxonomy of the genus; however, there are still several species groups that have not yet been studied in such an integrated approach. The present study aimed to reconsider the taxonomy of the *Poecilimon luschani* species group using an integrated approach that included morphology, male calling song, and a DNA phylogeny based on two mitochondrial markers.

Ramme (1933) suggested that *P. luschani* and *Poecilimon ledereri* constitute a related species group (Group VI). The later-described species *Poecilimon birandi* and *Poecilimon tuncayi* were suggested to be closely related to *P. luschani* (Karabağ, 1950, 1953). Ünal (2004) suggested that *Poecilimon tuncayi* is similar to *P. luschani* although later (Ünal, 2005) he synonymized it with *P. ledereri.* Additionally, Ünal (2005) described a subspecies *Poecilimon luschani egrigozi*. Recently, a molecular phylogeny by Ullrich *et al*. (2010) demonstrated that a Balkan species *Poecilimon orbelicus* Pančić, 1883, is closely related to the *P. luschani* group; therefore, it should be considered in a study of this group. Another recent study by Kaya, Gündüz & Çıplak (2012b) using a molecular marker indicated complex genetic variability within the populations of the *P. luschani* group located in the west of Antalya and east of the Muğla provinces of Turkey. All available data together suggest a considerable rectification for taxonomy of this group.

The present taxonomy of the genus *Poecilimon* is mainly based on qualitative morphology. Some recent studies have indicated the necessity of testing the validity of morphology as a taxonomic tool in defining species within *Poecilimon* (e.g. Kaya *et al*., 2012a). Testing this is especially important for two reasons. First, members of *Poecilimon* are short winged/wingless (short wings function as stridulatory organ in males; Heller, 1988), and therefore have limited dispersal ability. Second, several species in the genus prefer mountainous cold habitats, and their radiations were thus possibly affected by the glacial cycles of the Quaternary (La Greca, 1999; Çıplak, 2004; Heller *et al*., 2006; Kaya *et al*., 2012a). Limited dispersal ability and habitat preference may have led to the evolution of local morphs (Çıplak *et al*., 2008), some of which may have been erroneously defined as distinct species. Thus, most of the existing species need to be tested to determine whether or not they are truly isolated reproductive units. As members of the *P. luschani* group are recorded from sea level up to 2300 m and they show altitudinal clines (Çıplak *et al*., 2008; Kaya *et al*., 2012b), we felt it to be an appropriate group with which to test the addressed issue.

This study had two main goals. The first was to reconsider the taxonomy of the *P. luschani* species group from an integrated perspective. Knowledge of the phylogenetic relationships of the species in the group was required to achieve this goal. Additionally, formulation of a phylogenetic hypothesis allowed us to determine homological and homoplastic characters and to evaluate their evolution. As producing data on the basis of previously proposed species/subspecies may cause a bias, a taxonomically neutral approach was followed during data production. Taxonomic decisions were thus made later in the light of combined data. The second goal was to evaluate the appropriateness of qualitative morphology in the delimitation of species within *Poecilimon* based on the information obtained from the *P. luschani* species group.

MATERIAL AND METHODS

Sampling

Most of the specimens used in this study were obtained during different projects conducted by the research team. Samples of *P. orbelicus* were provided by Dr Dragan Chobanov (Sofia, Bulgaria). Collection sites, including type localities of taxa in the species group, and specimens examined are presented in Table 1 (see also Fig. 1). Specimens were prepared as dry museum specimens or preserved in 96% alcohol kept at –20 °C for molecular studies. Specimens examined during this study are preserved at Akdeniz University, Department of Biology, Zoological Museum, Antalya, Turkey (AUZM). We also benefited from the photos presented in *Orthoptera Species File Online* Version 2.0/4.1 (Eades *et al*., 2012). Although we did not examine these, specimens from the private collection of K. G. Heller (Magdeburg, Germany) are also listed in order to define the range of species more precisely.

Morphology

Morphological structures were examined, measured, and photographed using a Leica M6 stereomicroscope

Table 1. Collection details (see Fig. 1) of specimens used for morphology, song, and DNA studies

Population	Male (*N*)	Female (*N*)	Coordinates	Altitude (m asl)	Vegetation	Collection dates
İzmir	46	42	38°17′39.2″N, 028°01′06.6″E	768	High grasses	06/06/2010
Aydın	59	56	37°25′04.4″N, 028°08′59.4″E	77	High grasses	05/06/2010, 15/05/2011
Balkan (Bulgaria)	18	7	41°48′16.0″N, 023°28′06.0″E	2070	Alpine	24/08/2011
Kütahya	22	25	39°24′10.1″N, 029°08′46.3″E	1698	Alpine	04/07/2011
Balıkesir	25	7	39°42′19.6″N, 026°53′44.3″E	1688	Alpine	05/07/2011
Akdağ*	23	12	36°34′56.8″N, 029°34′59.2″E	2247	Alpine	15/07/2005
Erentepe*	16	8	36°44′38.3″N, 029°38′45.0″E	1982	Alpine	15/07/2005
Eşen1	37	10	36°42′01.6″N, 029°24′59.0″E	672	Maquis opening	15/05/2005, 14/05/2011
Eşen2	61	33	36°33′29.4″N, 029°25′17.4″E	470	Maquis opening	08/05/2005, 14/05/2011
Eşen3	22	17	36°25′33.5″N, 029°16′24.1″E	85	Maquis opening	20/05/2006, 14/05/2011
Kalkan	26	22	36°15′16.2″N, 029°27′55.1″E	857	Maquis opening	15/05/2005, 13/05/2011
Patara	29	31	36°17′22.5″N, 029°20′03.5″E	110	Maquis opening	12/04/2007, 14/05/2011
Demre	58	45	36°16′32.1″N, 030°03′26.5″E	120	Maquis opening	19/05/2006, 14/05/2011
Olympos	48	38	36°27′05.2″N, 030°25′41.5″E	398	Maquis -Timberline	19/05/2006, 13/05/2011
Kemer	16	17	36°22′40.0″N, 030°15′06.2″E	1110	Subalpine	02/06/2006
Tahtalıdağ	17	3	36°19′02.4″N, 030°15′03.1″E	1850	Subalpine	10/07/2006
Termessos	29	8	36°35′34.0″N, 030°16′49.1″E	878	Maquis -Timberline	11/06/2005
Bakırlıdağ	26	24	36°49′09.6″N, 030°21′19.1″E	1819	Alpine	03/07/2004
Outgroups						
Poecilimon obtusicercus			37°22′27.0″N, 029°08′14.0″E	1175		07/06/2010
Poecilimon obtusicercus			36°56′06.8″N, 030°05′49.4″E	1309		08/06/2010
Poecilimon izmirensis			39°24′10.1″N, 029°08′46.3″E	1698		04/07/2011
Poecilimon cervus			40°58′48.0″N, 031°33′16.0″E	499		08/07/2011

*DNA sequences were not available.

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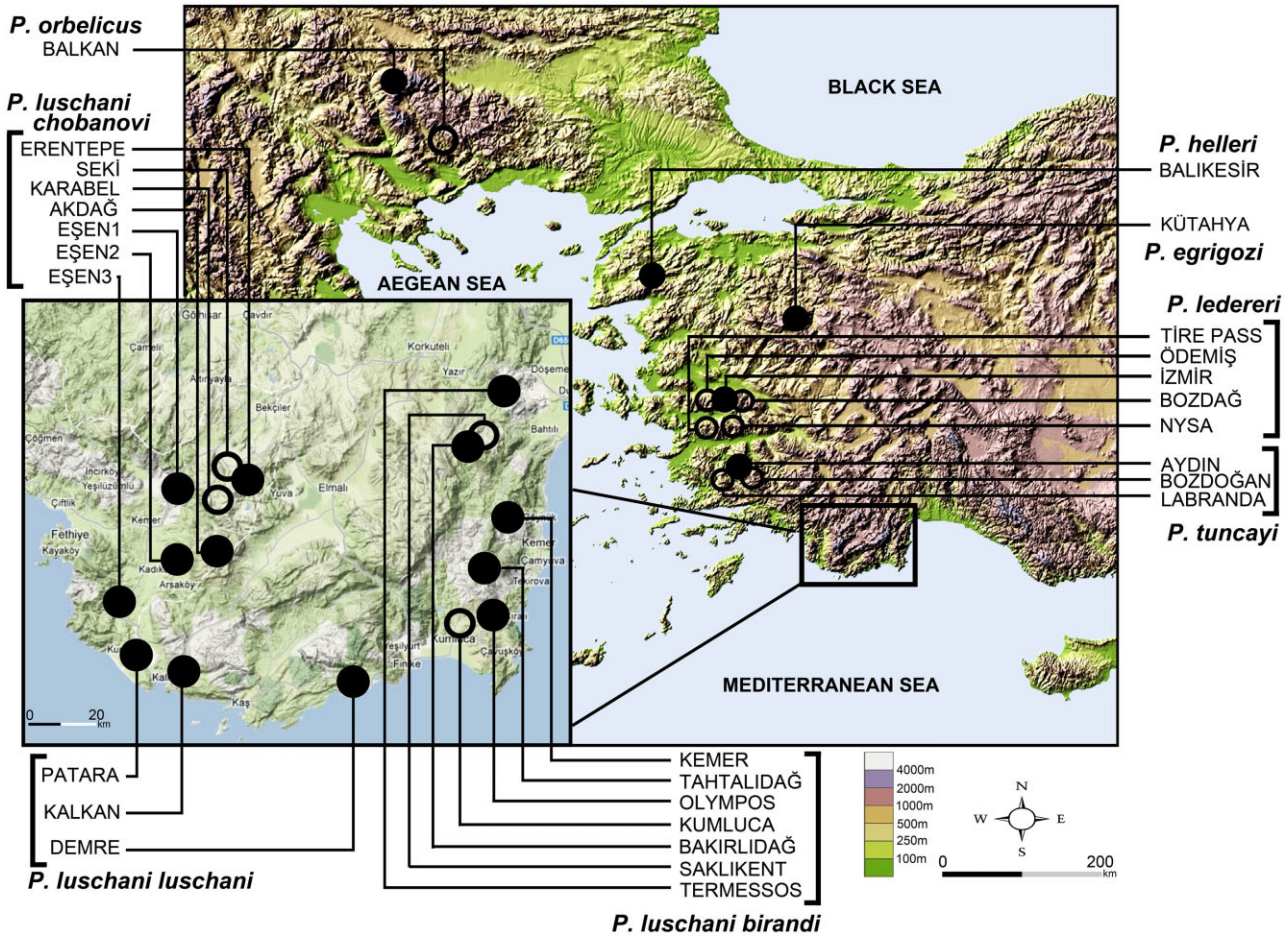


Figure 1. Distribution of the *Poecilimon luschani* species group (filled circles indicate the specimens used for morphology, song, and DNA studies, see Table 1; open circles indicate the material in the collection of K.G. Heller; taxa names are according to the results of this study).

and a Leica DC200 digital imaging system. The structures traditionally used in taxonomy of *Poecilimon* (vertex, pronotum, and subgenital plate in males and females, cerci and stridulatory file in males, and gonangulum in females) were examined, necessary illustrations representing inter- and intrapopulation variation were provided. The traditional terminology given in Bei-Bienko (1954) and Harz (1969) was followed (Fig. 2).

Song

Male calling songs were recorded in room conditions during field studies or in the laboratory (for recording temperatures see Table 2). For sound recording, a FOSTEX FR-2 digital recorder was used with a G.R.A.S. type 40BF microphone (frequency response 10 Hz–40 kHz ± 1.0 dB, 4 Hz–100 kHz ± 2.0 dB). Song files of *P. orbelicus* (files pooe7901–7905, pooe79k1–79k3) were downloaded from the SysTax website (uploaded by K. G. Heller). Oscillograms and sound measuring were carried out using a PC with the software programs TURBOLAB (Stemmer AG) and CoolEdit Pro. v. 2.0 (Syntrillium Software Corporation). In song descriptions, bioacoustic terminology by Heller (1988) and Ragge & Reynolds (1998) was used: calling song – spontaneous song produced by an isolated male; syllable – the song produced by one opening + closing movement cycle of the tegmina; syllable duration – the time interval starting from the beginning of one syllable to its end; impulse – a simple, undivided transient train of sound waves; impulse period – the time interval starting from the beginning of one impulse to that of the following; s – second; ms – millisecond (Fig. 3).

Following early examinations of the songs, we saw that three parameters showed variation and may potentially be useful for diagnosing populations. Apart from the song pattern syllable duration, impulse number per syllable and impulse period

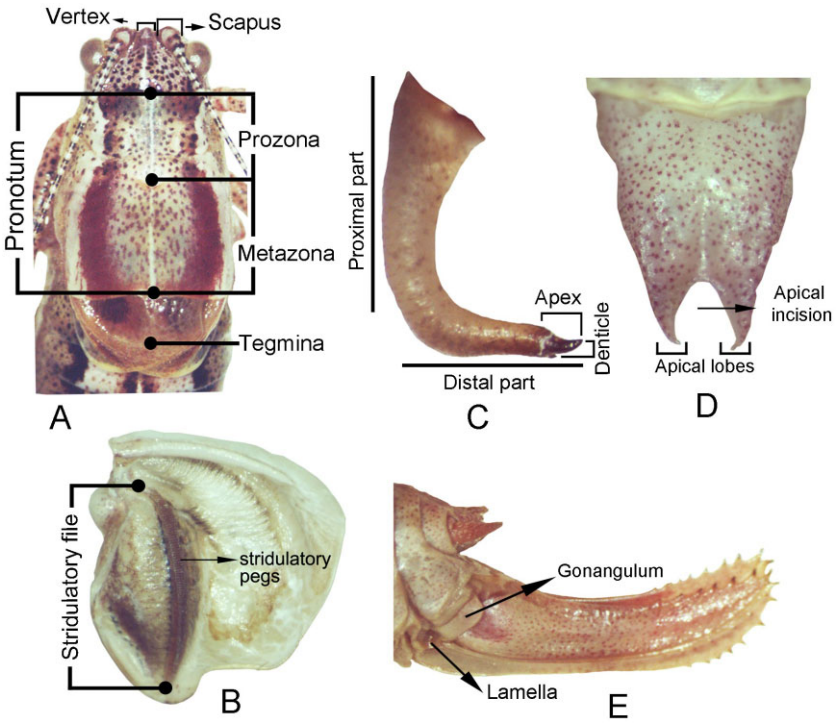


Figure 2. Morphological terminology followed for *Poecilimon luschani* species group. A, male head pronotum and tegmina from above; B, tegmina from ventral side; C, male cerci; D, male subgenital plate; E, female genitalia from profile.

(mean over whole syllable) were measured and analysed. To determine the possible effect of the recording temperature on temporal parameters of the song (syllable duration and impulse period) and to exclude it, a series of statistical analyses was performed. For traits for which the effect of temperature was found to be statistically significant ($P < 0.05$), multiple regression analysis was performed including the temperature as a linear covariate. k–1 (k, the number of populations) dummy variables were created in order to investigate the effect of each population. The empirical distribution of residuals was used to check the normality assumption of the error term, equal variance assumption, model over-fitting, model under-fitting, and outlier detection. Estimation of the outliers and influential observations were determined via Cook's distance (Cook, 1977) and leverage statistics (HAT matrix) provided by the software SAS v. 9.1 (SAS Institute, http://www.sas.com/). When an influential observation was detected, it was removed from the analysis. Significant dummy variables ($P < 0.05$) for the populations were taken as evidence of differences between the populations (Suits, 1957). A determination coefficient was used to assess model adequacy. To determine the most suitable regression model from the possible competing models, we used Mallows' Cp criterion (Mallows, 1973), the Akaike information criterion (AIC) (Akaike, 1973), and the Bayesian information criterion [BIC; also known as the Schwarz information criterion (Schwarz, 1978)]. The parameters obtained from the regression analysis were used to calibrate the temperature effect and eliminate the population differences for each of the song characters. Standardized variables were analysed using one-way ANOVA. The pairwise comparison of populations for these five characters was carried out using a Tukey's test. All statistical analyses of song data were performed in SAS v. 9.1.

Sequence data and phylogenetic analyses

In this study, 16S rDNA and cytochrome *c* oxidase subunit I (COI) fragments of mitochondrial DNA (mtDNA) were targeted. We used either public (GenBank accession nos: JX6668990–JX6669008; published in Kaya *et al*., 2012b) or newly obtained sequences of 16S rDNA whereas all COI sequences were produced in this study. Total DNA was extracted from the muscle tissue using proteinase K digestion followed by a standard salt/isopropanol method (Aljanabi & Martinez, 1997). The 16S rDNA fragments were amplified using the universal primers forward LR-J-12887 and reverse LR-N-13398 for the majority of samples. As we were unsuccessful in amplifying 16S rDNA from specimens of the Kütahya population using these primers, we amplified the

Table 2. Song parameters (transformed according to 25 °C) per population [first row, minimum-maximum (number of samples); second row, mean ± standard deviation]

Population	Recording temperature (°C)	Syllable duration (ms)	Impulse number per syllable	Impulse period (ms)
İzmir	18.9–19.8	92.21–228.17 (N = 355)	14–67 (N = 355)	1.68–5.83 (N = 355)
		150.52 ± 21.73	42.56 ± 7.23	3.08 ± 0.66
Balkan (Greece)	22.0–25.0	135.85–275.95 (N = 196)	30–56 (N = 196)	2.83–5.76 (N = 196)
		195.94 ± 32.46	43.23 ± 5.92	4.46 ± 0.57
Balıkesir	23.5–25.8	59.30–126.19 (N = 67)	21–45 (N = 67)	1.46–3.63 (N = 67)
		89.81 ± 15.10	33.05 ± 5.70	2.36 ± 0.54
Kütahya	25.5–26.9	117.91–173.98 (N = 70)	27–49 (N = 70)	3.10–5.85 (N = 70)
		157.92 ± 11.19	40.88 ± 4.85	3.95 ± 0.60
Aydın	22.0–22.5	96.26–141.26 (N = 105)	9–43 (N = 105)	3.30–8.01 (N = 105)
		115.81 ± 10.00	26.54 ± 7.89	4.09 ± 0.67
Akdağ	32.0	87.31–163.31 (N = 95)	37–56 (N = 95)	1.99–3.35 (N = 95)
		124.30 ± 12.56	49.79 ± 3.57	2.39 ± 0.32
Erentepe	32.0	100.28–132.28 (N = 26)	41–56 (N = 26)	2.22–2.34 (N = 26)
		120.01 ± 9.32	49.46 ± 4.21	2.28 ± 0.08
Eşen1	22.4–30.0	107.03–201.80 (N = 79)	34–59 (N = 79)	1.87–4.09 (N = 79)
		164.48 ± 26.90	52.98 ± 4.59	2.87 ± 0.49
Eşen2	23.1–30.0	72.93–180.67 (N = 124)	16–61 (N = 120)	1.93–5.13 (N = 120)
		137.34 ± 17.82	46.01 ± 10.60	2.83 ± 0.64
Eşen3	22.5–30.0	75.05–229.67 (N = 106)	30–57 (N = 106)	1.77–4.54 (N = 106)
		153.58 ± 45.90	45.52 ± 5.92	3.04 ± 0.72
Patara	22.0–31.7	91.01–169.63 (N = 99)	38–51 (N = 99)	1.80–3.22 (N = 99)
		129.81 ± 22.19	45.44 ± 2.53	2.56 ± 0.42
Kalkan	22.6–30.0	110.12–171.92 (N = 101)	31–63 (N = 101)	2.29–4.03 (N = 101)
		145.10 ± 14.60	43.98 ± 4.32	3.13 ± 0.37
Demre	17.2–25.0	111.84–296.44 (N = 305)	23–60 (N = 305)	2.67–5.90 (N = 305)
		180.96 ± 38.79	44.17 ± 8.77	4.02 ± 0.75
Olympos	22.0–26.0	119.92–250.82 (N = 273)	25–65 (N = 270)	2.31–6.80 (N = 270)
		176.25 ± 21.96	45.49 ± 9.47	3.77 ± 0.67
Kemer	27.0	121.93–155.93 (N = 80)	41–58 (N = 80)	1.19–2.89 (N = 80)
		141.14 ± 8.94	46.30 ± 4.80	2.11 ± 0.38
Tahtalıdağ	27.5	72.80–126.20 (N = 20)	35–41 (N = 20)	1.48–3.18 (N = 20)
		118.45 ± 11.20	38.55 ± 1.79	2.75 ± 0.37
Termessos	27.0–28.0	109.98–175.98 (N = 90)	38–56 (N = 90)	1.94–3.95 (N = 90)
		148.07 ± 13.17	50.29 ± 4.16	2.74 ± 0.42
Bakırlıdağ	26.5	117.92–168.92 (N = 50)	36–52 (N = 50)	2.57–3.92 (N = 50)
		147.94 ± 12.64	43.58 ± 2.93	3.28 ± 0.31

Nad1-L-16S fragment using the primers forward 12533F and reverse 13176R to obtain a partial sequence of 16S rDNA. The universal primers forward C1-J-1718 or C1-J-1751 and reverse TL2-N-3014 were been used to amplify the COI fragments (for details on primers see Simon *et al*., 1994).

Amplification of both markers was performed in a 50-µl volume containing 0.3 µl of each primer (100 µM), 1 µl deoxyribonucleotide triphosphate mix (10 mM), 2 µl 50 mM $MgCl_2$, 5 µl 10X Platinum PCR buffer [containing 200 mM Tris–HCl (pH 8.4), 500 mM KCl], 1.25 U Platinum TaqDNA polymerase (Invitrogen), and 0.5–1 µl of 50–70 ng template DNA. Temperature cycling was carried out in an Eppendorf Mastercycler Personal. Amplification of the two fragments involved an initial cycle of denaturation at 94 °C for 1 min, and 35 subsequent cycles at 94 °C for 40 s, annealing temperature at 49 °C for 30 s, extension temperature 72 °C for 60 s and 90 s for 16S rDNA and COI, respectively, followed by a final extension step of 72 °C for 10 min for both fragments. Double-stranded sequence analysis (performed on a 23 ABI 3730XL DNA Analyzer) and purifications, using the above-mentioned primers, were carried out via the Macrogen sequencing service (Macrogen Inc., Amsterdam, the Netherlands). Nucleotide sequences of each unique haplotype identified in this study have been deposited in the GenBank database under the

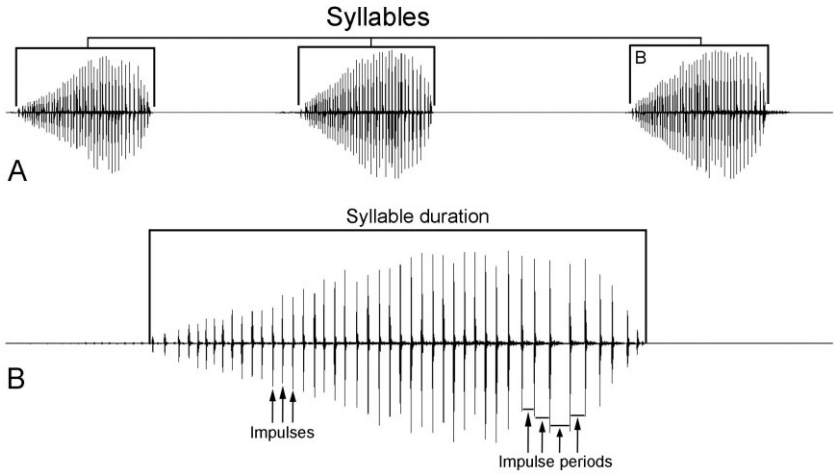


Figure 3. Bioacoustic terminology followed for *Poecilimon luschani* species group: A, a syllable sequence; B, impulses in a single syllable.

accession numbers KF288932, KF288933, KF286946-KF286984 for 16S rDNA and KF379500-KF379583 for COI.

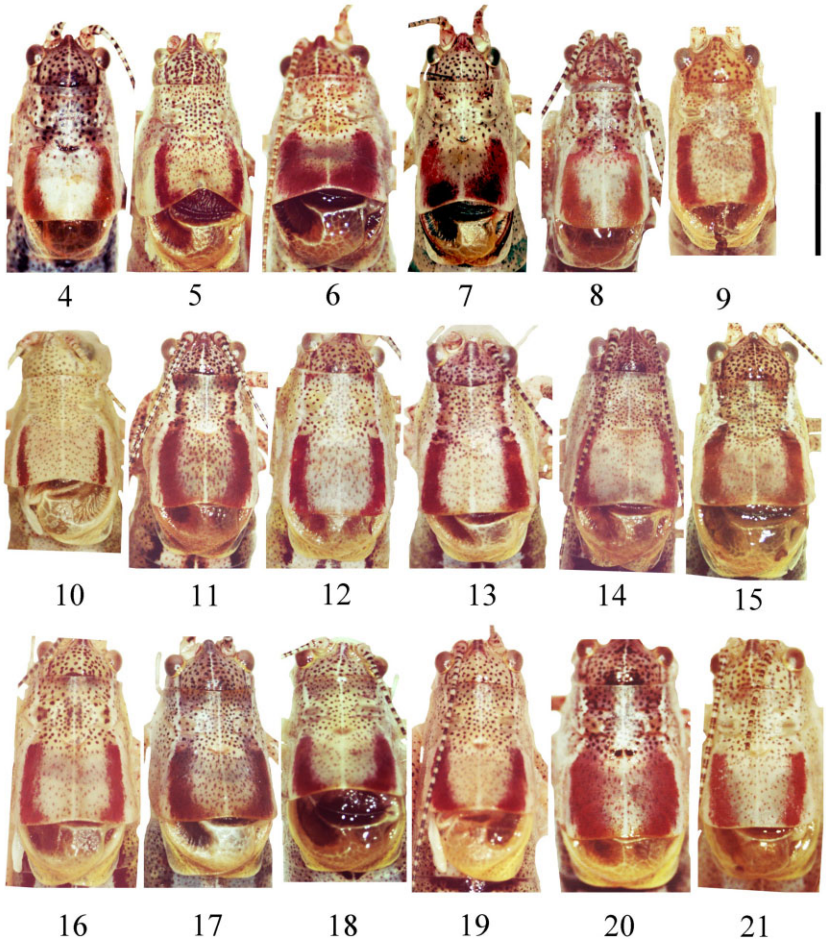
The sequences were aligned in SEQUENCHER v. 4.1 (Gene Codes Corporation) and checked manually by eye. After testing for homogeneity using PAUP v. 4.0b10 (Swofford, 2000), sequences of 16S rDNA and COI were concatenated into a final data set. DnaSP v. 5 (Librado & Rozas, 2009) was used to determine unique haplotypes. The haplotype matrix was prepared using MEGA v. 5 (Tamura *et al*., 2011). The 12 gap positions observed in 16S rDNA were removed from the matrix before analyses. A maximum parsimony (MP) analysis was carried out using 100 random additions following the heuristic search approach and using the tree bisection-reconnection algorithm. A 10 000 nonparametric bootstrap resampling was applied to assess the branch confidences (Felsenstein, 1985). The parameters and best fit model were estimated using MODELTEST v. 3.06 (Posada & Crandall, 1998). The selected model was implemented in the maximum likelihood (ML) analysis. Nonparametric bootstrapping (Felsenstein, 1985) was used to evaluate the support of nodes based on 1000 pseudoreplicates analysed by ML. A Bayesian phylogenetic inference (BI) search was carried out, implemented in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003; Ronquist, Huelsenbeck & van der Mark, 2005), using four simulations of Markov chains, 10 000 000 generations and sampling every 100 generations. The software tool TRACER v. 1.5 (Rambout & Drummond, 2003) was used to examine the parameters and to determine the number of trees needed to reach stationarity. BI posterior branch probabilities were calculated by the majority rule consensus of the sampled trees, excluding the first 3000 trees as burn-in. MP and ML analyses were conducted using PAUP v. 4.0b10 (Swofford, 2000) and BI by MrBayes v. 3.1.2. Branches that received a bootstrap or posterior probability support higher than 50% from each of the MP, ML, and BI analyses were indicated by their support value on the resulting tree. Three different outgroups, *Poecilimon izmirensis*, *Poecilimon obtusicercus*, and *Poecilimon cervus*, were chosen for the phylogenetic analyses.

RESULTS

MORPHOLOGY

The vertex of the fastigium is one to two times as wide as the scapus and the distribution does not exhibit any gaps in variation that could be useful for internal taxonomy of the *P. luschani* species group (Table S1). The pronotum and tegmen are another two structures that are similar at group level. The raising of the metazona according to prozona and the location of the sulcus show a small amount of variation but such variations are also observed among individuals of the same population (Figs 4–29). The Balıkesir population exhibits a slightly swollen metazona. Structures belonging to the female genitalia are also very similar at group level (Figs 66–73). The basal part of the ventral valves (gonangulum) is not or only indistinctly extended, the subgenital plate is almost triangular, and ovipositors are relatively stout. Thus, females cannot be diagnosed by the morphological phenotype.

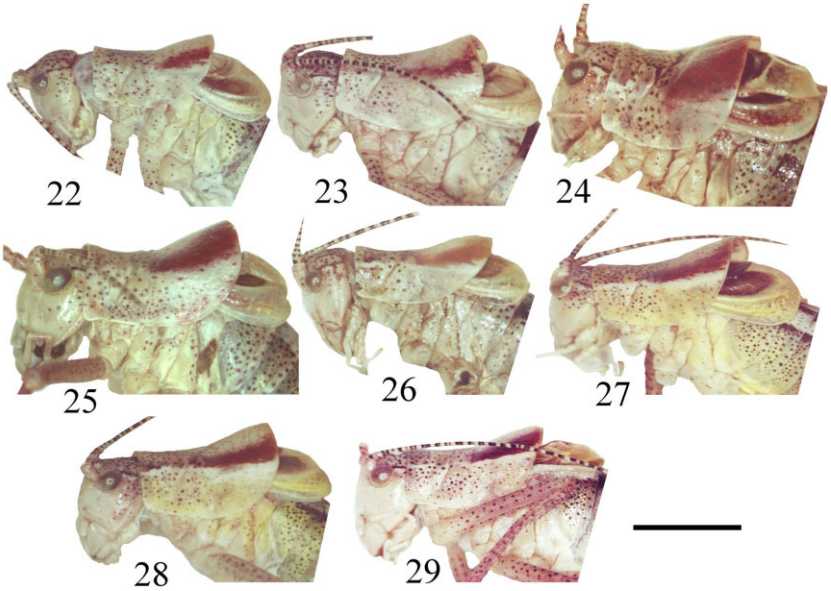
Two structures exhibiting considerable variation and offering some diagnostic characters are the male cerci (Figs 30–47) and the subgenital plate (Figs 48–65). The Balkan, Balıkesir, and Kütahya populations can be distinguished from all others by the cerci having a widened apex and by the absence of a prominent denticle (Figs 31–33). In all other populations examined, the apex of the cerci is not widened



Figures 4–21. Male pronotum from dorsal in *Poecilimon luschani* species group, each representing a population (scale bar = 2 mm): 4, İzmir; 5, Balkan; 6, Balıkesir; 7, Kütahya; 8, Aydın; 9, Akdağ; 10, Erentepe; 11, Eşen1; 12, Eşen2; 13, Eşen3; 14, Patara; 15, Kalkan; 16, Demre; 17, Olympos; 18, Kemer; 19, Tahtalıdağ; 20, Termessos; 21, Bakırlıdağ.

and the apical denticle located toward the outer margin of the apex is distinct (Figs 30, 34–47). The curvature of the cerci also exhibits considerable variation and can be used in species diagnoses. The populations other than Balkan, Balıkesir, and Kütahya constitute two groups according to the curvature of the cerci. The İzmir and Aydın populations differ from the other populations by having the cerci incurved with an obtuse angle (Figs 30, 34). In the remaining populations, the cerci are curved with almost a rectangle. The number of cercal denticles is variable amongst and within populations and even sometimes between the left and right cerci of the same individual (Table 2). Although the number is variable, the structure and location of the denticles, together with the shape of distal part exhibit useful characters for distinguishing amongst populations located in the southern part of the group's range (Antalya + Muğla Provinces). The Termessos, Bakırlıdağ, Kemer, and Olympos populations constitute a cluster (named as a morphological unit, MU-1) because the cerci always have a single, spine-like denticle (Figs 43–47) specific to this group. A second group (MU-2), containing the Demre, Patara, and Kalkan populations, is diagnosable by a high number of denticles (two to six), their location, and the group-specific shape of the distal part (Figs 40–42). The third group (MU-3) includes the Akdağ, Erentepe, Eşen1, Eşen2, and Eşen3 populations and is characterized by the specific shape of the distal part and the hook-like, strong denticle (Figs 35–39). In this group, there may sometimes be a small accessory denticle at the base of the prominent one.

The male subgenital plate also exhibits considerable variation offering diagnostic characters, although not as easily applicable as those of the cerci (Figs 48–65). The first definable character is the absence or presence of a caudal incision, and the Balkan and Kütahya populations differ from all others by presenting the first state (Figs 49, 51). The second character



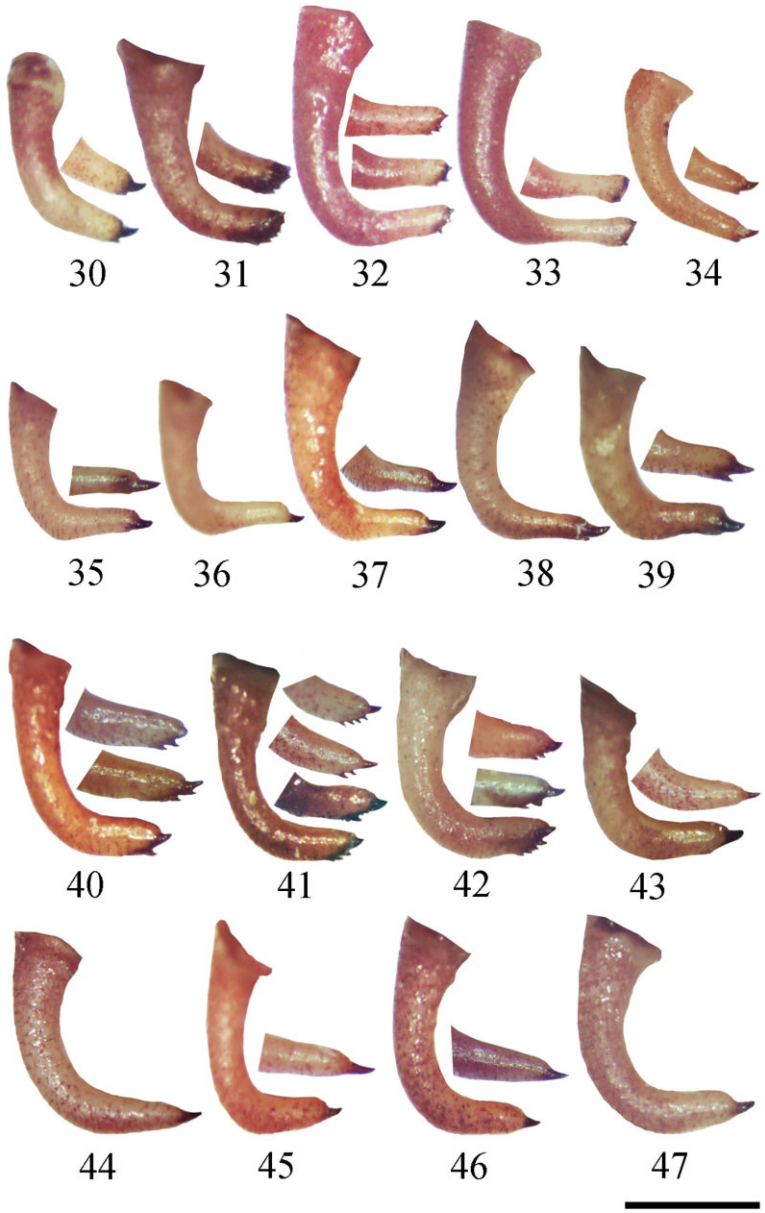
Figures 22–29. Male pronotum from profile in *Poecilimon luschani* species group, each representing a phylogroup suggested by the phylogenetic tree (scale bar = 4 mm): 22, İzmir; 23, Balkan; 24, Balıkesir; 25, Kütahya; 26, Aydın; 27, Demre; 28, west phylogroup of the Antalya + Muğla range; 29, east phylogroup of the Antalya + Muğla range.

is the depth of incision as two states. The Balıkesir, İzmir, and Aydın populations differ from the populations in the southern part of the group's range by having an incision that is one-third of the subgenital plate in length (Figs 48, 50, 52). However, populations located in the Antalya + Muğla range still can be distinguished by the depth of incision (although not very prominent); it is shallower in the populations from the eastern part (Termessos, Bakırlıdağ, Tahtalıdağ, Kemer, and Olympos; Figs 61–65) and deeper in those in the western part (Demre, Kalkan, Patara, Eşen1, Eşen2, Eşen3, Akdag, and Erentepe; Figs 53–60). The third character concerns the margin of the incision – for the populations with an incision. The margins of the incision are divergent proximally and then convergent distally. This state is especially prominent for the İzmir and Aydın populations (Figs 48, 52). The Balıkesir (Fig. 50B), and some populations in the western part of the Antalya + Muğla range (Figs 55–57, 60), also share the same character state, at least in some individuals. In the Termessos, Bakırlıdağ, Tahtalıdağ, Kemer, Olympos, Kalkan, and Patara populations margins are divergent along their total length.

Some meristic (counted or measured) data were gathered from the structures commonly measured in Orthoptera. The meristic data are rather similar in the *P. luschani* group and thus uninformative for internal taxonomy (Table S1). Although the number of stridulatory pegs varies between 89 and 197 within the group, the limits of variation overlap between populations. On average, the peg number is lower than the others (89–114) in the Balkan, Balıkesir, Kütahya, İzmir, and Aydın populations, which are located in the north part of the group's range. On average, this number is higher in the populations occupying the southern part of the range of the group's range, where it varies between 100 and 197 (Table 2). The variation limits per population overlap and the pattern does not correlate with the geography or existing species; thus, it is uninformative for the internal taxonomy of the group. Although a one-way ANOVA suggested considerable variation within the group, a Tukey's test did not separate any population(s) from each other ($F = 46.78$, $P < 0.001$).

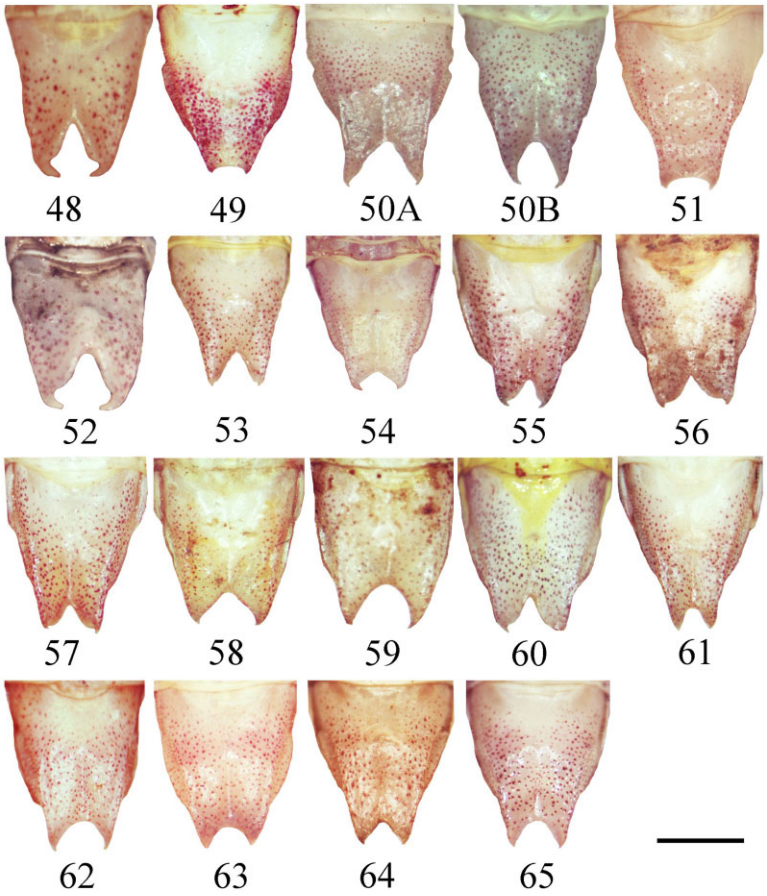
SONG

The male calling song is similar in pattern at group level and consists of isolated syllables (Figs 74–81), which are produced during the closing movement of the tegmina (see Heller, 1988 for *P. orbelicus* and Heller, 1990 for *P. ledereri*). A male produces irregular syllables during the initial period of singing, and a relatively regular series follows in the later period. Independent of the song pattern, three parameters of the song were examined at population level (Table 2). The multiple regression analyses suggested a significant correlation between the temperature and the values of syllable duration and impulse period. After effect of temperature removed for these two characters, data for each of three parameters were analysed using ANOVA.

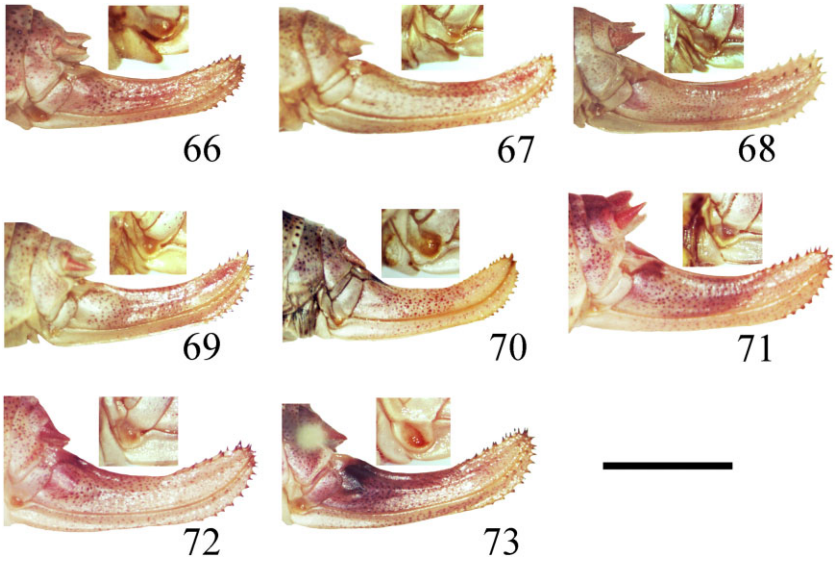


Figures 30–47. Male cerci in *Poecilimon luschani* species group, each representing a population (scale bar = 2 mm): 30, İzmir; 31, Balkan; 32, Balıkesir; 33, Kütahya; 34, Aydın; 35, Akdağ; 36, Erentepe; 37, Eşen1; 38, Eşen2; 39, Eşen3; 40, Patara; 41, Kalkan; 42, Demre; 43, Olympos; 44, Kemer; 45, Tahtalıdağ; 46, Termessos; 47, Bakırlıdağ.

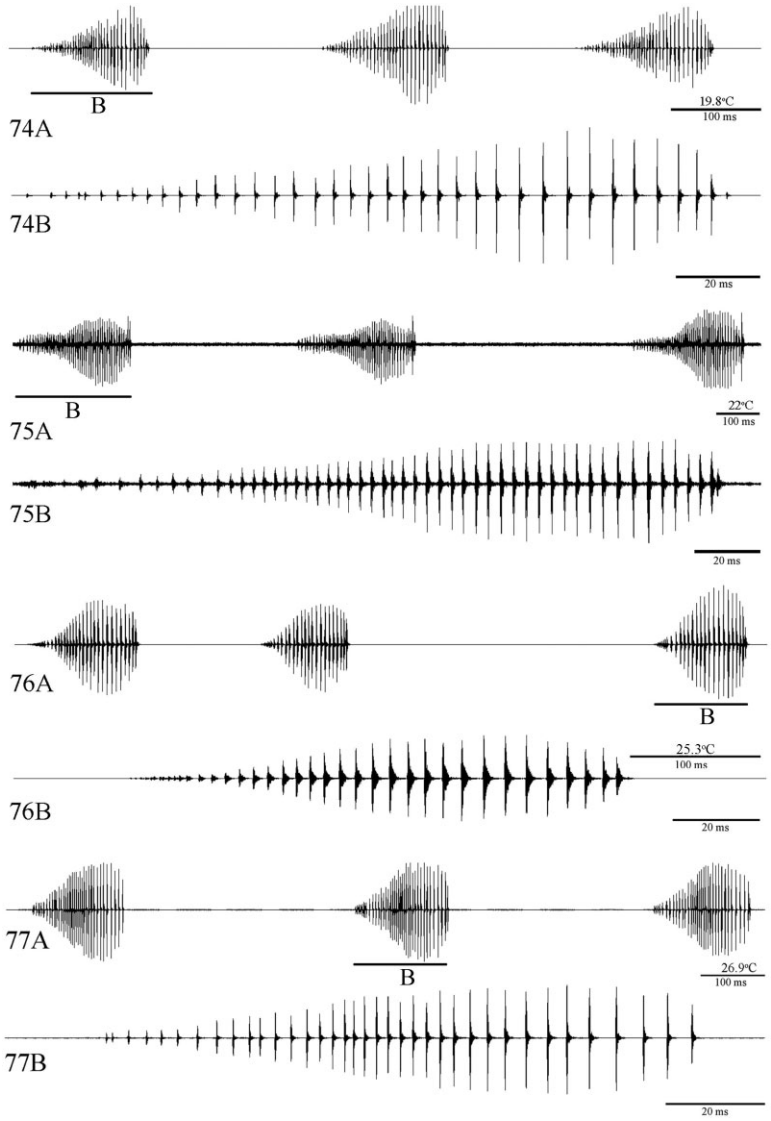
The results suggested little variation amongst populations for all three characters (Figs 82–84; Tables 2, 3). At group level, the mean syllable duration varied from 89.81 ms (Balıkesir population) to 195.94 ms (Greek population), largely overlapping between populations. A Tukey's test performed using the calibrated syllable duration data distinguished the Balıkesir population from all others (Fig. 82). Each syllable consists of a group of impulses, the numbers of which, as means of the population, ranged from 27 (in the Aydın population) to 50 (in the Akdağ and Termessos populations). A Tukey's test applied to impulse number per syllable suggested three clusters: (1) the Aydın; (2) Balıkesir; and (3) the remaining 16 populations (Fig. 83). The impulse period, as means of the population varied between 2.28 ms (in the Erentepe population) and 4.46 ms (in the Greek population). A Tukey's test applied to impulse period suggested two clusters: (1) the Olympos, Demre, Kütahya, Aydın, and Balkan; and (2) the remaining 13 populations (Fig. 84). This clustering pattern is inconsistent with the existing taxonomy.



Figures 48–65. Male subgenital plate in *Poecilimon luschani* species group, each representing a population (scale bar = 2 mm): 48, İzmir; 49, Balkan; 50, Balıkesir; 51, Kütahya; 52, Aydın; 53, Akdağ; 54, Erentepe; 55, Eşen1; 56, Eşen2; 57, Eşen3; 58, Patara; 59, Kalkan; 60, Demre; 61, Olympos; 62, Kemer; 63, Tahtalıdağ; 64, Termessos; 65, Bakırlıdağ.



Figures 66–73. Female genitalia from profile in *Poecilimon luschani* species group, each representing a phylogroup suggested by the phylogenetic tree (scale bar = 4 mm): 66, İzmir; 67, Balkan; 68, Balıkesir; 69, Kütahya; 70, Aydın; 71, Demre; 72, west phylogroup of the Antalya + Muğla range; 73, east phylogroup of the Antalya + Muğla range.

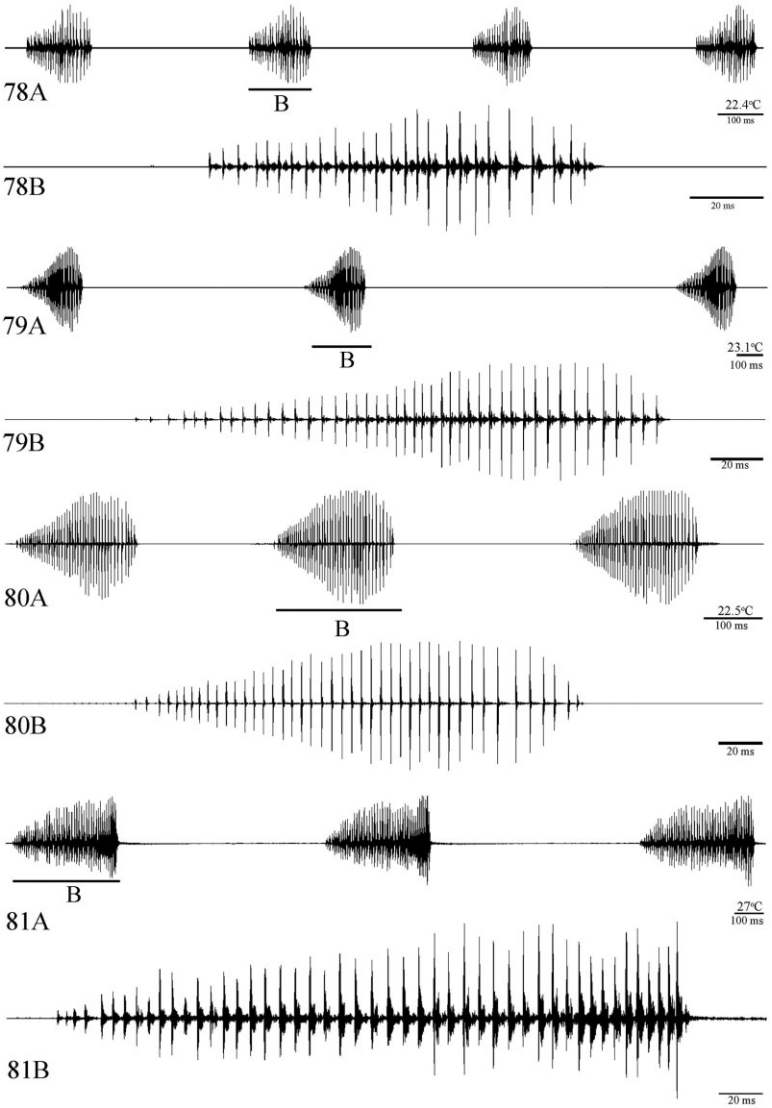


Figures 74–81. Male calling song in *Poecilimon luschani* species group, each representing a phylogroup suggested by the phylogenetic tree (A, a syllable sequence; B, details of a syllable): 74, İzmir; 75, Balkan; 76, Balıkesir; 77, Kütahya; 78, Aydın; 79, Demre; 80, west phylogroup of the Antalya + Muğla range; 81, east phylogroup of the Antalya + Muğla range.

PHYLOGENY

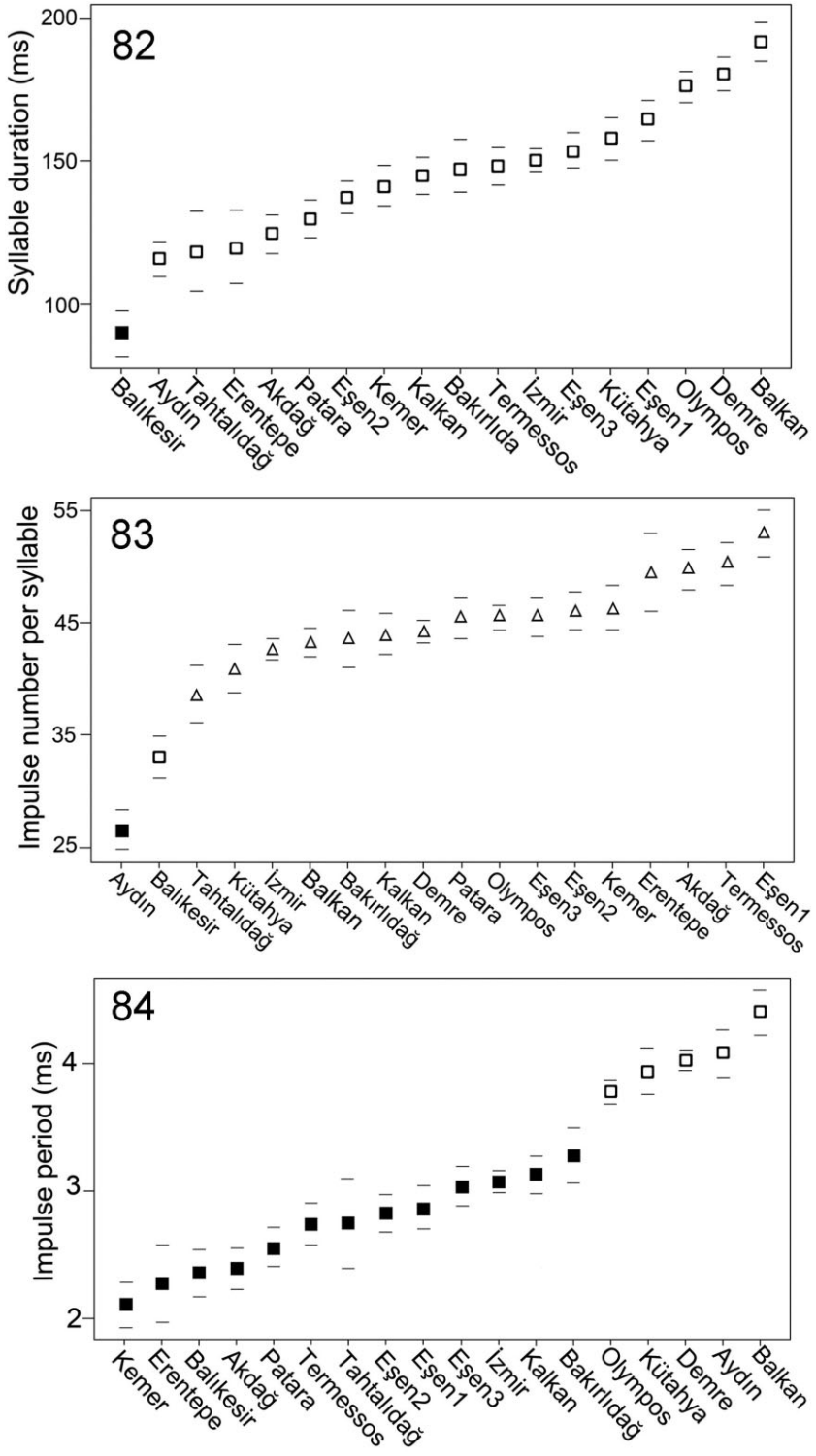
After alignment, trimming, and removal of 12 gaps, the final length of 250 sequences of 16S rDNA was 301 bp and that of 283 sequences of COI (with no gaps) was 1100 bp. The homogeneity test suggested a similar evolutionary pattern ($P = 0.167$) for the sequences of both markers and therefore we concatenated both into a single matrix. The total length of the concatenated sequences was 1401 bp. Of these, 1011 sites were constant, 390 were variable, and 326 were parsimony informative. Seventy-two different haplotypes were defined from the concatenated matrix. Of these 72 haplotypes, four belonged to outgroups (two from *P. obtusicercus*, and one from each of *P. cervus* and *P. izmirensis*) and 68 to the ingroup. MODELTEST v.3.6 applied to the concatenated data matrix suggested a general time reversible model with gamma correction (Γ) and invariable sites (I) according to AIC, with $\Gamma = 1.2528$ and $I = 0.6110$. These parameters were set in PAUP v. 4.0b10 and MrBayes v. 3.1 for ML and BI, respectively. The MP phylogenetic analysis applied to the 72 haplotypes resulted in 1007 equally parsimonious trees (tree length = 938, consistency index = 0.519, retention index = 0.872, rescaled consistency index = 0.453).

All of the MP, ML, and BI analyses resulted in trees with similar topologies (Fig. 85). The monophyly of the ingroup haplotypes was suggested by all trees.



Figures 74–81. *Continued*

The occurrence of the İzmir phylogroup, consisting of eight unique haplotypes, at the base of all ingroup haplotypes was also supported by all analyses. MP, ML, and BI did not agree for the step next to the İzmir phylogroup. The MP strict consensus tree suggested the Aydın phylogroup (containing eight unique haplotypes) as a basal clade leading to others and the Balkan phylogroup at the base of the Balıkesir + Kütahya phylogroup. However, the MP bootstrap test (1000 replications) did not support this, and resulted in a tree topology similar to the ML and BI trees. The node above the İzmir phylogroup is a dichotomy consisting of the Balkan phylogroup (two haplotypes) and the phylogroup of the remaining haplotypes. Next to this step on the tree, there are three phylogroups: (1) the Aydın phylogroup (eight haplotypes); (2) the Balıkesir (four haplotypes) + Kütahya (eight haplotypes) phylogroup; and (3) the Antalya + Muğla phylogroup (44 haplotypes). Each of the last two phylogroups consists of two infraphylogroups. The infraclades of the second phylogroup are Balıkesir and Kütahya. The Antalya + Muğla phylogroups consist of two infraphylogroups (named as PG-1 and PG-2 hereafter). PG-1 consists of 11 haplotypes unique to the populations in the east part of the Antalya + Muğla range (Olympos, Kemer, Bakırlıdağ, and Termessos), in addition to a basal clade containing six haplotypes unique to the Demre population. PG-2 consists of 24 haplotypes from the populations located in the west part of the Antalya + Muğla range (Eşen1, Eşen2, Eşen3, Kalkan, and Patara) and a basal clade consisting of three haplotypes unique to



Figures 82–84. Plots showing results of Tukey's analyses applied to four song parameters from *Poecilimon luschani* species group given in Table 2. 82, syllable duration; 83, impulse number per syllable; 84, impulse period.

Table 3. Results of the regression (for effects of temperature) and one-way ANOVA analyses applied to song parameters given in Table 2

Characters	Regression								ANOVA		
	Trns	df_M	df_E	*F*	C_P	Adj-r^2	AIC	BIC	df_P	df_E	*F*
Syllable duration	Ln	18	2196	251.70**	19	0.6709	13569.5998	13571.9284	17	2197	126.72
Impulse number per syllable	–	–	–	–	–	–	–	–	17	2218	68.80
Impulse period	–	17	2185	134.27**	19	0.5071	–2269.3480	–2267.0175	17	2185	160.72
Impulse number per 100 ms	–	–	–	–	–	–	–	–	17	2133	98.46

**$P < 0.001$.

Trns, transformation; df_M, degrees of freedom of model; df_P, degrees of freedom of population; df_E, degrees of freedom of error; *F*, *F* test; C_P, Mallow's C_P; Adj-r^2, adjusted r^2; AIC, Akaike information criterion; BIC, Bayesian or Swardz information criterion.

the Demre population. The last haplotype unique to the Demre population (Demre 4) is within PG-2. Within PG-2 there are two infraphylogroups apart from the basal Demre clade, one of which includes five haplotypes unique to Eşen3 and the second the others. This second phylogroup includes three further infraphylogroups; the first includes ten haplotypes unique to Eşen1 plus Eşen2, and the second and third consist of haplotypes unique to Kalkan and Patara plus a single haplotype unique to Demre.

TAXONOMY

The *P. luschani* group can be defined by the combination of the following characters: (1) tegmina in female rudimentary, not touching each other; (2) fastigium one to two times as wide as scapus; (3) tegmina yellow peripherally and dark brown in the middle; (4) pronotum moderately constricted in prozona and slightly widened/elevated in metazona; (5) characteristic coloration of the pronotum with red lateral corners of metazona of disc; (6) relatively long male cerci with one or a few apical denticles, one of which is prominent and located in the externo-apical corner; (7) male subgenital plate deeply incised at caudal margin; (8) female lamella (basal fold of the lower ovipositor valve) indistinct and not protruded laterally; and (9) song consisting of isolated syllables but separated by only relatively small intervals (A in Figs 74–81). Ramme (1933), when first proposing the *P. luschani* group (without *P. orbelicus*), mentioned characters 6 and 7. However, *P. orbelicus*, *P. egrigozi*, and *Poecilimon helleri* sp. nov. do not possess character 6, and the first two also do not have character 7. However, the present DNA data, in addition to those published by Ullrich *et al*. (2010), support the monophyly of the group together with the uniform song pattern.

None of the three data sets, morphology, song, and DNA, given separately on the basis of populations, agreed on the same taxonomic arrangement. Male calling song in particular is similar in all populations and only poorly diagnoses the Balıkesir (by the short syllable duration) and Aydın (by the least impulse number per syllable) populations congruently with phylogeny and morphology (see Discussion). The male cerci and subgenital plate are still the two most important morphological structures offering diagnostic characters. However, comparison between DNA phylogeny and the characters defined from the male subgenital plate and cerci (Fig. 86) indicates parallel or secondary losses/gains within the species group. Taxonomic decisions were made according to both phylogeny and the diagnostic phenotypic characters from morphology and/or song (Fig. 86). This perspective confirmed the species status of *P. ledereri* Ramme, 1933, and *P. orbelicus* Pančić, 1883. The

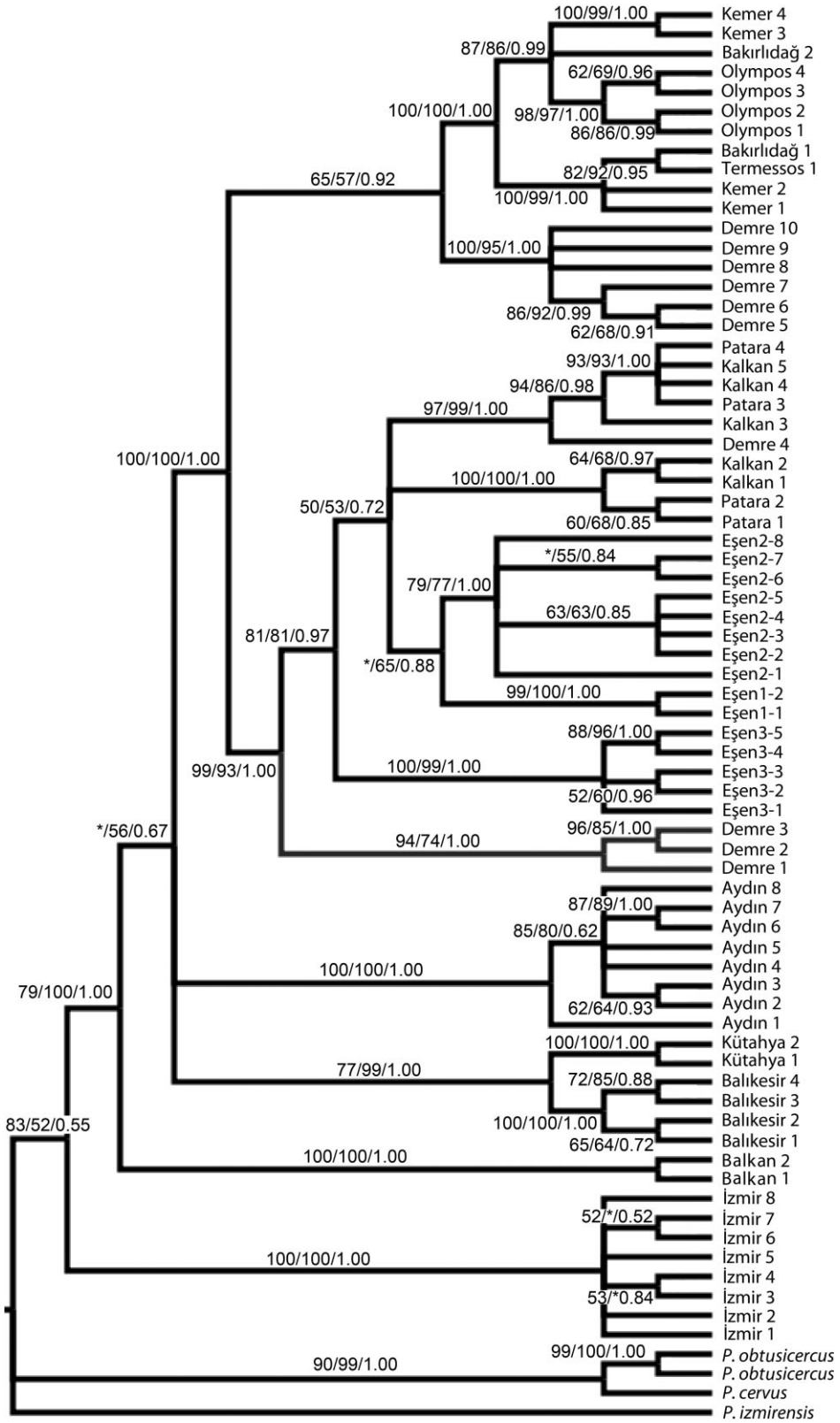


Figure 85. Phylogenetic tree combined from maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses for *Poecilimon luschani* species group. Numbers on the nodes show bootstrap (MP and ML) or posterior probability (BI) values for MP, ML, and BI, respectively. * indicates node not supported by the respective analysis.

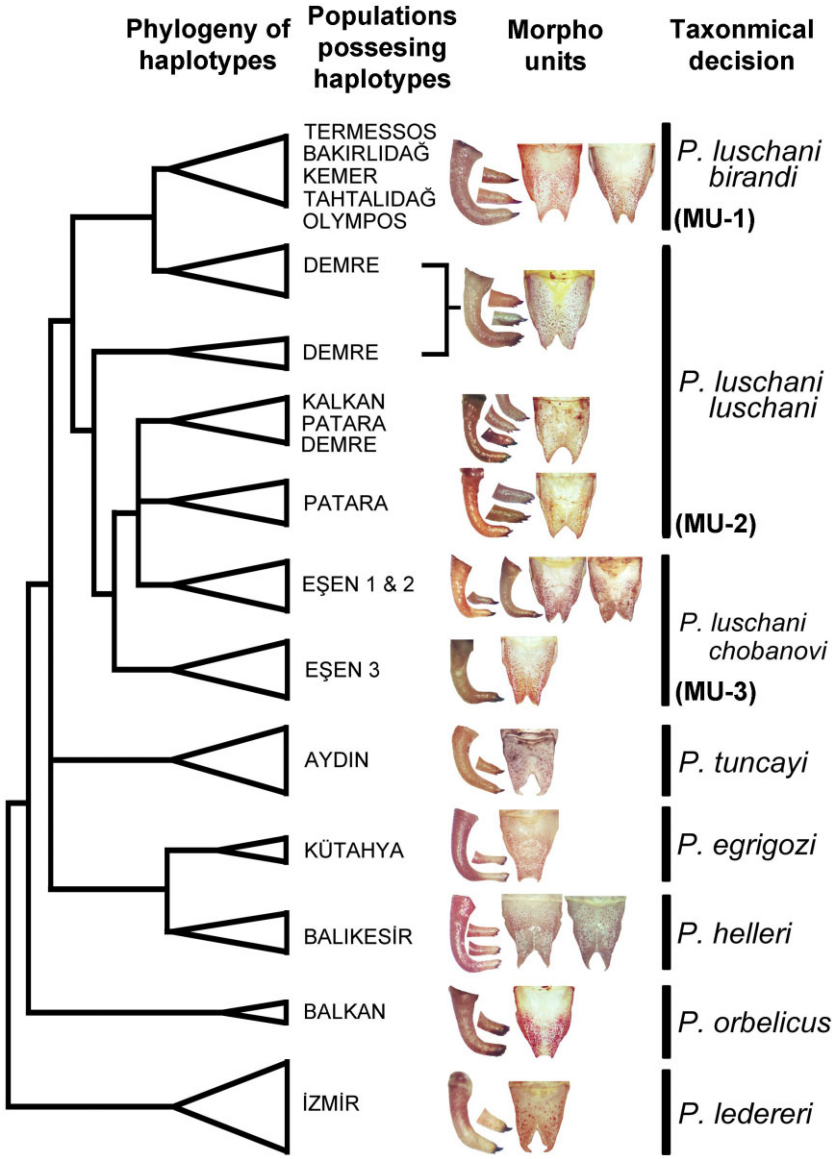


Figure 86. Evolution of the male cerci and subgenital plate in the light of molecular phylogeny and the background for taxonomic decisions for *Poecilimon luschani* species group. MU, morphological unit.

available data support the uniqueness of the Aydın population as a distinct species; thus, the re-establishment of *P. tuncayi* Karabağ, 1953, stat. rev., formerly synonymized with *P. ledereri* by Ünal (2005), is required. The population from Kütahya was described as a subspecies, *P. luschani egrigozi*, by Ünal (2005); however, our present data suggest that it is a distinct species, elevated herein as *P. egrigozi* Ünal, 2005, stat. nov. Another geographically discrete population is that of Balıkesir; all our data suggest that it is a distinct, independent reproductive unit. Thus, we establish a new species named *P. helleri* Boztepe, Kaya & Çıplak sp. nov. (Fig. 86).

Applying this approach to the populations in the southern part of the group's range requires further evaluation. The gene tree suggests that all populations in the southern part of the group's range constitute a single species as chains of populations. However, the phylogenetic pattern conflicts with the morphological units. According to cercal morphology, there are three units (MU-1, MU-2, and MU-3) in the area, but they constitute two main phylogroups, named PG-1 and PG-2. Although the Demre population belongs to MU-2, ten haplotypes unique to it constitute two independent subphylogroups: one at the base of PG-1 and the other at the base of PG-2.

KEY TO SPECIES/SUBSPECIES (MALE ONLY)

1a. Subgenital plate with a distinct incision (Figs 48, 50, 52–65)....3
1b. Subgenital plate without or with an indistinct incision (Figs 49, 51)....2
2a. Cerci almost the same width throughout its length and not narrowed at distal part (Fig. 31)....*Poecilimon orbelicus*
2b. Cerci narrowed at distal part (Fig. 33)....*Poecilimon egrigozi*
3a. Cerci curved with an obtuse angle that is > 110 (Figs 30, 34)....4
3b. Cerci curved, almost forming a rectangle (Figs 32, 35–47)....5
4a. Impulse number per syllable on average is 42 (14–67); cerci with a short distal part compared to *P. tuncayi* (Fig. 30)....*Poecilimon ledereri*
4b. Impulse number per syllable on average is 27 (9–43) (Fig. 83); cerci with a long distal part compared to *P. ledereri* (Fig. 34)....*Poecilimon tuncayi*
5a. Apex of cerci not wider than its proceeding part (Figs 35–47); subgenital plate with a shallow incision, if the incision is deep then the apical lobes not strongly tapered (Figs 53–65)....6 (*Poecilimon luschani*)
5b. Apex of cerci wider than its proceeding part (Fig. 32); subgenital plate with a deep triangular incision and long, strongly tapered lobes (Fig. 50)....*Poecilimon helleri*
6a. Subgenital plate with a relatively deep incision margins of which diverges at proximal part and converges at distal part (Figs 53–60); if not convergent distally, then cerci with two or more denticles; cerci with two to six apical denticles; if with a single denticle then the outer margin of its distal part is concave (Figs 35–42)....7
6b. Subgenital plate with relatively shallow, triangular incision margins of which diverge along total length (Figs 61–65); cerci as in Figures 43–47....*Poecilimon luschani birandi*
7a. Cerci with two or more denticles each located separately; the distal part of cerci is short and robust (Figs 40–42)....*Poecilimon luschani luschani*
7b. Cerci with a single, hook-like denticle; if there is a second small denticle it is just at the base of the large one. The distal part of cerci is slender and has a slightly concave outer margin (Figs 35–39)....*Poecilimon luschani chobanovi*

The Eşen3 population exhibits another case of gene tree and phenotypic clustering conflict. It belongs to MU-3, but the haplotypes unique to this population constitute an infraphylogroup out of the other members of MU-3 within PG-2.

We considered each of the morphological units as a separate subspecies, although such a taxonomic decision results in paraphyletic taxa. These taxonomic arrangements were made for three reasons: (1) the morphological units are robust in definition; (2) the possibility of potential reproduction amongst these units cannot be excluded at present as none is monophyletic; and (iii) the conflict between gene and (sub-)species is not rare in nature (see Discussion for further details). This decision requires the following nomenclatural changes. There are two existing species, *P. luschani* and *P. birandi*, corresponding to PG-1 and PG-2, respectively. According to ICZN (1999), *P. luschani* Ramme has priority. Therefore, we suggest the establishment of two subspecies corresponding to these existing names. The nominate subspecies *P. luschani luschani* corresponds to MU-2 as its type locality Kale, Antalya, is within the range of this unit (see Ramme, 1933; Heller, 2004; Ünal 2005 for type locality). Thus, this subspecies is represented by the lowland populations of Demre, Kalkan, and Patara. The second subspecies is *P. luschani birandi* stat. rev., corresponding to MU-1 and including the topotypical *P. birandi* from the Tahtalıdağ population (Karabağ, 1950). Other populations are known from Termessos, Bakırlıdağ, Tahtalıdağ, Kemer, and Olympos. MU-3 includes the Eşen1, Eşen2, Eşen3, Akdağ, and Erentepe populations and represents a new subspecies, described here as *Poecilimon luschani chobanovi* ssp. nov. (Fig. 86).

POECILIMON LEDERERI RAMME, 1933

Poecilimon ledereri Ramme, 1933: 540–541.

Type information: Holotype – male; paratypes two males and one female; Turkey – İzmir, Bozdağ (Naturhistorisches Museum, Vienna) (not examined).

Material examined: Specimens used for morphological, bioacoustic, and molecular studies are listed in Table 1 and shown in Figure 1. Further specimens identified as *P. ledereri* in the collection of K. G. Heller (K. G. Heller, pers. comm.) are as follows (Fig. 1). Four males and four females, Turkey, Aydın Province, Nyssa, 37°54′N, 28°9′E, 5.v.1985 (legitat K. G. Heller); one male, Turkey – Izmir Province, Zeytinlik, 38 km south-east of Turgutlu (near Ödemiş), 38°16′59″N, 28°0′44″E, 3.vi.2005 (legitat Klaus Reinhold); nine males, Turkey – Izmir Province, Aydin Dagları, about 20 km south of Tire, pass,

38°1′N, 27°47′E, 900 m, 31.v.2010, (legitat Maryanska-Nadachowska); three males and one female, Turkey – Izmir Province, Boz Dağ, 38°21′N, 28°5′E, 7.v.1985 (legitat K. G. Heller); four males, Turkey – Izmir Province, Gölcük, Boz Dağları, *c.* 18 km north of Ödemiş, 38°19′N, 28°0′E, 1100 m, 3.vi.2010 (legitat Maryanska-Nadachowska).

Description: For morphology see Table S1, Figures 4, 22, 30, 48, and 66; for song see Table 2 and Figures 74, 82–84. Additional description can be found in Ramme (1933) and Bei-Bienko (1954).

Distribution (Fig. 1): Endemic to Aegean Anatolia; mainly associated with the Bozdaglar and Aydın Dağları mountain belts in İzmir and Aydın Provinces of Turkey. The southern end of the range is bordered by the Büyük Menderes River. Previous records can be found in Ramme (1933), Karabağ (1958), and Heller (1990). The records of *P. ledereri* from Aydın Province (1, Bozdoğan, Kemer; 2, Çakirbeyli; and 3, Çamköy) by Ünal (2005) correspond to the range of *P. tuncayi*.

Remarks: Although morphological differences between *P. ledereri* and *P. tuncayi* are not prominent, the first species is distinguishable by shorter and weakly incurved male cerci. The impulse number per syllable on average is higher in *P. ledereri*. Although the phenotypic differences are not large, genetic data clearly separate it from *P. tuncayi* and all other species.

POECILIMON ORBELICUS PANČIĆ, 1883

Poecilimon orbelicus Pančić, 1883*:* 163.

Type information: Syntypes, lost (?); Rila Mt., Elenin Vrukh Peak, Bulgaria.

Material examined: See Table 1.

Description: For morphology see Table S1, Figures 5, 23, 31, 49, 67; for song see Table 2 and Figures 75, 82–84. Additional description can be found mainly in Ramme (1933), Bei-Bienko (1954), and Harz (1969).

Distribution (Fig. 1): Bulgaria, Macedonia, and Greece (Harz, 1969; Peshev, 1975; Willemse, 1984; Chobanov, 2009).

POECILIMON TUNCAYI KARABAĞ, 1953 **STAT. REV.**

Poecilimon tuncayi Karabağ, 1953*:* 179.

Poecilimon ledereri Ramme; Ünal, 2005*:* 439.

Type information: Holotype – male; Turkey, Aydın Province, Bölüntü (Natural History Museum, London) (not examined).

Material examined: Specimens used for morphological, bioacoustic, and molecular studies are listed in Table 1 and shown in Figure 1. Further specimens identified as *P. tuncayi* in the collection of K. G. Heller (K. G. Heller, pers. comm.) are as follows (Fig. 1). Eight males and two females, Turkey – Muğla Province, Labranda, 37°24′N, 27°42′E, 2.v.1985 (legitat K. G. Heller); two males, Turkey – Aydın Province, 10 km south of Bozdoğan, 37°35′41″N, 28°20′52″E, 3.vi.2005 (legitat K. Reinhold).

Description: For morphology see Table S1, Figures 8, 26, 34, 52, 70; for song see Table 2A Figures 78, 82–84. Additional description can be found in Karabağ (1953) and Bei-Bienko (1954).

Distribution (Fig. 1): In addition to the type locality, this species has been recorded from Savaştepe, Balıkesir (Karabağ, 1964), and the road to Zeytinköy, Aydın (Karabağ, Gümüşsuyu & Tutkun, 1981). The records by Ünal (2005) from Aydın (1, Bozdoğan; Kemer; 2, Çakirbeyli; and 3, Çamköy) are also indicative of *P. tuncayi*. Both earlier records and localities listed in the Material examined section indicate that this species has a narrow range in the Aegean lowlands in the southern parts of Aydın Province (bordered by the Büyük Menderes River and Aydın Dağları mountains in the north) and northern parts of Mugla Province. We think that the record by Karabağ (1964) from Savaştepe (Balıkesir Province) is possibly not *P. tuncayi* as this locality is in the north of Bozdağ and the Aydın Dağları mountain ranges are the region inhabited by *P. ledereri*.

Remarks: Ünal (2004) suggested that this species is very similar, and possibly synonymous, to *P. luschani*; later, he synonymized it with *P. ledereri* (Ünal, 2005). As presented in the Results section, the Aydın population constitutes a distinct phylogroup. Additionally, this population differs from the others by having the lowest average impulse number per syllable. Thus, we think it is a distinct and independent reproductive unit and we re-establish it at a species rank. For diagnosis, see *P. ledereri* and *P. luschani*.

POECILIMON HELLERI BOZTEPE, KAYA & ÇIPLAK **SP. NOV.**

Holotype: Male; Turkey – Balıkesir Province, Akçay, Kaz Dağı, Sarıkız District, 39°42′19.6″N, 026°53′44.3″E, 1688 m, 05.vii.2011 (legitat B. Çıplak *et al.*) (AUZM).

Paratypes: Twenty-four males and seven females; same data as holotype.

Diagnosis: Genetic data suggest that *P. helleri* is a sister species to *P. egrigozi*. It is similar to *P. egrigozi* and *P. orbelicus* by the L-shaped and apically wide male cerci. However, it differs from these species and other members of the group by the male's subgenital plate with a deep triangular incision and tapered triangular apical lobes. Additionally, this species has the shortest syllable duration within the species group.

Etymology: Over several years, Dr Klaus-Gerhard Heller has made a considerable contribution to the taxonomy of Orthoptera. His contributions are especially prominent for the genus *Poecilimon*.

Description: For morphology see Table S1, Figures 6, 24, 32, 50, 68; for song see Table 2 and Figures 76, 82–84.

Male (holotype): Small to medium sized for the genus, over medium sized for the group.

Head: fastigium of vertex prominently produced anteriorly with a groove, its lateral margins parallel or slightly converging, wider than the scapus.

Thorax: pronotum (Fig. 6) short, moderately constricted in prozona, metazona gradually but weakly widened backward and considerably risen, with its surface slightly domed; metazona covering the tegmina beyond cubital vein. Posterior margin of pronotum gently concave. Lower margin of pronotal lateral lobes slightly wavy and with small, nose-like projection in the frontal part. Tegmina, reach to hind edge of first abdominal tergite. The stridulatory file has comparatively dense pegs, gradually increasing in size from the base and becoming largest in the middle of the file. Peg number varies between 89 and 105. Fore femora slightly longer than pronotum. Hind femora with ventral spinules.

Abdomen: cerci (Fig. 32) wide at the base but comparatively robust and long for the group; they are almost straight in the basal third to quarter and incurved with a rectangle distally; the apex as wide as or wider of its former part; the apical end of the cerci truncate and with three to five small denticles. Subgenital plate (Fig. 50) long, extending beyond the tip of cerci; with a deep triangular incision and strongly tapered lobes.

Coloration: general coloration green to dark green with black dots. Antennae yellowish-green, annulated with dark rings. Head and pronotal dorsum with two lateral and one medial thin pale stripes. Dorsolateral corners of pronotum in metazona with big, reddish-pink spots almost touching each other at the hind margin of metazona. Tegmina yellow with dark brownish-black stridulatory area. Femora and tibiae green getting yellowish apically. Abdomen green with the first and sometimes all tergites with a black spot mediobasally, which constitute two black stripes along the dorsal side. Cerci reddish-brown, with black denticles.

Song: in general similar to that of the group (Figs 76, 82–84; Table 2).

Female: Pronotum almost cylindrical but slightly flattened and widened in metazona, with a straight or insignificantly concave hind margin. Pronotal lateral plates as in male and straight dorsally with a transverse sulcus cutting the median line just behind the middle of pronotum. Tegmina fully covered by pronotum, reduced to scale-like appendages. Subgenital plate short, transverse, widely oval, apically blunt. Ovipositor roughly one and a half times the pronotum in length; apical part with short, stout teeth dorsally, ventrally and laterally, as in allied species. Lamella (basal fold of dorsal margin of lower ovipositor valve) (Fig. 68) short, flattened, laterally indistinctly widened.

Coloration: as in male.

Distribution: The new species occurs in the subalpine and alpine zones of the Kazdağları Mountains in north-west Turkey (Fig. 1). This population was first reported by Ünal (2004; as *P. luschani*) and Sevgili, Demirsoy & Durmuş (2011; as *P. luschani egrigozi*). However, both records refer to the same locality on the Kazdağları Mountains. By contrast, the record by Karabağ (1964) as *P. tuncayi* from Balıkesir, Savaştepe, requires confirmation; it is unclear whether it belongs to this species.

POECILIMON EGRIGOZI ÜNAL, 2005 STAT. NOV.

Poecilimon luschani egrigozi Ünal, 2005*:* 440, 441.

Type information: Holotype – male; Turkey – Kütahya, Emet, Eğrigöz Mountain (Collection of M. Ünal in Abant İzzet Baysal University, Bolu-Turkey) (Ünal, 2005).

Material examined: See Table 1.

Description: For morphology see Table S1, Figures 7, 25, 33, 51, 69; for song see Table 2 and Figures 77, 82–84. Additional description can be found mainly in Ünal (2005) and Sevgili *et al*. (2011).

Distribution: According to the present data, this species is restricted to the alpine zone of the Egrigoz Mountain in the Kütahya Province of Turkey (Fig. 1).

Remarks: Ünal described this population as a subspecies under *P. luschani*. The present data show prominent differences between this population and the other members of the group. An apically widened cercus is shared by *P. orbelicus* and *P. helleri* sp. nov. However, cercal structure still exhibits some specific aspects. In addition, the male's subgenital plate with no or indistinct incision is typical for *P. egrigozi* and *P. orbelicus*. More importantly, the molecular phylogeny, based on sequences of two different markers, suggested the species' close relationships with *P. helleri* sp. nov. and also its independence as a reproductive unit. Therefore, we consider it as an independent species.

POECILIMON LUSCHANI RAMME, 1933

Poecilimon luschani Ramme, 1933*:* 539–540.

All populations in the southern part of the group's range constitute a single species with three different morphological units. Each of these units is considered as a subspecies. Of these morphounits, two correspond to the existing species *P. luschani* and *P. birandi*. Based on priority, *P. luschani* is retained as the species name and *P. birandi* is treated as a subspecies of *P. luschani*. The third morphounit is named as a new subspecies (Figs 1, 86). In two recent studies the entire Antalya + Muğla range was considered as a single species under the name of *P. birandi* (Çıplak *et al*., 2008; Kaya *et al*., 2012b). These records correspond to three subspecies of *P. luschani*.

POECILIMON LUSCHANI LUSCHANI RAMME, 1933

Poecilimon luschani Ramme, 1933*:* 539–540.

Type information: Holotype – male; Kleinasien (Turkey), Göllbakti (Naturhistorisches Museum, Vienna; not examined). In the original description, the type locality was given as 'Göllbakti (Kleinasien)' by Ramme (1933). Later, Karabağ (1958) gave this locality as 'Gölbaşı-Ankara (?)', with uncertainty. However, members of this species group are present along a line in west Anatolia (plus the Balkans) and no record has been mentioned from central Anatolia since that date. Heller (2004) and later Ünal (2005) (also V. Fet, pers. comm.) reported that Luschan collected around Kale (Antalya) in south-west Anatolia and thus very probably Kale is the type locality.

Material examined: See Table 1.

Description: For morphology see Table S1, Figures 14–16, 27, 40–42, 58–60, 72; for song see Table 2 and Figures 79, 82–84. Additional description can be found in Ramme (1933) and Bei-Bienko (1954).

Distribution: Endemic to south-west Anatolia, in the western part of Antalya Province and the most eastern end of Muğla Province, Turkey (Fig. 1). All populations belonging to this subspecies are recorded from lowlands in a range starting from Demre (Antalya Province) and extending to the most eastern end of Muğla Province in the Eşen Valley.

POECILIMON LUSCHANI BIRANDI KARABAĞ, 1950 **STAT. NOV.**

Poecilimon birandi Karabağ, 1950*:* 150.

Type information: Holotype – male; Turkey – Antalya Province, Tahtalıdağ (Natural History Museum, London). Photos given in OSF2 (Orthoptera Species File v.2.0) were examined.

Material examined: Specimens used for morphological, bioacoustic, and molecular studies are listed in Table 1 and shown in Figure 1. Further specimens identified as *P. luschani birandi* (as *P. birandi*) in the collection of K. G. Heller (K. G. Heller, pers. comm.) are as follows. Two males and two females, Turkey – Antalya Province, Bakırlı Dağ, 2020 m, 24.vii.2011 (legitat Dragan Chobanov); three males and two females, Turkey – Antalya Province, Saklıkent (*c.* 10 km below the village), 36°53′N, 30°24′E, 26.vi.2002 (legitat K. G. Heller); ten males and one female, Turkey – Antalya Province, Termessos (*c*. 25 km north-west of Antalya), 36°58′N, 30°30′E, 31.v.2000 (legitat K. G. Heller); three males and three females, Turkey – Antalya Province, 10 km east of Kumluca (*c*. 70 km south-west of Antalya) 36°23′N, 30°22′E, 300 m, 4.vi.2000 (legitat K. G. Heller).

Description: For morphology see Table S1, Figures 17–21, 29, 43–47, 61–65, 73; for song see Table 2 and Figures 81, 82–84. Further description can be found in Karabağ (1950), Bei-Bienko (1954), and Sevgili (2001).

Distribution: This subspecies is endemic to the western part of Antalya Province (Fig. 1). Ünal (2004) reported this subspecies (as *P. birandi*) from the Isparta (road to Senirkent) Province of Turkey; however, no specimens of *P. birandi* were observed although we visited the locality (on 03–04.vii.2011, searching an altitudinal range of 1000–1750 m). The *P. birandi* record by Karabağ (1958) from Muğla Province, Fethiye, and that by Sevgili (2001) from Burdur Province, Altınyayla, are probably *P. luschani chobanovi* ssp. nov. From our extensive field studies we conclude that *P. luschani birandi* occurs only on the

southern slopes of Western Taurus, not in the north of Antalya Province.

POECILIMON LUSCHANI CHOBANOVI BOZTEPE, KAYA & ÇIPLAK SSP. NOV.

Holotype: Male, Turkey – Muğla Province, Fethiye, middle parts of Eşen Valley, around Tylos, 36°33′29.4″N, 029°25′17.4″E, 470 m, 14.v.2011 (legitat B. Çıplak *et al*.) (AUZM).

Paratypes: Sixty-one males and 33 females, Turkey – Muğla Province, Fethiye, middle parts of Eşen Valley, around Tylos, 36°33′29.4″N, 029°25′17.4″E, 470 m, 08.v.2005, 14.v.2011 (legitat B. Çıplak *et al*.); 23 males, 12 females, Antalya Province, Elmalı, Akdağ, 36°34′56.8″N, 029°34′99.2″E, 2247 m, 15.vii.2005 (legitat B. Çıplak *et al*.); 16 males and eight females, Turkey – Antalya Province, Elmalı, Erentepe, 36°44′38.3″N, 029°38′45.0″E, 1982 m, 15.vii.2005 (legitat B. Çıplak *et al*.); 16 males and eight females, Turkey – Muğla Province, Fethiye, upper parts of Eşen Valley, 36°42′01.6″N, 029°24′66.9″E, 672 m, 15.v.2005, 14.v.2011 (legitat B. Çıplak *et al*.); 22 males and 17 females, Turkey – Muğla Province, Fethiye, lower parts of Eşen Valley, around Xanthos, 36°25′71.8″N, 029°16′32.3″E, 85 m, 20.v.2006, 14.v. 2011 (legitat B. Çıplak *et al*.) (all in AUZM).

Apart from the specimens in AUZM, there are further specimens of this subspecies in the collection of K. G. Heller (K. G. Heller, pers. comm.) as follows. Four males and one female, Turkey – Antalya Province, Xanthos (*c*. 130 km south-west of Antalya), 36°23′N, 29°17′E, 5.vi.2000 (legitat K. G. Heller); one male, Turkey – Muğla Province, Karabel Pass (*c*. 30 km east of Fethiye), 36°43′N, 29°42′E, 800 m, 7.vi.2000 (legitat K. G. Heller); one male and one female, Turkey – Muğla Province, Seki, 36°48′N, 29°39′E, 1400 m, 7.vi.2000 (legitat K. G. Heller); six males and one female, Turkey – Muğla Province, Tlos (*c*. 20 km east of Fethiye), 36°34′N, 29°23′E, 6.vi.2000 (legitat K. G. Heller).

Etymology: This new subspecies is dedicated to the young orthopterist Dr Dragan Chobanov. He has recently conducted impressive studies on Barbitistini and has assisted us by sending us specimens of *P. orbelicus*.

Diagnosis: In our opinion, in the southern part of its range (i.e. Antalya and Muğla Provinces), the *P. luschani* group is represented by a single species that includes three morphological units (MU-1, MU-2, and MU-3); the last unit is described here as *P. luschani chobanovi* ssp. nov. This subspecies clearly has genetic affinity to the nominate subspecies as both constitute a phylogroup; the existing data do not indicate reproductive isolation between the two. Apart from its genetic properties, *P. luschani chobanovi* ssp. nov. can be easily distinguished from other subspecies by its specific male cerci.

Description: For morphology see Table S1, Figures 9–13, 28, 35–39, 53–57, 72; for song see Table 2 and Figures 80, 82–84.

Male: Head: fastigium of vertex well produced anteriorly with a groove, its lateral margins parallel or slightly converging, roughly 1.3 times wider than scapus.

Thorax: pronotum short, moderately constricted in prozona, metazona gradually but weakly widened backward and weakly raised; metazona covering the tegmina beyond cubital vein. Posterior margin of pronotum truncate or gently concave. Lower margin of pronotal lateral lobes slightly wavy with small, nose-like projection in the frontal part. Tegmina reach to hind edge of first abdominal tergite. The stridulatory file has comparatively dense pegs, gradually increasing in size from the base and becoming largest in the middle of the file; with 100–196 pegs. Fore femora slightly longer than pronotum. Hind femora with ventral spinules.

Abdomen: cerci (Figs 35–39) comparatively slender and long for the group; they are almost straight in the basal two-fifths to three-fifths and curved inward with a rectangle distally, constituting a typical 'L' shape; the distal part of cerci gradually tapering, especially from its outer margin; the apex ends with a single, hook-like robust denticle or sometimes with a second small accessory one at the base of the large one. Subgenital plate (see Figs 53–57) long, extending beyond the tip of cerci; with a triangular incision, margins of incision divergent along their total length or divergent distally then divergent over a short distance apically.

Coloration: general coloration green with several black dots especially on prozona, legs, and abdomen. Antennae whitish-green, annulated with dark rings. Head and pronotal dorsum with lateral and medial thin pale stripes. Dorsolateral corners of pronotum in metazona with big, reddish-pink spots almost touching each other at the hind margin. Tegmina yellow with dark brownish-black stridulatory area. Femora and tibiae dark green with several black dots. Abdomen green with three black stripes, one mediodorsally and each of the others dorsolaterally. Cerci reddish-brown, with black denticles.

Female: Pronotum almost cylindrical but slightly flattened and slightly widened in metazona and with a truncate or insignificantly concave hind margin.

Pronotal lateral plates as in male and straight dorsally with a transverse sulcus cutting the median line just behind the middle of pronotum. Tegmina fully covered by pronotum, reduced to scale-like appendages. Subgenital plate short, transverse, widely oval, apically blunt. Ovipositor roughly one and a half times the pronotum in length, its apical part with short, stout teeth dorsally, ventrally, and laterally, as in allied species. Lamella (basal fold of dorsal margin of lower ovipositor valve; Fig. 72) short, flattened, and indistinctly widened laterally.

Coloration: as in the male.

Distribution: The new subspecies occurs over a wide altitudinal range starting nearly from sea level in the Eşen Valley and extending to beyond 2000 m on Akdağ Mountain (Table 1, Fig. 1). It occurs in openings amongst Mediterranean forest or maquis vegetation in the lowlands and in the alpine zone in the highlands.

DISCUSSION

Three different data sources – male calling song, morphology, and two DNA markers from the matrilineal genome – were used to revise the *P. luschani* group. In the group, males produce single syllables and the female is mute. However, it is suggested that the unidirectional communication system in the group evolved secondarily (Heller, 1990; Heller & von Helversen, 1993), as in the *Poecilimon ampliatus* (Heller & Lehmann, 2004) and *Poecilimon propinquus* species groups (Heller, 2006). Male calling song functions in mate recognition and has thus been assumed to be important in the definition of reproductively isolated units (Ragge & Reynolds, 1998; Heller, 2006 and others). Contrary to this assumption, in the present study the male calling song appeared to be the most uninformative character source amongst the three data sources. Some species exhibit certain differences that can be used as auxiliary characters to delimit species. Sometimes, differences in songs contradicted the grouping pattern suggested by other character sources. For example, the Tukey's test applied to the impulse period suggested that the Kütahya, Aydın, Balkan, Olympos, and Demre populations constitute a separate cluster. Each of the first three populations represents a distinct species. Both the Olympos and Demre populations also represent separate species, but genetic data do not support this clustering. However, this may not be exceptional because divergence in song may be slow in the case of allopatric speciation (Heller, 2006; Çıplak, Heller & Willemse, 2009). The species of the *P. luschani* group are allopatric; our data indicate a divergence in allopatry. Possibly, this kind of diversification pattern is connected with slow divergence, resulting in poorly differentiated song within the *P. luschani* group.

The morphological variation pattern at group level suggests, at least partially, a different taxonomic scheme than that based on song pattern and (sometimes) than that based on molecular phylogeny. Some structures are invariable and some are variable among individuals of the same population. For example, contrary to some other species group in the genus (Chobanov & Heller, 2010), the number of stridulatory pegs is very variable, both at population and at group level, ranging from 89 to 197. The number is relatively low in populations occupying basal branches of the phylogenetic tree and higher in those in the crown group and this indicates a phylogenetically gradual increase. However, when all populations were considered together the variation in peg number overlapped between populations; thus the number of pegs does not support any logical grouping. This is the case for some other characters as well, such as the width of the fastigium, the elevation of the metazona, the expansion of the lamella, and the length/structure of the ovipositor. The metric characters are also uninformative taxonomically (Table S1), although we did not carry out any geometric analysis. Contrary to these, the structure of the male cerci and male subgenital plate are variable and offer diagnostic characters to be used in establishing a phenotypic key to species. However, a close consideration of variation in these two structures indicates a more complex case. Evaluation of character states defined from these two structures in the light of phylogeny suggests that they are not homologous (Fig. 86). For example, the Balkan and Kütahya populations share a male subgenital plate without incision, but according to the molecular phylogeny both species gained it independently. This is also the case for the curvature of the margins of incision (the proximally divergent and apically convergent state is shared by *P. ledereri*, *P. tuncayi*, and some members of *P. luschani*) and the number of cercal denticles (presence of a single denticle in *P. luschani birandi* and in some populations of *P. l. chobanovi*, and presence of two denticles in *P. ledereri*, *P. tuncayi*, and some members of *P. luschani*). Thus, definition of characters from these structures, as is common for the genus, requires caution.

According to the integrated data, the geographically discrete İzmir, Balkan, Aydın, Kütahya, and Balıkesir populations fit the criteria of the biological and phylogenetic species concepts in addition to being morphospecies. Therefore, each deserves to be considered as a distinct species. However, the populations in the southern part of the generic range (Antalya and Muğla Provinces of Turkey) present a more complex case, both in morphology and in DNA data. According to cercal morphology, 13 populations in this part of

the range constitute three distinct units (named MU-1, MU-2, and MU-3). MU-1 includes the Termessos, Bakırlıdağ, Tahtalıdağ, Kemer, and Olympos populations located in the east (see Figs 43–47). MU-2 contains the Patara, Kalkan, and Demre populations occupying lowlands in the central part of the Antalya + Muğla range (see Figs 40–42). MU-3 is represented by the Akdağ, Erentepe, Eşen1, Eşen2, and Eşen3 populations, located in the west/north-west of this range (see Figs 35–39). MU-1 is also somewhat definable by the incision of the male subgenital plate whereas MU-2 and MU-3 are not. However, a phylogeny based on the DNA sequences of the two markers indicates another pattern that is partially congruent with that obtained from cercal morphology (the COI sequences from Akdağ and Erentepe were not available to be included in the concatenated data set). However, some genetic data for these populations are given in Kaya *et al*., 2012b). The monophyly of these three morphounits is well supported by the phylogenetic tree, which includes two main phylogroups (named PG-1 and PG-2; Fig. 85). Of these two, PG-1 contains all the haplotypes from the members of MU-1, and a sister group including an infraclade of six haplotypes unique to the Demre population belonging to MU-2. Omitting the Demre population, the phylogenetic tree suggests MU-1 to be a distinct phylogenetic species. The monophyly of PG-2 is well supported by high bootstrap/posterior probability values and the group includes haplotypes only from the populations in MU-2 and MU-3. As in PG-1, three haplotypes unique to the Demre population constitute an infraphylogroup at the base of PG-2. The five haplotypes unique to the Eşen3 population from MU-3 constitute a phylogroup next to the basal Demre phylogroup in PG-2. The relationships of the remaining haplotypes are more confusing and their monophyly received low bootstrap/posterior probability values. All haplotypes from Eşen1 and Eşen2, both belonging to MU-2, constitute a single internal phylogroup. The haplotypes from Patara and Kalkan, which are in MU-2, constitute two independent infraphylogroups. One of a total of ten haplotypes unique to the Demre population also occurs together with haplotypes of the Kalkan and Demre populations. This phylogenetic pattern indicates a conflict between morphological and genetic data, and makes a taxonomic decision difficult. It also can be concluded that the Demre phylogroups merge PG-1 and PG-2 and the Eşen3 phylogroup merges the infraphylogroups in PG-2.

Data related to the genetic structure of these populations by Kaya *et al*. (2012b) present some other important aspects. The pairwise F_{ST} values of these populations (Akdağ, Erentepe, Eşen1, Eşen2, Eşen3, Patara, Kalkan, and Demre) are very high, in general indicating a considerable amount of fixed genetic differences. However, the F_{ST} values between pairs from Akdağ, Erentepe, Eşen1, and Eşen2, all from MU-3, are relatively low. Although Eşen3 has cerci specific to the MU-3 group, the F_{ST} values between Eşen3 and each of the above-mentioned four populations are very high. The pairwise F_{ST} value between the Kalkan and Patara populations is very low; however, the values between these two and Demre are very high, thus contradicting the uniqueness of MU-2. Additionally, the F_{ST} values for the population pairs in MU-1 are very low.

Judging from the combined genetic data presented here and in Kaya *et al*. (2012b), there are three plausible statements that can be made in the absence of a taxonomic decision. First, a complex speciation pattern occurs in this small area (roughly 120×70 km) in south-western Anatolia. Second, there are five genetically distinct units in the Antalya + Muğla complex: (1) Termessos, Bakırlıdağ, Tahtalıdağ, Kemer, plus Olympos; (2) Eşen1, Eşen2, Akdağ, plus Erentepe; (3) Eşen3; (4) Kalkan and Patara; and (5) Demre. Third, the molecular phylogenetic pattern is inconsistent with the geographical-genetic structuring. This indicates that the evolutionary histories of the markers studied are different from those of the populations (Edwards, 2009). This inconsistency, as a working hypothesis, may be because of introgression events between partially diverged populations, either in the past or at present. The existing data do not suggest any gene flow at present as each population has only its own unique haplotypes, in addition to the high F_{ST} values (Kaya *et al*., 2012b). Additionally, Kaya *et al*. (2012b) estimated that the Demre population started diverging 546 Kyr ago. Therefore, if there has been an introgression it should have happened around or before this time, which might have led to paraphyly.

Combining all these data makes taxonomic decisions difficult. According to the existing classification, there are two species, *P. luschani* and *P. birandi*. However, our phylogenetic tree suggests these species to be paraphyletic, and MU-3 remains outside of these formerly described morphospecies. Furthermore, the phylogenetic tree does not suggest any robust infraphylogroups, corresponding to either phenotypic units or geographical fragmentation, to be considered in a further taxonomic rearrangement. By contrast, the morphological data present robust clusters that correspond well to geographical ranges. Although the genetic units described above appear to be genetically isolated, the potential for reproduction between these populations/units cannot be excluded. Thus, the most plausible assumption is to consider the entire Antalya + Muğla range to be inhabited by a single 'chain species' including three 'chain links', or

subspecies, each corresponding to one morphological unit (see Figs 1 and 86 for taxonomic revision).

CONCLUSIONS

An important objective of systematic biology is to define and name natural units. This requires inclusive data from present populations and reconstruction of their evolutionary history. The data integrated from song, morphology, and matrilineal DNA suggested a considerable revision of the classification of the *P. luschani* group. The traditional taxonomy of the genus *Poecilimon* is mainly based on morphology. Our study has shown, however, that the characters defined from these structures may not be homologous. Therefore, these morphological characters should be used with caution. Despite this, the male genitalia offer useful diagnostic characters, in contrast to some other species-groups of the genus (Kaya *et al*., 2012a). Male calling song, which has recently been widely applied in systematics, is not variable in this case, possibly because of speciation through allopatry (Heller, 2006; Çıplak *et al*., 2009). An important issue concerning the *P. luschani* complex in the southern part of the group's range is the considerable radiation that is present in a small area in the west part of Antalya Province. A similar case was also observed within salamanders in the *Lyciasalamandra luschani* complex (Veith *et al*., 2008). Such radiations are possibly caused by the geographical/tectonic evolution of this area (Kaya *et al*., 2012b). A radiation mediated by geographic evolution of the area indicates further aspects for evolution of *Poecilimon* in general, and for the *P. luschani* group specifically. For example, all haplotypes are unique to the populations they observed but they mix on the phylogenetic tree. Possibly this is a result of hybridization of partially diverged populations. This assumption offers two intriguing hypotheses to be tested. First, the current data and our observations do not indicate any actual gene flow, and the assumption that these hybridization events have occurred in the past needs to be tested. Kaya *et al*. (2012b) presented molecular clock estimations for the time of divergence of these populations based on a single marker gene. This assumption still needs to be tested using different markers and longer DNA sequences (Kaya *et al*., unpubl. data). All these indicate that hybridization in the history of the *Poecilimon* lineage may not be rare and this should be considered in future studies on the genus. Second, if partially diverged populations can reproduce as stated above, then the question to be answered is 'how much morphological divergence is required for reproductive isolation in *Poecilimon*?' Analysing morphology geometrically in correlation with population genetic data may provide some clues.

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AUTHOR CONTRIBUTIONS

B. Ç. conceived the ideas; Z. B., S. K., and B. Ç. collected the specimens and recorded the songs; Z. B. and S. K. conducted laboratory studies and produced the data; S. K., Z. B., and B. Ç. analysed the data; and B. Ç. led the writing.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Some measurements per population of the *Poecilimon luschani* group [in mm, minimum–maximum, means, and SD] for males (A) and females (B).