Evolution and systematics of Green Bush-crickets (Orthoptera: Tettigoniidae: *Tettigonia*) in the Western Palaearctic: testing concordance between molecular, acoustic, and morphological data

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Abstract The genus *Tettigonia* includes 26 species distributed in the Palaearctic region. Though the Green Bush-crickets are widespread in Europe and common in a variety of habitats throughout the Palaearctic ecozone, the genus is still in need of scientific attention due to the presence of a multitude of poorly explored taxa. In the present study, we sought to clarify the evolutionary relationships of Green Bush-crickets and the composition of taxa occurring in the Western Palaearctic. Based on populations from 24 disjunct localities, the phylogeny of the group was estimated using sequences of the cytochrome oxidase subunit I (COI) and the internal transcribed spacers 1 and 2 (ITS1 and ITS2). Morphological and acoustic variation documented for the examined populations and taxa was interpreted in the context of phylogenetic relationships inferred from our genetic analyses. The trees generated in the present study supported the existence of three main lineages: “A”—composed of all sampled populations of *Tettigonia viridissima* and the *Tettigonia vaucheriana* complex, “B”—comprising *Tettigonia caudata*, *Tettigonia uvarovi*, and the *Tettigonia armeniaca* complex, and “C”—consisting of *Tettigonia cantans*. The present study provides the first phylogenetic foundation for reviewing the systematics of *Tettigonia* (currently classified mostly according to morphological characteristics), proposing seven new synonymies.

Keywords *Tettigonia* · mtDNA · rDNA · Phylogeny · Bioacoustics

Introduction

Genus *Tettigonia* Linnaeus, 1758 presently includes 26 recognized species (Eades et al. 2016) distributed in the Palaearctic ecozone and belongs to the long-horned orthopterans or the bush-crickets (Ensifera, Tettigonioida). *Tettigonia*, popularly known as the Green Bush-crickets, are generally large green orthopterans with moderately slender body and legs and well-developed wings that inhabit the plant cover searching for their food (usually smaller insects or plant tissues). *Tettigonia* is one of the most notable Old World example with two centers of diversity: one in the Mediterranean–Pontic region (see, e.g., Ramme 1951; Pinedo 1985; Chobanov et al. 2014) and another in the Japanese archipelago (see Ichikawa et al. 2006; Kim et al. 2016). Both regions are characterized by a similar number of endemic taxa and insufficient knowledge regarding the taxonomy and systematics of Green Bush-crickets (Ichikawa et al. 2006; Chobanov et al. 2014; own unpublished data).

Despite the fact that several species of Green Bush-crickets are quite well known and have been the subject of detailed neuro-ethological studies (e.g., Zhantiev and Korsunovskaya 1978; Schul 1998), others remain poorly known from single specimens, and even nowadays, the discovery of new species continues (Ogawa 2003; Ichikawa et al. 2006; Chobanov et al. 2014; Storozhenko et al. 2015). Data on the systematics of this genus involve piecemeal morpho-acoustic studies conducted for geographically restricted areas or focused on...
morphological groups of species (e.g., Heller 1988; Rhe 2013; Chobanov et al. 2014). Our recent morphological and acoustic studies on Tettigonia, concentrated on the Western Palearctic, revealed a number of conflicts within the published data when trying to identify certain populations and develop hypotheses about the systematics of the group (own unpublished data). The latter further supports the need to use new markers to test the systematic position of some taxa and unravel the evolutionary history of this genus.

The evolution of acoustic communication systems in orthopterans has led to high levels of acoustic specialization. As acoustic signals are important for intraspecific and sex recognition as well as for interspecific isolation (Paterson 1985; Hochkirch and Lemke 2011), they are of great significance for studying the processes underlying evolutionary radiation. Acoustic diversity within bush-cricket genera varies from very low with a more or less uniform pattern of the male calling song (cf. Heller 2006; Čiplak et al. 2009) to very high with a great variety of song types, especially in sympatric taxa, even within groups of closely related species (cf. Heller 1988, 1990, 2006; Chobanov and Heller 2010, etc.).

In Tettigonia, song differences between species (especially well expressed in sympatric taxa) may express in different syllable arrangement and repetition rate, echeme length, and duty cycle. Some differences are also found in the carrier frequency of the song. In some species (e.g., Tettigonia cantans), females are not very sensitive to the conspecific structure of the song and thus may respond to heterospecific males (Schul et al. 1998), while in other species (i.e., Tettigonia caudata), females rely on the minimum duty cycle of the echemes, thus neglecting the fine song structure (Schul 1998). In Tettigonia viridissima, song recognition based on temporal clues has been shown to be more complicated. Here, females evaluate the pause within disyllabic echemes and respond only to the species-specific echeme structure (Schul 1998).

In the present study, we aim to evaluate phylogenetic relationships within Tettigonia. We based our study on a genetic dataset that was used as a basis for mapping acoustic and morphological characters in an attempt to track the evolutionary paths of the acoustic communication in this genus. For these purposes, sequences of the mitochondrial cytochrome oxidase subunit I (COI) gene and the nuclear internal transcribed spacers 1 and 2 (ITS1 and ITS2) were used that have previously been widely employed in phylogenetic studies of grasshoppers (e.g., Cooper et al. 1995; Chapeco and Litiengerber 2002) and bush-crickets (Ullrich et al. 2010; Allegrucci et al. 2011; Boztepe et al. 2013; Čiplak et al. 2015). The DNA sequences selected for the present study have different modes of evolution and inheritance history, and thus, they may reveal different aspects of the speciation history of the examined lineages.

In bush-crickets, the songs of closely related species, especially those that speciated in allopatry, usually have a lineage-specific amplitude–temporal pattern that enables recognition for systematic purposes and for drawing conclusions about paths of speciation (e.g., Heller 1990, 2006; Chobanov and Heller 2010). In Tettigonia, differences between species have been observed in the time and frequency domains, while particular song-recognizing mechanisms may depend on the geographic and ecological preferences of the species (Schul 1994; Schul et al. 1998). Hence, we use the male calling song as an additional clue for evolutionary assumptions as well as for testing the variation of song types according to genetic or morphological units. Thus, the present study indirectly vindicates the significance of acoustic recognition systems and song specialization patterns in this genus.

**Material and methods**

**Taxon sampling and morphological identification**

The species used in this study and their sampling localities are presented in Table 1 and Fig. 1. This dataset contains 66 Tettigonia specimens from 33 disjunct localities/populations and five outgroup taxa representing two tettigonid subfamilies (Tettigoniiinae: Amphiestris Fieber, 1853 (Tettigonini), Onconotus Fischer von Waldheim, 1839, Paratlanticus Ramme, 1939, Platycleis Fieber, 1853; Saginae: Saga pedo (Pallas, 1771)).

The properties of the chosen DNA fragments (high amount of interspecific variation providing good phylogenetic signal at a generic level but some risk of false results at a higher systematic level due to convergencies and phenomena like long branch attraction), we choose taxa that, according to published data, are closely related and/or have close ancestral position to Tettigonia (in the case of Saginae) (e.g., Gorochov 1995; Song et al. 2015).

Used samples of Tettigonia have been identified using original descriptions and published reviews (e.g., Bolivar 1914; Chopard 1943; Ramme 1951; Harz 1969; Massa 1998; Chobanov et al. 2014 and references therein). All specimens listed in Table 1 were morphologically related to existing taxa based on available literature and museum specimens. Apart from own material, the following specimens from public collections that refer to the studied taxa were studied:

* Tettigonia acutipennis Ebner, 1946—male, holotype, “Kleinasien 1914 | Marasch, Tölg. | coll. R. Ebner” (Naturhistorisches Museum Wien (NHMW)); male, Hakkari (the Natural History Museum London (NHM)); two males, “Turkey: | Gumusane, | Soganli Gecidi, 7-7500’.” | 25. vii.
| Voucher ID | Species | Location | Geographical position | GenBank accession nos. |
|-----------|---------|----------|-----------------------|-----------------------|
| out1      | *Saga pedo* (Pallas, 1771) | Bulgaria: E Stara Planina Mts, Zeravna vill, 900 m | 42.8427 N 26.4519 E | KT936310 KT823256 KT823233 |
| out2      | *Platyceles (Squamiana)* sp. | Turkey: Zara-Sudehi road, 1650 m | 39.5556 N 37.9161 E | KT936311 KT358278 KT358337 |
| out3      | *Onconus servillei* Fisher von Waldheim, 1846 | Bulgaria: Kapitan Dimitrovo vill. | 43.85 N 27.7 E | KT936312 KT358279 KT358338 |
| out4      | *Amphiesistis baetica* (Rambur, 1838) | Spain: Cultivo hija de otra de los Barrios, Cadiz | 36.32 N 6.17 W | KT936313 KT358280 KT358339 |
| out5      | *Paratlanticus assimilis* (Uvarov, 1926) | Russia: Primorsky Krai, Lazovskii Natural Reserve, Korpad | 43.55 N 133.57 E | KT936314 KT358281 KT358340 |
| tam1a     | *T. armeniaca* complex | Turkey: Horasan-Agri, Saclidag Pass, 2160 m | 39.8747 N 42.3856 E | KT358223 KT358282 KT358341 |
| tam1b     | *T. armeniaca* complex | Turkey: Horasan-Agri, Saclidag Pass, 2160 m | 39.8747 N 42.3856 E | KT358224 KT358283 KT358342 |
| tam2a     | *T. armeniaca* complex | Turkey: Horasan-Agri, Savsat-Ardahan road, 1630 m | 41.2312 N 42.4338 E | KT358225 KT358284 KT358343 |
| –         | *T. armeniaca* complex | Turkey: Pulmur, 1818 m | 39.5134 N 40.883 E | – – – |
| –         | *T. armeniaca* complex | Turkey: Ispir, 1900 m | 39.8365 N 45.6933 E | – – – |
| –         | *T. armeniaca* complex | Armenia: above Djemruk, 2400 m | 39.6933 N 45.7080 E | – – – |
| –         | *T. armeniaca* complex | Armenia: E Saravan, 2290 m | 39.6831 N 45.3027 E | – – – |
| –         | *T. armeniaca* complex | Armenia: Shora near Sevan Lake, 1965 m | 40.5039 N 44.6613 E | – – – |
| –         | *T. armeniaca* complex | Armenia: Lernmontovo vill., 1850 m | 40.9947 N 43.8879 E | – – – |
| tca1      | *T. cantans* (Fuessly, 1775) | Hungary: Borzsony Mts | 47.55 N 19.00 E | KT358226 KT358284 KT358344 |
| tca2a     | *T. cantans* (Fuessly, 1775) | Poland: OPN, Dolina Sapowska | 50.1236 N 19.4854 E | KT358227 KT358285 KT358345 |
| tca2b     | *T. cantans* (Fuessly, 1775) | Poland: OPN, Dolina Sapowska | 50.1236 N 19.4854 E | KT358228 KT358286 KT358346 |
| tca2c     | *T. cantans* (Fuessly, 1775) | Poland: OPN, Dolina Sapowska | 50.1236 N 19.4854 E | KT358229 KT358287 KT358347 |
| tca2d     | *T. cantans* (Fuessly, 1775) | Poland: OPN, Dolina Sapowska | 50.1236 N 19.4854 E | KT358230 KT358288 KT358348 |
| tca2e     | *T. cantans* (Fuessly, 1775) | Poland: OPN, Dolina Sapowska | 50.1236 N 19.4854 E | KT358231 KT358289 KT358349 |
| tca2f     | *T. cantans* (Fuessly, 1775) | Poland: OPN, Dolina Sapowska | 50.1236 N 19.4854 E | KT358232 KT358290 KT358350 |
| tca3a     | *T. cantans* (Fuessly, 1775) | Romania: Lepsa | 45.57 N 26.34 E | KT358244 KT358291 KT358351 |
| tca3b     | *T. cantans* (Fuessly, 1775) | Romania: Lepsa | 45.57 N 26.34 E | KT358245 KT358292 KT358352 |
| tca3c     | *T. cantans* (Fuessly, 1775) | Romania: Lepsa | 45.57 N 26.34 E | KT358247 KT358293 KT358353 |
| tca3d     | *T. cantans* (Fuessly, 1775) | Romania: Lepsa | 45.57 N 26.34 E | KT358246 KT358294 KT358357 |
| tct3      | *T.cantans* (Fuessly, 1775) | China: Xinjiang, near Tianshi (or Tienchi/Heaven Lake) in Tianshan Mts, near mountain of Bogda Feng, 2000 m | 43.9 N 88.117 E | KT358235 KT358297 KT358357 |
| tct1      | *T. cantans* (Fuessly, 1775) | Kyrgyzstan: Isik Ata | 42.53 N 74.51 E | KT358233 KT358295 KT358355 |
| –         | *T. caudata* (Charpentier, 1842) | Turkey: Ispir, 1900 m | 40.583 N 40.883 E | – – – |
| Voucher ID | Species                  | Location                                                                 | Geographical position | GenBank accession nos. |
|-----------|--------------------------|--------------------------------------------------------------------------|-----------------------|------------------------|
| –         | *T. caudata* (Charpentier, 1842) | Armenia: Gorhajk near Vorotan Dam, 2120 m                             | 39.68521 N 45.78486 E | – – –                  |
| tet2      | *T. caudata* (Charpentier, 1842) | Bulgaria: Byala                                                          | 43.4717 N 23.7696 E  | KT358234 KT358296 KT358356 |
| tdm       | *T. uvarovi* Ebner, 1946 | Russia: Primorsky Krai, Ussuri River, Gornye Kluchi (Shamkovka)         | 45.20 N 134.40 E     | KT358236 KT358298 KT358358 |
| tmo1a     | *T. cf. longealata*        | Morocco: S Ajabo, 1360 m                                               | 33.0659 N 5.4086 W   | KT358254 KT358299 KT358359 |
| tmo1b     | *T. cf. longealata*        | Morocco: S Ajabo, 1360 m                                               | 33.0659 N 5.4086 W   | KT358252 KT358300 KT358360 |
| tmo1c     | *T. cf. longealata*        | Morocco: S Ajabo, 1360 m                                               | 33.0659 N 5.4086 W   | KT358253 KT358301 KT358361 |
| tmo2a     | *T. cf. longealata*        | Morocco: NW Khenifra, 1100 m                                           | 33.1377 N 5.9235 W   | KT358261 KT358302 KT358362 |
| tmo2b     | *T. cf. longealata*        | Morocco: NW Khenifra, 1100 m                                           | 33.1377 N 5.9235 W   | KT358262 KT358303 KT358363 |
| tmo2c     | *T. cf. vaucheriana*       | Morocco: NW Khenifra, 1100 m                                           | 33.1377 N 5.9235 W   | KT358248 KT358304 KT358364 |
| tmo3a     | *T. cf. vaucheriana*       | Morocco: near El Kebab, 966 m                                         | 32.7569 N 5.6451 W   | KT358257 KT358305 KT358365 |
| tmo3b     | *T. cf. vaucheriana*       | Morocco: near El Kebab, 966 m                                         | 32.7569 N 5.6451 W   | KT358251 KT358306 KT358366 |
| tmo3c     | *T. cf. vaucheriana*       | Morocco: near El Kebab, 966 m                                         | 32.7569 N 5.6451 W   | KT358249 KT358307 KT358367 |
| tmo3d     | *T. cf. vaucheriana*       | Morocco: near El Kebab, 966 m                                         | 32.7569 N 5.6451 W   | KT358250 KT358308 KT358368 |
| tmo3e     | *T. cf. vaucheriana*       | Morocco: near El Kebab, 966 m                                         | 32.7569 N 5.6451 W   | KT358242 KT358309 KT358369 |
| tmo4a     | *T. cf. vaucheriana*       | Morocco: SE Thar Es-Souk, 650 m                                       | 34.6585 N 4.2417 W   | KT358258 KT358310 KT358370 |
| tmo4b     | *T. cf. vaucheriana*       | Morocco: SE Thar Es-Souk, 650 m                                       | 34.6585 N 4.2417 W   | KT358260 KT358311 KT358371 |
| tmo5a     | *T. cf. viridissima*       | Morocco: S Ain Zora, 835 m                                            | 34.5708 N 3.6657 W   | KT358256 KT358312 KT358372 |
| tmo5b     | *T. cf. viridissima*       | Morocco: S Ain Zora, 835 m                                            | 34.5708 N 3.6657 W   | KT358263 KT358313 KT358373 |
| tmo5c     | *T. cf. viridissima*       | Morocco: S Ain Zora, 835 m                                            | 34.5708 N 3.6657 W   | KT358255 KT358314 KT358374 |
| tmo5d     | *T. cf. viridissima*       | Morocco: S Ain Zora, 835 m                                            | 34.5708 N 3.6657 W   | KT358265 KT358315 KT358375 |
| tmo5e     | *T. cf. viridissima*       | Morocco: S Ain Zora, 835 m                                            | 34.5708 N 3.6657 W   | KT358264 KT358316 KT358376 |
| tmo6a     | *T. cf. vaucheriana*       | Morocco: Bouchfia W Taza, 675 m                                       | 34.0830 N 4.2996 W   | KT358239 KT358317 KT358377 |
| tmo6b     | *T. cf. vaucheriana*       | Morocco: Bouchfia W Taza, 675 m                                       | 34.0830 N 4.2996 W   | KT358238 KT358318 KT358378 |
| tmo7a     | *T. sp. aff. viridissima*  | Morocco: E Azrou, 1520 m                                              | 33.4259 N 5.1926 W   | KT358268 KT358319 KT358379 |
| tmo7b     | *T. sp. aff. viridissima*  | Morocco: E Azrou, 1520 m                                              | 33.4259 N 5.1926 W   | KT358266 KT358320 KT358380 |
| tmo7c     | *T. sp. aff. viridissima*  | Morocco: E Azrou, 1520 m                                              | 33.4259 N 5.1926 W   | KT358267 KT358321 KT358381 |
| tmo7d     | *T. sp. aff. viridissima*  | Morocco: E Azrou, 1520 m                                              | 33.4259 N 5.1926 W   | KT358243 KT358322 KT358382 |
| tmo8a     | *T. cf. vaucheriana*       | Morocco: Tilougguite Pass, 1570 m                                     | 32.0852 N 6.3003 W   | KT358241 KT358323 KT358383 |
| tmo8b     | *T. cf. vaucheriana*       | Morocco: Tilougguite Pass, 1570 m                                     | 32.0852 N 6.3003 W   | KT358240 KT358324 KT358384 |
| tmo9      | *T. cf. vaucheriana*       | Morocco: SW Derrada, 400 m                                           | 35.0896 N 5.3074 W   | KT358259 KT358325 KT358385 |
Table 1 (continued)

| Voucher ID | Species               | Location                        | Geographical position | GenBank accession nos. |
|------------|-----------------------|---------------------------------|-----------------------|------------------------|
|            | T. cf. vauchariana    | Morocco: N Fes, 20 m            | 34.47138 N 5.38195 W  | – – –                  |
| tvi1       | T. viridissima        | Kyrgyzstn: Ata Arche           | 42.3842 N 74.2848 E   | KT358273 KT358326 KT358386 |
| tvi2       | T. viridissima        | Spain: Isik Ata, Montes de Toledo | 39.3045 N 04.4353 W | KT358237 KT358327 KT358387 |
| tvi3a      | T. viridissima        | Ukraine: Donetsk Region         | 48.14 N 37.74 E       | KT358274 KT358328 KT358388 |
| tvi3b      | T. viridissina        | Ukraine: Donetsk Region         | 48.14 N 37.74 E       | KT358275 KT358329 KT358389 |
| tvi3c      | T. viridissina        | Ukraine: Donetsk Region         | 48.14 N 37.74 E       | KT358276 KT358330 KT358390 |
| tvi3d      | T. viridissina        | Ukraine: Donetsk Region         | 48.14 N 37.74 E       | KT358277 KT358331 KT358391 |
| tvi4       | T. viridissina        | Turkey: Yanikcay, 1920 m        | 38.2547 N 42.8978 E   | KT358269 KT358332 KT358392 |
| tvi5a      | T. viridissina        | Bulgaria: Varna, Botanical Garden | 43.2374 N 28.003 E | KT358272 KT358333 KT358393 |
| tvi5b      | T. viridissina        | Bulgaria: Haskovo, Perperikon Ruins | 41.715 N 25.4657 E | KT358270 KT358334 KT358394 |
| tvi5c      | T. viridissina        | Bulgaria: Dobrich, Bola Bay     | 43.3838 N 28.4715 E   | KT358271 KT358335 KT358395 |

All sequences are submitted to the NCBI GenBank

1960. | K. M. Guichard | & D. H. Harvey. | B.M. 1960-364” (NHM).

*Tettigonia armeniaca* stat. nov.—two females (not identified), “Ibisu (Gov. Eriwan)” (NHMW); female (not identified), “Bakurian” [Georgia] (NHMW); male (not identified), “Kasikoparan” [Turkey] (NHMW); male (not identified), “Soganli Gecidi” [Turkey] (NHMW)

*T. cantans* (Fuessly, 1775)—male, “Karnten 1927 | Vellacher Toschna, 3. ix. | coll. R. Ebner” (NHMW)

*T. caudata* (Charpentier, 1842)—male, “Jasenova, Jugoslawien” (NHMW); male, “Pirot” (NHMW); male, “Walouiki, R. m. | Velitchkovsky” [Ukraine] (NHMW); male, “Gegend v. Wien | Von Hn. Turk | Coll. Br. v. W.” (NHMW); male, “Eriwan-Tiflis” (NHMW); male, “Poin-Shaval, Elbrus | Funke leg.” (NHMW); male, “Persia s.- | Elburs | Rehne-Demavend | ca. 2700–3600 m | 20–27. vii. 1936” (NHMW); male, “Sabzawaran | 12. v. 50 / Ø stern. | Iran exped. 1950” (NHMW); male, “Afghanistan | Chira | Hr. v. Pleson | Coll. Br. v. W.” (NHMW)

*Tettigonia lozanoi* (Bolivar, 1914)—male, Aguelman (NHM)

*Tettigonia uvarovi* Ebner, 1946—male, holotype, Siberia (NHMW)

*Tettigonia vauchariana* Pictet, 1888—male, “Morocco, Azrou, 1200–1400 m, 28. v.-1. vi. 1930. Ebner” (NHMW); female, “Atlas, Asni | 1200 m, 23–30. vi.’ 30. | Ebner” (NHMW)

Fig. 1 Map showing the sampling sites for *Tettigonia*
According to the state of knowledge, taxonomic recognition and morphological similarities, we divided *Tettigonia* taxa into three groups:

1. **Commonly recognized taxa**: well-studied *T. viridissima* (Linnaeus, 1758), *T. caudata* (Charpentier, 1842) s. str., and *T. cantans* (Fuessly, 1775) of the Western Palaearctic.

2. **Taxa that have been recently described and only partially studied**: *T. dolichoptera maritima* Storozenko, 1994 = *T. uvarovi* Ebner, 1946 (see Storozenko et al. 2015). Described from the Russian Far East, this subspecies was thought to differ in the length of the pronotum and tegmina from the nominotypical form from South Korea. However, many South Korean specimens are similar to *T. uvarovi* in their dimensions (Rhee 2013), and only the most long-winged ones are now assumed to represent *T. dolichoptera* (Storozenko et al. 2015). In any case, this representative of the Eastern Palaearctic fauna may provide clues as to the relationships and phylogeographic connections of the east and west Palaearctic lineages of *Tettigonia*.

3. **Poorly known sibling species termed here as follows**:

   (a) The *T. armeniaca* complex. Upon sampling of a specific shorter-winged *Tettigonia*, resembling *T. caudata* in terms of many morphological features, which occurs from Eastern Anatolia to Southern Caucasus and possibly further to Kyrgyzstan (own unpublished information), we failed to definitely outline morpho-units that fit each of the taxa *T. caudata armeniaca* Tarbinsky, 1940 (presently a synonym of *T. caudata* s. str.), *T. acutipennis* Ebner, 1946, and *Tettigonia turcica* Ramme, 1951. This complex has been previously defined by weak (but present) black coloration at the base of ventral post-femoral spines and more or less shortened wings.

   (b) The *T. vaucheriana* complex. A multitude of forms has been described from northwestern Africa, differing mostly in size, length of the forewings, and relative width of the scapus (front border of the vertex bordering the frons) (*T. vaucheriana* Pictet, 1888 = *Tettigonia maroccana* Bolivar, 1893, syn.; *T. lozanoi* (Bolivar, 1914); *Tettigonia langealata* Chopard, 1937; *Tettigonia krugeri* Massa, 1998). Some of them resemble *T. viridissima*, which has been recorded from North Africa. Upon extensive sampling in Morocco and comparison of museum specimens, we observed a significant overlap between populations, with extreme examples ranging from a slender body shape with long wings (*T. viridissima* type) to a stout body with long wings (*T. langealata*) or short wings (*T. vaucheriana, *T. lozanoi*) as this has already been noted by Pinedo (1985).

**Genomic sampling**

DNA extraction was performed using NucleoSpin® Tissue Kits (Macherey-Nagel, Düren, Germany) according to the standard protocol. DNA was used as a PCR template to amplify four genetic markers, including mitochondrial and nuclear genes. These were (1) partial cytochrome c oxidase subunit I (COI), (2) partial sequences of the first internal transcribed spacer (ITS1) of the nuclear ribosomal gene cluster, and (3) partial sequences of the second internal transcribed spacer (ITS2) of the nuclear ribosomal gene cluster. The COI gene was amplified with the primers LCO [5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′] and HCO [5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′] (Folmer et al. 1994). For nuclear DNA, ITS1 regions were PCR amplified using the primers 18S-28S [5′-TAG AGG AAG TAA AAG TCG-3′] (Weekers et al. 2001) and ITS-R1 [5′-CAT TGA CCC ACG AGC C-3′] (Ulrich et al. 2010), whereas ITS2 regions were amplified using the primers ITS2-28S [5′-GGA TCG ATG AAC AAC G-3′] and 28S-18S [5′-GCT TAA ATT CAG CGG-3′] (Weekers et al. 2001).

PCR was performed in 30-μL reaction volumes, which comprised 10 pmol of each primer, 10 mM of each dNTP, 25 mM MgCl₂, 2.5 μL 10× PCR buffer, 1 U Taq polymerase (EURx, Gdańsk, Poland), and sterile H₂O.

To amplify COI, we used the following PCR protocol: 35 cycles at 95 °C for 50 s, 50 °C for 1 min and 72 °C for 1 min, with the final extension at 72 °C for 6 min. PCR amplification of ITS1 and ITS2 consisted of 25 cycles at 95 °C for 1 min, 52 °C for 1 min 50 s, and 72 °C for 2 min, with the final extension at 72 °C for 10 min. PCR products were purified with the Gene MATRIX PCR/DNA Clean-Up Purification Kit (EURx, Poland, following the standard protocol) and sequenced directly. Purified DNA was sequenced in both directions using the same primers as for PCR and the Big Dye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer’s instructions.

**Phylogenetic analyses**

DNA sequences were edited and compiled using Muscle (Edgar 2004). To test for pseudogenes, coding sequences (COI) were translated into protein with MEGA 6 (Tamura et al. 2013) using the standard invertebrate mitochondrial genetic code. No stop codons were observed. Nucleotide composition homogeneity within genes was tested with PAUP* 4.0b10 (Swofford 2002). Mean net genetic distances among clades were calculated using MEGA 6 (Tamura et al. 2013).
within the Kimura two-parameter model (K2P; standard errors (SE) were obtained by bootstrapping with 1000 replicates).

Two different phylogenetic methods, maximum likelihood (ML) and Bayesian inference (BI), were used to infer evolutionary relationships. Following independent analysis for each COI, ITS1, and ITS2 dataset, the COI and ITS1 + ITS2 datasets were concatenated and further analyses were performed using the combined matrix. Evolutionary models for each dataset and combined dataset were selected using MrModeltest 2.3 (Nylander 2004) with the Akaike information criterion (Akaile 1974). Support for nodes in ML analysis was assessed with non-parametric bootstrapping (BP) using Phylm (Guindon and Gascuel 2003) with 1000 pseudoreplicates and ten random BioNJ trees, and parameters were estimated from each dataset within the model selected for the original dataset. BI of phylogenetic relationships using Metropolis-coupled Monte Carlo Markov chain (mcmc) simulation was performed with MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2001). Posterior probabilities were based on two independent MCM runs, each composed of four chains (three heated chains and one cold chain). The mcmc simulations were run for 10,000,000 generations with sampling every 100 generations. The convergence of analyses was validated by monitoring likelihood values graphically using Tracer (Rambaut and Drummond 2007), and trees prior to stationarity were discarded as burn-in. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PPs). Phylogenetic trees were produced using TreeView (Page 1996) and FigTree software (Rambaut 2008).

Bioacoustic evaluations

Male songs were recorded under different environmental conditions using the following equipment: (1) Knowles BT-1759-000 electret condenser microphone with a sensitivity of −60 ± 3 dB re 1 V/μbar at 1 kHz and with a frequency response roll-off of about 10 kHz and cutoff at over 45 kHz (data combined from Irie 1995 and W. Schulze, FriedrichAlexander-Universität Erlangen-Nürnberg, personal communication), equipped with a custom-made preamplifier connected to a PC through an external soundcard (Transit USB, “M-Audio”) (48/96-kHz sampling rate), used in the lab; (2) Pettersson D500 external microphone with a frequency range corresponding to that of the Pettersson D500x recorder, being between 1 (−6 dB)–2 kHz (−3 dB) and 190 kHz (500-kHz sampling rate) (Lars Pettersson, personal communication), connected to a ZOOM H2 or ZOOM H4 handy recorder (Zoom Corporation) (96-kHz sampling rate), used in captivity and in nature; (3) UHER M645 microphone with a frequency response flat up to 20 kHz connected to a UHER 4200 IC tape recorder; and (4) Bruel and Kjaer 4135 microphone connected to a RACAL store 4DS tape recorder.

The bioacoustic terminology used in this study is as follows (based on Ragge and Reynolds 1998, modified from Chobanov et al. 2014): calling song—the song produced by an isolated male; echeme—a first-order grouping of syllables; echeme duration—the time measured from the beginning of the first to the end of the last syllable; echeme period—the span including an echeme and the following interval; syllable—the sound produced by one opening-and-closing movement of the tegmina; syllable period—the span including a syllable and the following interval, usually measured between syllable peaks; syllable repetition rate—reciprocal of the syllable period (unit Hz = 1/s); diplosyllables = disyllabic echemes—two syllables separated from the neighboring diplosyllables by longer silent intervals than those within each diplosyllable; duty cycle—during singing activity, the proportion of time spent actually singing: echeme duration divided by the echeme period (in T. viridissima duration of echeme sequences divided by duration of acoustic activity); and chirp—an isolated acoustic event regardless of its structure. Temperature during the recordings varied, but for evaluation, we used only temperature-independent structures (duty cycle) and relationships (relationship between temperature-dependent chirp duration and temperature-dependent interval duration). Differences between a daytime song consisting of chirps and a continuous nighttime song as in T. cantans were not observed in T. armeniaca complex nor in any other Tettigonia species.

We concentrate to the poorly studied groups of the here named T. armeniaca complex and T. vaucheriana complex. Altogether, nine continuous recordings under different conditions (temperature, time of the day, different male individual) from six remote localities of males, representing different morphotypes of the T. vaucheriana complex, were studied (see Fig. 2). From the T. armeniaca complex, we studied, respectively, 34 recordings from ten localities (partly represented in Fig. 3). Own data for the rest of the studied taxa were supplemented with published recordings and song measurements. Recordings of T. cantans and T. caudata caudata from Massa et al. (2012) are used for comparative purposes in the figures (see “Results” section).

The recordings used for duty cycle measurements included those made by the present authors as well as recordings from several published sound sources (Grein 1984; Bonnet 1995; Kleuckers et al. 1997; Ragge and Reynolds 1998; Nielsen 2000; Odé and Fontana 2002; Bellmann 2004; Barataud 2007; Roesti and Keist 2009; Massa et al. 2012; Kocarek et al. 2013; Gomboc and Segula 2014) and from the Internet (SYSTAX 2015; data provided by G. Schmidt).

Processing of sound files, measurements, and preparation of oscillograms were performed with Audacity 2.0.3 (Audacity team 1999–2013), BatSound 4.1.4 (Pettersson Electronics and Acoustics AB 1996–2010), and Amadeus II (Martin Hairer; http://www.hairersoft.com).
Results

Phylogenetic reconstruction

COI, ITS1, and ITS2 genes are standard markers used in phylogenetic studies of insects, which in many cases have proved informative on a specific and generic level. We obtained the following fragments: 547 bp for COI, 400 bp for ITS1, and 420 bp for ITS2 (including gaps and variable regions). No indels were observed in the COI fragment.

The congruency of COI, ITS1, and ITS2 \( (p = 0.99) \) allowed for these markers to be combined into a single matrix. The resulting 1367 bp matrix was obtained after alignment and trimming, containing 20% variable and 13% parsimony informative sites. MrModeltest identified the GTR + G model (gamma distribution shape parameter \( G = 0.94; -\ln L = 12,691.23; AIC = 25,400.47 \)) as the best nucleotide substitution model for ML and BI analyses.

Phylogenies reconstructed based on the combined data using ML and Bayesian methods (Fig. 4) showed similar topologies. The tree inferred from COI + ITS1 + ITS2 sequences (Fig. 4) showed that *Tettigonia* taxa are grouped into three main clades. Clade “A” includes all sampled populations of *T. viridissima* and all northwestern African specimens of the taxon referred to in this paper as the *T. vaucheriana* complex. Clade “B” is composed of *T. caudata*, *T. uvarovi*, and what is here referred to as the *T. armeniaca* complex. Clade “C” is composed of all the *T. cantans* samples in our dataset.

The genetic distances between and within clades for all genes are presented in Tables 2 and 3, respectively. Genetic distances between major clades were similar for COI (1–4%) and for ITS (1–3%). The genetic distances between ingroup species were very low for COI (0–2%).

![Oscillograms of the song of the *Tettigonia viridissima* group (1–9) and *T. cantans* (10) recorded at two speeds: 1 T. cf. longealata (MO: Ajabo, \( T = 20 \) °C), 2 T. cf. vaucheriana (MO: N Fes, \( T = 20 \) °C), 3 T. cf. vaucheriana (MO: Bouchkaa W of Taza, \( T = 21 \) °C), 4 T. cf. vaucheriana (MO: Tikouguite Pass, \( T = 23 \) °C), 5 T. cf. vaucheriana and cf. longealata (MO: El Kebab, \( T = 25 \) °C), 6 T. cf. vaucheriana (MO: El Kebab, \( T = 28–30 \) °C), 7 T. cf. viridissima (MO: S Ain Zora, \( T = 22 \) °C), 8 T. cf. viridissima (MO: S Ain Zora, \( T = 25 \) °C), 9 T. viridissima (BG: Sofia, \( T = 27 \) °C), and 10 T. cantans (IT: Val Malene; from Massa et al. 2012, \( T = 15 \) °C).

Scale bar for A is 10 s and for B 2 s.
Bioacoustic evaluation and morphological characters

The three bioacoustically well-distinguished species (see “Introduction” section) belong to three well-outlined clusters in our study. In addition to the previously known acoustic diversity in Central Europe, we found striking examples of variation among populations with uniform morphology, as well as uniform song patterns among morphologically distinct populations hitherto classified as different species.

Clade A (Figs. 4 and 5j–m) comprises haplotypes with low (COI) to very low (ITS) ingroup genetic distances. This clade includes populations from a large portion of the range of...
T. viridissima as well as all populations sampled in Northwestern Africa that may be identified as one of T. vaucheriana, T. lozanoi, T. longealata, T. krugeri, and T. viridissima. All these taxa were described in terms of...
differences in body size, relative length of the tegmina (see open and closed symbols designating each specimen in Fig. 4), and some additional features, such as the width of the fastigium of the vertex (according to our observations in this group, a wide fastigium corresponds to large, stout body and vice versa). Despite these “strict” differences, we failed to clearly outline taxa as wide variation was observed between populations, and animals with both long and short wings occurred together in some areas. All sampled populations and studied museum specimens were compared (also with descriptions), and we did not find differences in the shape of male cerci and genitalia (titillators), female subgenital plate, or other species-specific characters.

All studied individuals from the sampled populations of clade A showed the same song pattern—sequences of disyllabic echemes of variable length separated by short intervals (see Fig. 2). Large intra-individual variation in the echeme sequence length was also observed. The fine structure of the song fully corresponded to that of *T. viridissima* though the latter species usually produced longer echeme sequences. Yet, a large overlap was detected between the song duty cycles (calculated using echemes and echeme intervals) of the northwestern African populations and *T. viridissima* (Fig. 6). The genetic data, showing low genetic diversification, support the phenotypic similarities.

Clade B (Fig. 4) is formed by *T. uvarovi* (Fig. 5c, d), *T. caudata* (Fig. 5e), and the *T. armeniaca* complex (Fig. 5f–i). *T. uvarovi* is a well-characterized species, morphologically resembling *T. viridissima*, with a song more similar to that of *T. cantans* (Rhee 2013). *T. caudata* is also a well-studied species, though this only applies to its nominotypical form, while its relationships with subspecific taxa are vague. Its typical song, consisting of long echemes (1–10 s), was recorded from Switzerland in the west (Roesti and Keist 2009), through Europe and Anatolia, to China (Xinjiang) in the east (Fan et al. 2013). The *T. armeniaca* complex as here regarded concerns populations sampled in the Transcaucasus and Eastern Anatolia (see Table 1) with specimens fitting either *T. caudata* armeniaca, T. acutipennis, or T. turcica. All of the latter individuals were characterized in comparison with *T. caudata* s. str. by more or less shortened wings, as well as shorter hind femora, smaller body size, and weaker development of black dots at the base of the ventral spines of the hind femora. Interestingly, although we could not discriminate between populations morphologically, specimens from different localities exhibited a wide variety of song types. The latter caused confusion not only for the fact that specimens with different songs looked the same, but due to the lack of a geographic structuring of the songs. Song types varied from long sequences of isolated syllables to sequences of disyllabic or polysyllabic echemes. However, all songs exhibited approximately the same relationship between chirp duration and chirp interval. Compared with the songs of *T. caudata*, the latter has higher absolute values in both aspects, still preserving the ratio between chirp duration and interval (Fig. 7). Part of the variation is certainly due to different temperatures during recording, but the groups did not overlap despite similar temperature ranges (*T. armeniaca*: 13–27.5 °C; *T. caudata*: 14–27 °C). The duty cycle of the armeniaca song (Fig. 6) varied significantly and partly overlapped with that of *T. caudata*.

After observation of dense populations of the *T. armeniaca* complex, we found that the songs of different individuals within the same population may vary to almost the same extent as in general (see Figs. 3 and 7). Rarely, single individuals may also produce a different song by alternating monosyllabic, disyllabic, or polysyllabic echemes within one performance (see Fig. 3 (5Ba, Bb, 9A, 9B)). The genetic structure also showed very low

### Table 2 Net mean genetic distances (%) between *Tettigonia* clades for mitochondrial (COI) and nuclear (ITS1 + ITS2) genes

| Clades                      | T1  | T2  | T3  | T4  |
|----------------------------|-----|-----|-----|-----|
| COI clades                 |     |     |     |     |
| *T. viridissima + T vaucheria* complex | T1  |     |     |     |
| *T. uvarovi*               |     | T2  |     |     |
| *T. armeniaca* complex + *T. caudata* s. str. | T3  | 0.03 | 0.02 |     |
| *T. caudata* s. str.       |     | T4  | 0.04 | 0.03 | 0.02 |
| *T. cantans*               |     | T5  | 0.01 | 0.03 | 0.03 | 0.04 |
| ITS1 + ITS2 clades         |     |     |     |     |
| *T. viridissima + T vaucheria* complex | T1  |     |     |     |
| *T. uvarovi*               |     | T2  |     |     |
| *T. armeniaca* complex + *T. caudata* s. str. | T3  | 0.01 | 0.02 |     |
| *T. caudata* s. str.       |     | T4  | 0.02 | 0.02 | 0.01 |
| *T. cantans*               |     | T5  | 0.02 | 0.03 | 0.01 | 0.01 |

### Table 3 Net mean genetic distances (%) within *Tettigonia* clades for mitochondrial (COI) and nuclear (ITS1 + ITS2) genes

| Clades                                | COI clades | ITS1 + ITS2 clades |
|---------------------------------------|------------|--------------------|
| *T. viridissima + T vaucheria* complex | 0.02       | 0.01               |
| *T. uvarovi*                          | –          | –                  |
| *T. armeniaca* complex + *T. caudata* s. str. | 0.01 | 0.05               |
| *T. caudata* s. str.                  | 0          | 0.01               |
| *T. cantans*                          | 0          | 0.07               |
Fig. 5 Appearance of some taxa of Western Palaearctic *Tettigonia* (relative size proportions between photos not retained). a *T. cantans*, male, Germany, Gunzenhausen; b *T. cantans*, female, Germany, Gunzenhausen; c *T. uvarovi* Ebner, 1946—male, holotype, Siberia (NHMW), lateral view; d same, dorsal view; e *T. caudata*, male, Bulgaria, Russe district, Byala; f *T. acutipennis* Ebner, 1946—male, holotype, “Kleinasien 1914 | Marasch, Tölz. | coll. R. Ebner” (NHMW), dorsal view; g same, lateral view; h *T. armeniaca*, male, Armenia, Djermuk; i *T. armeniaca*, male, Turkey, Ispir; j *T. viridissima* morphotype of *longealata*, male, Morocco, El Kebab; k *T. viridissima* morphotype of *longealata*, female, Morocco, El Kebab; l *T. viridissima* morphotype of *vaucheriana*, male, Morocco, El Kebab; and m *T. viridissima*, male and female in copula, Bulgaria, Haskovo district, Kostilkovo village.
(almost zero) differentiation for the COI fragment within the *T. armeniaca* complex, while distances within the clade consisting of the *T. armeniaca* complex and *T. caudata* were from very low (COI) to moderate (ITS) (Table 3).

Clade C (Fig. 4) is here represented by a single taxon, *T. cantans* (Fig. 5a, b). Its basal position in the tree supports the suggestion that its song corresponds to an ancestral state for *Tettigonia* due to its simple structure and low female preference towards temporal song parameters (Schul 1998).

**Discussion**

The genus *Tettigonia* is one of the ecologically most successful Palaearctic groups of bush-crickets, distributed throughout the Palaearctic ecozone. The Green Bush-cricket species occur in a wide variety of habitats—from the semideserts of North Africa and Central Asia to Eurasian taiga and the lush meadows of the treeless mountain zone. The low mitochondrial genetic differentiation found in this study suggests relatively recent diversification and fast expansion throughout the Palaearctic and the occasional presence of hybrids, even between members of different clades (Schul 1995), are in support of the latter suggestion. The occurrence of the most basal branching resulting in the topology clade C (*T. cantans*) + (clades A, B) supports the ancestral state of the temporal song structure and the low female preference filter of *T. cantans* (Schul 1998). Furthermore, this type of song (although female acoustic preference is not known) occurs in *T. uvarovi*, the basal taxon in clade B. Interestingly, *T. uvarovi* has relatively short wings but is similar in overall habitus to *T. viridissima*, which is another piece of evidence suggesting a closer relationship between clades A and B. While both *T. cantans* and *T. uvarovi* are typically found in humid habitats with a moderate to cool climate and occupy the northernmost areas of the range of this genus, *T. caudata* and the *T. armeniaca* complex occur mostly in mountainous and steppe areas from southeastern Europe, through Anatolia and Iran, to Central Asia. Thus, song elaboration by changes in syllable length and the development of echmes may have been connected with the southwestern expansion from the Eastern Palaearctic towards drier open habitats in the mountains of Central Asia and/or Irano-Anatolia.

The uniform morphology, intraindividual and intrapopulation song variability, and low genetic distances suggest that all studied members of the *T. armeniaca* complex represent a single taxonomic unit. The variable song pattern within the complex is a unique phenomenon in bush-crickets. The incorporation of either long monosyllables or short disyllabic or polysyllabic echmes in communication indicates an unusual mechanism of song recognition. The recognition mechanism of females of the related *T. caudata* requires mainly echmes that do not contain long intervals, i.e., echmes composed of a fast sequence of syllables (high syllable repetition rate; Schul 1998). Those females accept even continuous songs without intervals (Schul 1998). Yet, the minimum acceptable duration of an echme has not been tested. Females of the *T. armeniaca* complex may use the same criterion, possibly accepting shorter echme durations than *T. caudata* and rejecting longer ones. In this case, the internal structure of a chirp can vary as long as there are no long intervals within a chirp. Populations of the *T. armeniaca* complex may use the same criterion, possibly accepting shorter echme durations than *T. caudata* and rejecting longer ones. In this case, the internal structure of a chirp can vary as long as there are no long intervals within a chirp. Populations of the *T. armeniaca* complex occur sympatrically with *T. caudata* (their suspected syntopic occurrence may be marginal or accidental), and thus, low selectivity could provoke hybridization. Yet, we found neither significant differences in the song frequency (unpublished data) and duty cycle (Fig. 6), nor evidence for hybrids between these groups. Thus, to elucidate the mechanisms of recognition and sexual selection in this group, it is necessary to conduct large-scale population genetic research combined with a behavioral study of female acoustic preferences.

Clade A is composed of a group of populations with relatively uniform song patterns, more or less typical of
T. viridissima. In contrast to the T. armeniaca complex discussed previously, here, a uniform song pattern was present within a rather wide range of morphotypes. However, variation concerned only body size and wing length, while the shape of the female external genitalia and the male internal and external genitalia did not differ across the morphotypes. Although the song pattern showed moderate geographical structuring (shorter schemes in North African populations), genetic data revealed a mixed pattern of all sampled populations. The duty cycle distribution (Fig. 6) largely overlapped between Eurasian and North African samples. The previous data suggest a recent expansion of T. viridissima populations to the African continent (possibly during the glacial maxima connected with the ocean level decreasing since the Pleistocene). Thus, isolated micropopulations may have specialized in certain microclimates prior to subsequent secondary contact and gene exchange. Similar morphological changes involving shorter wings have been observed in T. viridissima populations occurring in Great Britain (Cooper et al. 2012).

Taxonomic reconsiderations

Following the interdisciplinary study presented previously, we suggest the following taxonomic reconsiderations:

**T. viridissima (Linnaeus, 1758)**
- T. vaucheriana Piclet, 1888, syn.n.
- T. maroccana Bolivar, 1893, syn.n. (upon synonymy with T. vaucheriana)
- T. lozanoi (Bolivar, 1914), syn.n.
- T. longealata Chopard, 1937, syn.n.
- T. krugeri Massa, 1998, syn.n.

**Notes, diagnosis, and distribution** In Northern Africa, two morphological groups of species occur. The Tettigonia savignyi group contains T. savignyi (Lucas, 1849) and Tettigonia macr oxipha (Bolivar, 1914) (including also Tettigonia longispina Ingrisch, 1983, described from Sardinia) that characterizes with male cercus with a very big internal spine (longer than the width of cercus), titillators with short stout apical arms with a very small second apical hook and apically attenuated female subgenital plate with a narrow incision. This group has not been considered in the present study.

The second group involves species related to T. viridissima and here assigned to as T. vaucheriana complex (among the listed in Eades et al. 2016, valid taxa are T. vaucheriana, T. lozanoi, T. longealata, and T. krugeri). The latter characterizes with male cercus with a short internal spine (much shorter than the width of cercus), titillators with long gracile apical arms with two equal apical hooks, and apically widened female subgenital plate with a wide incision. Formerly, the latter taxa were considered distinct species on account of differences in the length of tegmina, body size, and width of scapus in relation to the first antennal segment and subtle differences in the female subgenital plate (based on dry specimens in which its shape may differ after deformation due to desiccation). With the present study, we found all these characters highly variable between and within populations. For example, width of scapus is in direct relation to the body size (the stouter the body is, the wider the scapus is in relation to the first antennal segment). Considering the low genetic distances, uniform male and female genitalia, and the uniform song of the studied populations subjectively referred to at least three of the mentioned taxa, we consider all members of the T. vaucheriana complex synonymous with T. viridissima.

Thus, the species distribution, known to be mostly restricted north of the Mediterranean, is now proved to cover all Western Palaearctic including the southern Mediterranean on the territories of northern Morocco, Algeria, and partly Libya (thus, without any doubt, also Tunisia).

**T. armeniaca** Tarbinsky, 1940, stat. nov.
- T. acutipennis Ebner, 1946, syn.n.
- T. turcica Ramme, 1951, syn.n.

**Notes, diagnosis, and distribution** Two species related to T. caudata and usually recognized by their shorter wings are known to occur in Anatolia (T. acutipennis and T. turcica) (Ebner 1946; Ramme 1951). Similarly to T. caudata, these taxa are characterized by well-visible black dots in the base of the ventral spines of the femora (most visible in the apical half of hind femora), strongly apically attenuated stridulatory file, long ovipositor (as long as or longer than body, while shorter in most other Tettigonia), short and apically outcurved male cerci, and male titillators with comparatively stout apical arms ending with two short hooks. T. acutipennis and T. turcica differ from T. caudata by the shorter (usually less than 33 mm, while over 33 in T. caudata) and apically tapering tegmina, shorter hind femora (usually less than 25 mm, while over 25 in T. caudata), and less expressed black dots ventrally on the femora. From the Caucasus area, the subspecies T. caudata armeniaca has been described by Tarbinsky (1940) and later synonymized with T. caudata caudata by Stolyarov (1983). Our samples showed that the morphotype of T. armeniaca complex is widely distributed in the Transcaucasus area. Summarizing the results of the current study, we prove that T. armeniaca complex represents a single well-outlined species, T. armeniaca, stat. nov., with two newly established synonyms, T. acutipennis, syn.n., and T. turcica, syn.n. T. armeniaca characterizes with the previously mentioned features, as well as by its unique variable song and low genetic distances between its populations. Acoustically, it differs from T. caudata by the shorter chirps (ecchemes) and chirp intervals, both being less than a second, while over 1 s in T. caudata.

**T. armeniaca** occurs in moderately humid grass and shrub associations in the high plateaus and mountains of Eastern
Anatolia and whole Transcaucasia (Georgia, Armenia, and Azerbaijan), as well as in the Northern Iran (at least in the Elburs range) (this study and own unpublished data). Its occurrence further east in the mountains of Central Asia is not excluded.

Conclusions

The taxonomy of *Tettigonia* was hitherto based only on morphological descriptions that frequently led to difficulties in outlining its systematics and relationships between taxa (see references in the “Introduction” section). In the present study, we partially revealed the phylogeny and relationships of the genus *Tettigonia*, with a focus on the major groups in the Continental Palaeartic. Three main lineages were outlined representing three distinct clades with unique morpho-acoustic evolution. The combination of variable morphology and uniform song in the *T. viridissima* lineage, and of variable song and uniform morphology in the *T. caudata/armenica* lineage, addressed a multitude of evolutionary and behavioral questions. This paper provides a foundation for future investigations into the evolution of the recognition mechanisms and female choice in *Tettigonia*, which led to this diversity of forms, being the cause or result of the ecological success of Green Bush-crickets.

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