



# Multiple origin of flightlessness in Phaneropterinae bushcrickets and redefinition of the tribus Odonturini (Orthoptera: Tettigonioidea: Phaneropteridae)

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## Abstract

The possession of wings and ability to fly are a unifying character of higher insects, but secondary loss of wings is widespread. Within the bushcrickets, the subfamily Phaneropterinae (Orthoptera: Tettigonioidea) comprises more than 2000 predominantly long-winged species in the tropics. However, the roughly 300 European representatives are mainly short-winged. The systematics of these radiations have been unclear, leading to their unreliable formal treatment, which has hindered analysis of the evolutionary patterns of flight loss. A molecular phylogeny is presented for 42 short-winged species and members of all European long-winged genera based on the combined data from three nuclear gene sequences (18S, H3, ITS2). We found four phylogenetic lineages: (i) the first included the short-wing species of the genus *Odontura*; (ii) a further branch is represented by the South-American short-winged *Cohnia andeana*; (iii) an assemblage of long-wing taxa with a deep branching pattern includes the members of the tribes Acrometopini, Ducetiini, Phaneropterini, and Tylopsidini; (iv) a large group contained all short-winged taxa of the tribe Barbitistini. Phaneropterinae flightlessness originated twice in the Western Palaearctic, with a number of mainly allo- and parapatrically distributed species of the Barbitistini in Southeastern Europe, and the Middle East and a limited number of *Odontura* species in Northern Africa and Southwestern Europe. Both short-winged lineages are well separated, which makes it necessary to restrict the tribe Odonturini to the West-Palaearctic genus *Odontura*. Other flightless genera previously included in the Odonturini are placed as incertae sedis until their phylogenetic position can be established.

**Keywords** Barbitistini · Odonturini · Molecular phylogeny · Flight loss · Wing reduction · Brachypterism

## Introduction

Insect flight evolved around 400 million years ago (Grimaldi and Engel 2005; Misof et al. 2014), probably only once

(Hovmöller et al. 2002; Misof et al. 2014). This way of moving around was a key evolutionary innovation in insects and is one of the reasons for their success (Engel et al. 2013; Nicholson et al. 2014). Flight is advantageous for dispersal and migration (Bowler and Benton 2005) as it allows the quick exploitation of a wide range of interspersed habitats (Kingsolver and Koehl 1994; Denno et al. 1996; Langellotto and Denno 2001). Well-developed wings enable insects to disperse widely and easily in search of mates, food, and new habitats. In some species, flight is primarily an adaption for dispersal, with important consequences for gene flow, speciation, and evolution. The ability to disperse likely makes a major contribution to the fitness of individuals (Mayhew 2007). Powered flight is energetically costly as individuals have to produce and sustain lift and overcome drag. Reviews of flight costs have revealed an energy partitioning conflict between reproduction and flight, resulting in a trade-off: the so-called oogenesis-flight syndrome (Dixon et al. 1993; Guerra 2011). Even at rest, long-winged insects (capable of flight) need extra energy for their flight muscles

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(Reinhold 1999) and store metabolic resources as readily available flight fuels (Zera 2005; Zera and Zhao 2006). In contrast, flightless individuals can invest more energy into reproduction (Guerra 2011; Steenman et al. 2015).

The development of wings and ability to fly are characteristic traits of all higher insect orders. Nonetheless, a multitude of insect taxa secondarily lost the ability to fly (Roff 1990, 1994a; Wagner and Liebherr 1992), which is interpreted as an evolutionary adaptation to environmental factors (Roff 1994b; Hodkinson 2005). There have been debates on the evolutionary forces driving the occurrence of regressive traits, including flightlessness in insects (Fong et al. 1995; Lahti et al. 2009). The development of flight organs and their physiological maintenance imposes metabolic costs, and any individual must weigh the benefits of flight against the costs. When dispersal does not bring ecological benefits, flightlessness is the natural consequence; this is the case for cave, subterranean or small island insects, and for phoretic species like fleas (Roff 1990, 1994a; Wagner and Liebherr 1992). Habitat stability in forests also seems to support the evolution of flightlessness (Roff 1994b), at least in females (Hunter 1995; Snaell et al. 2007). Furthermore, short-winged species are common in the temperate zones (north and south) and high mountains of the tropics (Roff 1990). Winglessness in these cases seems to be better explained by the costs, where losing the ability to fly is an adaptation for energy saving in less favorable climates. Regardless of the ecological reasons, flightlessness can boost species diversity (Ikeda et al. 2012) and correlates with relaxed molecular evolution in energy-related mitochondrial genes (Mitterboeck and Adamowicz 2013; Mitterboeck et al. 2017).

The bushcricket or katydid subfamily Phaneropterinae is a suitable taxon for studying the origins of flight loss. This subfamily is the most species-rich group within the Tettigonioidea and has approximately 2500 species distributed worldwide, mostly in the tropics (Cigliano et al. 2018). In line with the general pattern for wing reduction, short-winged Phaneropterinae occur in tropical mountains (Braun 2010; Massa 2015), where brachypterism increases with altitude (Braun 2011). In the Western Palaearctic, the situation is completely different: there are just a few long-winged Phaneropterinae and a very large number of short-winged species. All short-winged Phaneropterinae worldwide were originally placed in the single tribe Odonturini (Brunner von Wattenwyl 1878, 1891). The European perspective made it necessary to separate several genera into the tribe Barbitistini (Bey-Bienko 1954). These have radiated into a vast number of allo- and parapatric species (Lehmann 1998) with moderate to small distribution areas in the Eastern Mediterranean (Bey-Bienko 1954; Heller 1984; Willemse and Heller 1992), comprising around 300 described species in 15 genera (Cigliano et al. 2018). This radiation in the Eastern Mediterranean is linked to extraordinary diversification in acoustic communication

systems (Heller 1984, 1990, 2006) and corresponding sensory ecology (Stumpner and Heller 1992; Strauß et al. 2012, 2014). From the plesiomorphic state of bidirectional acoustic communication in Phaneropterinae, where males sing and females answer (Heller et al. 2015), some species within the Barbitistini genus *Poecilimon* FISCHER, 1853 reduced the female's wings, which thus became non-functional for acoustic communication (Heller 1984, 1992; Heller and von Helversen 1993; Anichini et al. 2017). Furthermore, one species, *P. intermedius* (FIEBER, 1853), has switched to obligate parthenogenesis (Lehmann et al. 2011), which occurs in less than ten bushcricket species worldwide, and also has sensory reduction due to its missing sexual communication (Lehmann et al. 2007; Strauß et al. 2014). The reproduction of the Barbitistini, especially the genus *Poecilimon* (McCartney et al. 2008, 2010), is well-studied due to the extremely large nuptial gifts transferred during mating (Lehmann 2012). One could speculate that flightlessness is a primer for the extraordinary, large nuptial gifts in this tribe, as variations in the sex that searches for mates (unidirectional versus bidirectional species) correlate with spermatophore size (McCartney et al. 2012). Moreover, no other Orthoptera group has been more intensively studied with respect to chromosomal evolution (both chromosome numbers and structures) (Warchałowska-Śliwa 1998; Warchałowska-Śliwa and Heller 1998; Warchałowska-Śliwa et al. 2000, 2008, 2011, 2013; Grzywacz et al. 2011). Astonishingly, all studies reveal low chromosome differentiation between species and genera (Warchałowska-Śliwa et al. 2013; Grzywacz et al. 2014a) and show small phylogenetic signal. The few published molecular studies on Barbitistini bushcrickets are limited to species groups (Lehmann 1998), included in barcoding analyses (Hawllitschek et al. 2017), or have mainly concentrated on the genera *Poecilimon* (Ullrich et al. 2010) and *Isophya* BRUNNER VON WATTENWYL, 1878 (Chobanov et al. 2017). In a previous study, we were unable to clarify the position of the Barbitistini relative to Odonturini, due to restricted taxon sampling (Grzywacz et al. 2014b).

Species that radiated in the Western Mediterranean show low lineage diversification, with 17 taxa placed in the single genus *Odontura* RAMBUR, 1838 (Supplementary Table 1). They show little variation in their bidirectional acoustic communication system (Heller 1988; Grzywacz et al. 2014b), with the exception of *O. microptera*; the tegmina in females of this species do not touch each other; therefore, this species might have secondarily returned to a unidirectional acoustic communication system. However, despite their restricted species number, their chromosome organization is very differentiated, with autosome numbers ranging from 26 to 30 in males and sex chromosomes having evolved multiple times (Warchałowska-Śliwa et al. 2011; Grzywacz et al. 2014b). There is little geographic overlap between the many Barbitistini genera in the east and the genus *Odontura* in the west (Harz 1969; Heller 1988). To add further complication, a multitude of short-

winged genera around the world without geographic overlap and very distinct morphologies are still included in the Odonturini, despite the cautionary comments of Braun (2011) and others (Supplementary Table 2, Cigliano et al. 2018).

Here, we performed a molecular phylogenetic analysis of the flightless West-Palaearctic Barbitistini and Odonturini alongside a diverse subset of long-winged genera, including all European genera. We were also able to include the short-winged *Cohnia andeana* (HEBARD, 1924) from South America. We selected three nuclear genes (small subunit ribosomal RNA gene-18S rDNA, histone 3-H3, internal transcribed spacer 2-ITS2), which have been used successfully to resolve Orthoptera phylogenies (Svenson and Whiting 2004; Jost and Shaw 2006; Ullrich et al. 2010; Mugleston et al. 2013; Song et al. 2015; Chobanov et al. 2017). The present study focuses on the evolutionary origin of flightlessness in Phaneropterinae and clarifies the status of the Odonturini.

## Material and methods

### Taxon sampling

A total of 101 specimens belonging to 42 species of 17 genera of the Phaneropterinae (Orthoptera, Tettigoniodea) were selected for this study (Table 1). From the tribe Barbitistini, which comprises only short-winged species, we sampled 31 species from 10 out of the 15 genera currently recognized. From the tribe Odonturini, which also only comprises short-winged species, we included four species from the genus *Odontura* and the tentatively placed South American species *Cohnia andeana*. We also added six long-winged Phaneropterinae species covering selected genera occurring in the Western Palaearctic and one from East Asia hypothesized to be closely related to *Odontura* (compare Ragge 1980). Three taxa from the genus *Tettigonia* LINNAEUS, 1758, representing a different bushcricket family (Orthoptera, Tettigoniodea, Tettigoniidae) were selected as the outgroup.

### DNA extractions, PCR amplification, and sequencing

Genomic DNA was extracted from a hind leg of individuals using the NucleoSpin® Tissue kit (Macherey-Nagel, Germany), following the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out to amplify three nuclear genes: a fragment of the small subunit ribosomal RNA (18S rDNA), histone 3 (H3), and internal transcribed spacer 2 (ITS2). The primers used for the amplifications were 18Sai [5'-CCT GAG AAA CGG CTA CCA CAT C-3'] and 18Sbi [5'-GAG TCT CGT TCG TTA TCG GA-3'] for 18S rDNA (Whiting et al. 1997), H3fwd [5'-ATG GCT CGT ACC AAG CAG ACG GC-3'] and H3rev [5'-ATA TCC TTG GGC ATG ATG GTG AC-3'] for H3 (Colgan et al. 1998), and ITS2-

28S [5'-GGA TCG ATG AAG AAC G-3'] and 28S-18S [5'-GCT TAA ATT CAG CGG-3'] for ITS2 (Weekers et al. 2001).

The PCR reaction was performed in a 30-μl reaction volume containing 3.0 μl of 10× PCR buffer, 25 mM MgCl<sub>2</sub>, 10 mM dNTP mixture, 15 μM forward and reverse primers, 1 μl of genomic DNA, 0.2 μl of Taq DNA polymerase (EURx, Poland), and sterile deionized water. The general PCR profile run on the Thermocycler Mastercycler EP (Eppendorf, Germany) consisted of an initial denaturation step at 95 °C for 4 min, followed by 34 cycles at 95 °C for 30 s, 50 °C for 1 min, and 72 °C for 2 min, and a final extension step of 10 min at 72 °C. The cycling conditions for the 18S rDNA amplification consisted of an initial denaturation for 3 min at 94 °C followed by 30 cycles at 94 °C for 1 min, 51 °C and 72 °C for 1.30 min, with a 10-min final extension at 72 °C. PCR products were purified with the GeneMATRIX PCR/DNA Clean-Up Purification kit (EURx, Poland; following the standard protocol) and were sequenced using the ABI Prism BigDye® Terminator kit version 3.1 (PE Applied Biosystems, Foster City, CA) and ABI 3730XL sequencer. DNA sequences for each gene were deposited in GenBank under the accession numbers listed in Table 1.

### Phylogenetic analyses

The obtained nucleotide sequences were aligned and edited in Sequencher v. 4.1 (Gene Codes Corporation). Ambiguously aligned regions were identified following the method proposed by Lutzoni et al. (2000). An unambiguous alignment of a 111 bp portion of ITS2 could not be achieved, similar to the ambiguous characters in those nuclear markers found in a previous study of Phaneropterinae bushcrickets (Ullrich et al. 2010). Therefore, this region was excluded from further analysis. The partition homogeneity test (Farris et al. 1995) implemented in PAUP 4.0a (Swofford 2002) was used to determine the validity of combining 18S, H3, and ITS2 genes into a single analysis.

Phylogenetic inference analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI). Best fit models for ML and BI analyses were calculated in MrModeltest (Nylander 2004) using the Akaike information criterion (AIC). SYM with gamma distribution (SYM + G) represented the best fitting model of nucleotide substitution for the combined datasets. Maximum likelihood (ML) analyses were conducted in PAUP 4.0a. Bootstrap support (BS) was calculated with 1000 replicates. Bayesian analysis was performed in MrBayes v 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with four independent runs, each having three heated and one cold chain. Analyses were run for 10 million generations with trees sampled every 100 generations. Bayesian posterior probabilities (PP) were calculated using a Metropolis-coupled, Markov Chain Monte Carlo (MCMC) sampling approach. The first 25% of each run was discarded as burn-in. Convergence among the runs was

**Table 1** Taxonomic information and GenBank accession numbers for taxa included in this study

Voucher	Tribe/Family	Species	Collection locality	GenBank Accession Numbers		
				18S	H3	ITS2
Barbitistini						
ani1a		<i>Ancistrura nigrovittata</i> (Brunner von Wattenwyl, 1878)	Greece, Meteora, Kalambaka	KM819577	KM982077	KM981967
ani2a			Bulgaria, Blagoevgrad, Maleshevska Mountains	KM819578	KM982089	KM981968
am1a		<i>Andreiniimon nuptialis</i> (Kamy, 1918)	Bulgaria, Haskovo, Glouhite Kamani	KM819579	KM982100	KM981969
am2a			Macedonia, Cma Reka Valley, Blashnica	KM819580	KM982111	KM981970
bco1a		<i>Barbitistes constrictus</i> Brunner von Wattenwyl, 1878	Bulgaria, Sliven, Zheravna	KM819588	KM982081	KM981978
boc1a		<i>Barbitistes ocskayi</i> Charpentier, 1850	Bulgaria, Vidin, Belogradchik	KM819589	KM982082	KM981979
boc2a			Croatia, Istria, Padna	KM819590	KM982083	KM981980
boc2b			Croatia, Istria, Padna	KM819591	KM982084	KM981981
bye1a		<i>Barbitistes yersini</i> Brunner von Wattenwyl, 1878	Slovenia, Lipiza	KM819592	KM982085	KM981982
bye1b			Slovenia, Lipiza	KM819593	KM982086	KM981983
ikr1a		<i>Isophya kraussii</i> Brunner von Wattenwyl, 1878	Germany, Bavaria	KM819599	KM982093	KM981989
ikr1b			Germany, Bavaria	KM819600	KM982094	KM981990
im1a		<i>Isophya major</i> Brunner von Wattenwyl, 1878	Turkey, Antalya, Kuruçay	KM819601	KM982095	KM981991
im1b			Turkey, Antalya, Kuruçay	KM819602	KM982096	KM981992
imv1a		<i>Isophya mavromoustakisi</i> Uvarov, 1936	Cyprus, Pentadactylos Range	KM819603	KM982097	KM981993
imv1b			Cyprus, Pentadactylos Range	KM819604	KM982098	KM981994
imv1c			Cyprus, Pentadactylos Range	KM819605	KM982099	KM981995
imo1a		<i>Isophya modestior</i> Brunner von Wattenwyl, 1882	Slovenia, Gabree	KM819606	KM982101	KM981996
imo1b			Slovenia, Gabree	KM819607	KM982102	KM981997
ino2a			Bulgaria, Vidin, Belogradchik	KM819608	KM982103	KM981998
ino3a			Serbia, Novi Sad, Kamenitsa	KM819609	KM982104	KM981999
inel1a		<i>Isophya nervosa</i> Ramme, 1931	Turkey, Kütahya, Tavşanlı	KM819610	KM982105	KM982000
ipa1a		<i>Isophya straubei paucidentis</i> Heller, 1988	Turkey, Isparta, Davraz	KM819611	KM982106	KM982001
isul1a		<i>Isophya</i> aff. <i>sureyai</i> Ramme, 1951	Turkey, Giresun, Tandere	KM819612	KM982107	KM982002
isul1b			Turkey, Giresun, Tandere	KM819613	KM982108	KM982003
ital1a		<i>Isophya taurica</i> Brunner von Wattenwyl, 1878	Ukraine, S Crimea, Babugan Yayla	KM819614	KM982109	KM982004
izel1a		<i>Isophya zernovi</i> Miram, 1938	Turkey, Artvin, Kafasor	KM819615	KM982110	KM982005
lal1a		<i>Leptophyes albovittata</i> (Kollar, 1833)	Russia, Semibalki	KM819616	KM982112	KM982006
lal1b			Russia, Semibalki	KM819617	KM982113	KM982007
lal2a			Russia, Semibalki	KM819618	KM982114	KM982008
lal2b			Russia, Semibalki	KM819619	KM982115	KM982009
lbo1a		<i>Leptophyes boscii</i> Fieber, 1853	Croatia, Istria, Buje	KM819620	KM982116	KM982010
lbo1b			Croatia, Istria, Buje	KM819621	KM982117	KM982011
lbo1c			Croatia, Istria, Buje	KM819622	KM982118	KM982012
ldil1a		<i>Leptophyes discoidalis</i> (Frivaldszky, 1868)	Serbia, Novi Sad, Kamenica	KM819623	KM982119	KM982013
ldil1b			Serbia, Novi Sad, Kamenica	KM819624	KM982120	KM982014
lpul1a		<i>Leptophyes punctatissima</i> (Bosc, 1792)	Bulgaria, Vama, Botanical Garden	KM819625	KM982121	KM982015
lpul1b			Bulgaria, Vama, Botanical Garden	KM819629	KM982126	KM982019
lpu2a			Germany, Brandenburg, Stahnsdorf	KM819626	KM982123	KM982016
lpu2b			Germany, Brandenburg, Stahnsdorf	KM819627	KM982124	KM982017
lpu2c			Germany, Brandenburg, Stahnsdorf	KM819628	KM982125	KM982018
lsp1a			Bulgaria, Bourgas, Kovach	KM819630	KM982127	KM982020
lsp1b			Bulgaria, Bourgas, Kovach	KM819631	KM982128	KM982021
lsp1c			Bulgaria, Bourgas, Kovach	KM819632	KM982129	KM982022

Table 1 (continued)

Voucher	Tribe/Family	Species	Collection locality	GenBank Accession Numbers		
				18S	H3	ITS2
mor1a		<i>Metaplastes ornatus</i> (Ramme, 1931)	Macedonia, Bitola, Konjarska Reka	KM819633	KM982130	KM982023
mor1b			Macedonia, Bitola, Konjarska Reka	KM819634	KM982131	KM982024
mor1c			Macedonia, Bitola, Konjarska Reka	KM819635	KM982132	KM982025
pan1a		<i>Parapoecilimon antalyensis</i> Karabag, 1975	Turkey, Antalya	KM819651	KM982150	KM982041
pan1b			Turkey, Antalya	KM819652	KM982151	KM982042
par1a		<i>Phonochorton arvinensis</i> Bey-Bienko, 1954	Turkey, Rize, Ikizdere	KM819677	KM982171	KM982067
par1b			Turkey, Rize, Ikizdere	KM819678	KM982172	KM982068
par1c			Turkey, Rize, Ikizdere	KM819679	KM982173	KM982069
pam1a		<i>Poecilimon ampliatus</i> Brunner von Wattenwyl, 1878	Slovenia, Gabcree	KM819648	KM982147	KM982038
pam1b			Slovenia, Gabcree	KM819649	KM982148	KM982039
pam1c			Slovenia, Gabcree	KM819650	KM982149	KM982040
pfula		<i>Poecilimon fustii</i> Fieber, 1878	Bulgaria, Plevna	KM819664	KM982159	KM982054
pgr1a		<i>Poecilimon gracilis</i> (Fieber, 1853)	Macedonia, Jablanica Mountain	KM819666	KM982161	KM982056
pgr1b			Macedonia, Jablanica Mountain	KM819667	KM982162	KM982057
pgr1c			Macedonia, Jablanica Mountain	KM819668	KM982163	KM982058
pml1a			Turkey (European part), Kırklareli, Mandrakoy	KM819669	KM982164	KM982059
por1a		<i>Poecilimon miramae</i> Ramme, 1933	Macedonia, Nidzhe Mountain	KM819671	KM981962	KM982061
por2a		<i>Poecilimon ornatus</i> (Schmidt, 1850)	Macedonia, Belasitsa Mountain	KM819672	KM981963	KM982062
pth1c		<i>Poecilimon thoracicus</i> (Fieber, 1853)	Bulgaria, Assenovgrad-Plovdiv	KM819676	KM982170	KM982066
pdel1a		<i>Polysarcus denticauda</i> (Charpentier, 1825)	Bulgaria, Smolyan, Stoykite	KM819653	KM982152	KM982066
pde2a			Macedonia, Jablanica Mountain, Goma Belica	KM819654	KM982153	KM982044
pde2b			Macedonia, Jablanica Mountain, Goma Belica	KM819655	KM982154	KM982045
pel1a		<i>Polysarcus zacharovi</i> (Sishelkanovtzev, 1910)	Turkey, Kars, Kars-Horasan road	KM819656	KM982156	KM982046
pel2a			Turkey, Van, Kuskunkran pass	KM819657	KM982157	KM982047
pel2b			Turkey, Van, Kuskunkran pass	KM819658	KM982158	KM982048
pse1a		<i>Polysarcus scutatus</i> (Brunner von Wattenwyl, 1882)	Greece, Ioannina, Epirus	KM819673	KM982167	KM982063
Odonturini						
oas1a		<i>Odontura (Odonturella) aspericauda</i> Rambur, 1838	Spain, Malaga, Serrania de Ronda	KM819636	KM982134	KM982026
oas1b			Spain, Malaga, Serrania de Ronda	KM819637	KM982135	KM982027
oas1c			Spain, Malaga, Serrania de Ronda	KM819638	KM982136	KM982028
oma1a		<i>Odontura (Odonturella) macphersoni</i> Morales-Agacino, 1943	Spain, Caceres, Puerto de Tornavacas	KM819642	KM982140	KM982032
oma1b			Spain, Caceres, Puerto de Tornavacas	KM819643	KM982141	KM982033
oma1c			Spain, Caceres, Puerto de Tornavacas	KM819644	KM982142	KM982034
ost1a		<i>Odontura (Odontura) stenoxypa stenoxypa</i> (Fieber, 1853)	Italy, Sicily, Eraclea Minoa, Riserva Nazionale Foce de Fiume del Platani,	KM819645	KM982143	KM982035
ogl1a		<i>Odontura (Odontura) glabricauda</i> (Charpentier, 1825)	Spain, Malaga, Serrania de Ronda	KM819639	KM982137	KM982029
ogl2a			Spain, Caceres, Puerto de Tornavacas	KM819641	KM982139	KM982031
ogl3a			Portugal, Faro, Monte da Rafoia	KM819640	KM982138	KM982030
can1a		<i>Cohnia andeana</i> (Hebard, 1924)	Peru, Cachaipojas	KM819594	KM982087	KM981984
can2a			Ecuador, Loja, Catamayo	KM819595	KM982088	KM981985
Phaneropterini						
pfa1a		<i>Phaneroptera falcata</i> (Poda, 1761)	Poland, OPN, Kolencin	KM819659	KM981957	KM982049
pfa2a			Poland, Biezanów	KM819660	KM981958	KM982050
pfa3a			Germany, Brandenburg, Prutzke	KM819661	KM981959	KM982051
pfa3b			Germany, Brandenburg, Prutzke	KM819662	KM981960	KM982052
pfa3c			Germany, Brandenburg, Prutzke	KM819663	KM981961	KM982053



Table 1 (continued)

Voucher	Tribe/Family	Species	Collection locality	GenBank Accession Numbers		
				18S	H3	ITS2
pn1a	Ducetiini	<i>Phaneroptera nana</i> Fieber, 1853	Macedonia, Brod Municipality, Slatina	KM819670	KM982165	KM982060
dj1a		<i>Ducetia japonica</i> (Thunberg, 1815)	South Korea, Seoul Nowon-ku Chung-Gye	KM819596	KM982090	KM981986
dj1b			South Korea, Seoul Nowon-ku Chung-Gye	KM819597	KM982091	KM981987
dj1c			South Korea, Seoul Nowon-ku Chung-Gye	KM819598	KM982092	KM981988
ase1a	Acrometopini	<i>Acrometopa servillea</i> (Brullé, 1832)	Bulgaria, Haskovo, Ivaylovgrad	KM819581	KM982122	KM981971
ase1b			Bulgaria, Haskovo, Ivaylovgrad	KM819582	KM982133	KM981972
ase1c			Bulgaria, Haskovo, Ivaylovgrad	KM819583	KM982144	KM981973
asy1a		<i>Acrometopa syriaca</i> Brunner von Wattenwyl, 1878	Cyprus, Pentadactylos-Range	KM819584	KM982155	KM981974
asy1b			Cyprus, Pentadactylos-Range	KM819585	KM982166	KM981975
asy1c			Cyprus, Pentadactylos-Range	KM819586	KM982078	KM981976
asy2a			Turkey, Antalya, Aspendos	KM819587	KM982080	KM981977
tl1a	Tylopsidini	<i>Tylopsis lilifolia</i> (Fabricius, 1793)	Italy, Abruzzo, Parco Naturale Regionale Sirente-Velin	KM819683	KM981964	KM982073
tl1b			Italy, Abruzzo, Parco Naturale Regionale Sirente-Velin	KM819684	KM981965	KM982074
tl1c			Italy, Abruzzo, Parco Naturale Regionale Sirente-Velin	KM819685	KM981966	KM982075
outgroup	Tettigoniidae					
tv1a		<i>Tettigonia viridissima</i> (Linnaeus, 1758)	Bulgaria, Dobrich, Bolata Bay	KM819686	KM982079	KM982076
tc1a		<i>Tettigonia caudata</i> (Charpentier, 1845)	Turkey, Van, Kuskunkran pass	KM819682	KM982176	KM982072
tc1a		<i>Tettigonia armeniaca</i> Tarbinsky, 1940	Turkey, Savsat-Ardahan	KM819681	KM982175	KM982071

**Table 2** Mean pairwise distances within studied tribes of Phaneropterinae calculated from combined three nuclear genes (18S + H3 + ITS2)

Taxa	Mean distance within group (%)
Barbitistini	34.4
Odonturini	33.8
Acrometopini	9.1
Phaneropterini	8.4
Tylopsidini	1.3
Ducetiini	0

assessed using Tracer v1.5 (Rambaut and Drummond 2009). Figtree (<http://tree.bio.ed.ac.uk/software/figtree>) was used to visualize the trees. Pairwise genetic distances were calculated in Mega v 6.0 (Tamura et al. 2013).

## Results

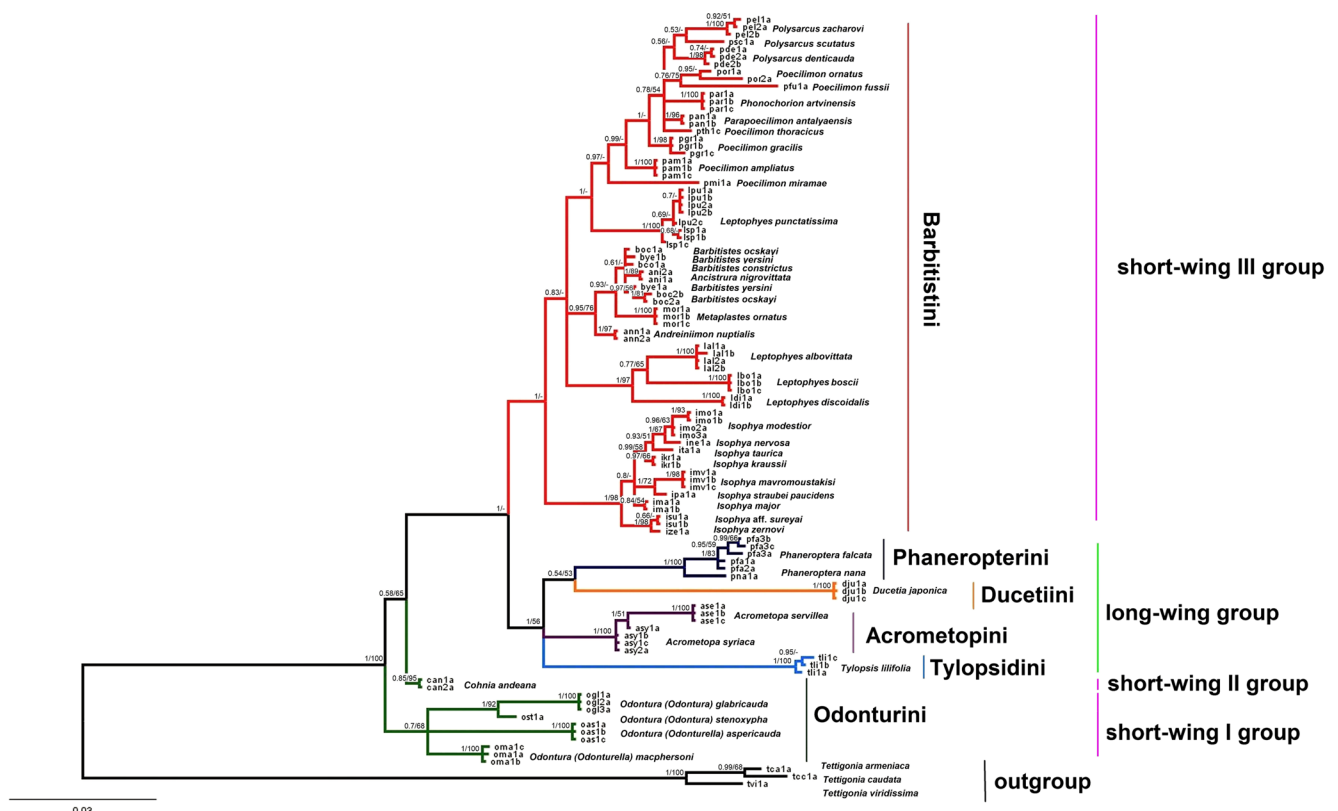
### Sequence data

The final DNA sequence dataset comprised 1177 bp. The sequences of the ITS2 gene (289 bp) were more polymorphic than those of H3 and 18S: for all taxa, 70% of sites

were variable and about 40% parsimony informative. The tribe Barbitistini had around 60% of sites variable and 47% parsimony informative for the ITS2. The sequences of the H3 gene (328 bp) were less variable: for all taxa, 47% of sites were variable and 18% parsimony informative. In this case, the tribe Barbitistini had 40% of sites variable and 7% parsimony informative. Sequences for 18S rDNA (560 bp) showed a much lower rate of polymorphism, of just 9% across all taxa with 4% of sites parsimony informative, and for Barbitistini, the values were 3 and 1%, respectively. The partition homogeneity test did not detect significant incongruence between genes; so, our analyses were conducted on the combined dataset. For the rDNA genes, in-group genetic distances within Phaneropterinae tribes were largest in Barbitistini (35%) and Odonturini (34%) (Table 2).

### Phylogenetic analyses

The analyses of maximum likelihood and Bayesian inference resulted in similar tree topologies (Fig. 1). The Bayesian posterior probability values for the nodes were generally higher than the bootstrap values. The outgroup taxon sampling with the three species of the genus *Tettigonia*, members of the bushcricket subfamily Tettigoniinae, clearly defined and



**Fig. 1** Bayesian tree from three nuclear gene analyses as performed in MrBayes. The two values on each branch represent the following: (1) Bayesian posterior probability (PP) and (2) maximum likelihood bootstrap support (BS) (only support values above 50%) as PP/BS

routed the tree. The phylogenetic analysis divided the subfamily Phaneropterinae into four lineages. The first major lineage (short-wing group I) is the sister group to all the rest and comprises solely the species of the genus *Odontura*, whose monophyly was supported in all analyses, with a posterior probability of 0.7 and bootstrap values of 68%. Our phylogeny suggests that the short-winged *Cohnia andeana* from the Andes of South America, tentatively placed within Odonturini (Braun 2011), is phylogenetically separated from *Odontura* and in our analysis represents the sole species of an additional, New World lineage (short-wing group II), paraphyletic to the rest of the Phaneropterinae. The “long-winged” taxa of Phaneropterinae included in this study do not form a homogenous group: they are separated by long branches and have unresolved basal relationships. The latter aspect shows their distinctiveness and supports their traditional placement in four separate tribes: the Acrometopini, Ducetiini, Phaneropterini, and Tylopsidini. Within the fourth major lineage, members of the species-rich tribe Barbitistini form a well-supported monophyletic lineage (short-wing group III) with 100% posterior probability but low bootstrap values (< 50%). The typically brachypterous groups, especially the Barbitistini and the Odonturini, are well separated, which suggests that these groups lost flight independently of each other.

## Taxonomic conclusions

Based on our analysis, we restrict the tribe Odonturini BRUNNER VON WATTENWYL, 1878 solely to the West-Palaearctic genus *Odontura* RAMBUR, 1838 (Supplement Table S1), well separated from the tribe Barbitistini. Other flightless genera from the Americas, tropical Africa and Papua New Guinea and previously included in Odonturini are placed here as incertae sedis (Supplement Table S2) until their phylogenetic position can be established.

## Discussion

The phylogenetic analyses of the sequences of the three nuclear DNA genes created a single most parsimonious tree for the Phaneropterinae. Within this tree, the genus *Odontura* is a clear monophyletic entity, which can be classified as the tribe Odonturini (Supplementary Table 1). However, the long-lasting inclusion of other short-winged genera from all over the world into the Odonturini is rejected here (Supplementary Table 2). The South American genus *Cohnia* branches outside the Odonturini, in full agreement with our previous phylogenetic results for this genus (Grzywacz et al. 2014b). The phylogenies of two additional genera formerly placed inside the Odonturini have been analyzed before by Mugleston et al.

(2013, 2016). Firstly, the *Austroodontura capensis* (WALKER, 1869) (Naskrecki and Bazelet 2011) from the Fynbos Flora of South Africa branches out with a Madagascan long-winged species *Parapyrrhicia dentipes* SAUSSURE, 1899 (Mugleston et al. 2016) arbitrarily placed in the Phaneropterini, but certainly belonging to an unnamed tribe (Hemp et al. 2017a). The second genus *Monticolaria* SJÖSTEDT, 1910 from the mountain arc of East Africa (Hemp et al. 2009; Massa 2015) clusters deep inside a branch containing a number of long-winged genera, traditionally embedded in the Phaneropterini and Tylopsidini (Mugleston et al. 2016). On the basis of our results in combination with the mentioned molecular phylogenetic studies, we formally restrict the tribe Odonturini to the 17 taxa of the genus *Odontura* and exclude all other short-winged genera from the Odonturini (see Supplementary Table 2). A future study with more comprehensive coverage from the megadiverse Phaneropterinae might clarify their phylogenetic affinities. The current knowledge suggests that the relatives of the short-winged Phaneropterinae may be best searched for in the fully winged genera of the regions of their occurrence. The wing reduction in *Odontura* and *Cohnia* is supposed to have evolved independently as both genera live on different continents, with *Odontura* in the Western Mediterranean, and *Cohnia* in the Central Andes of South America.

The Eastern Mediterranean genera form a third, well-supported monophyletic group of flightless species, which corresponds with their classification as the tribe Barbitistini. This tribe or at least a subset of genera belonging to it has been repeatedly supported as a monophyletic group (Mugleston et al. 2013, 2016). All species are short-winged and speciation may well have been related to the limited dispersal capacities in the oro-geographic diverse landscapes of Southeastern Europe and Anatolia (Lehmann 1998). This may have contributed to the sheer number of species (around 300), with little geographic overlap between the closely related taxa (Lehmann 1998; Boztepe et al. 2013; Kaya et al. 2015; Chobanov et al. 2017). Similarly to the western Odonturini, the nearest relatives of the Barbitistini are unknown: they probably also originated from some long-winged species that found refugia in the Eastern Mediterranean and radiated into allo- and parapatrically distributed species. The radiation resulting in the huge species number may be the result of geographic separation (discussed in Lehmann 1998) caused by climate cycles including multiple ice-ages (see Hewitt 2000, 2004). The genus *Leptophyes* has been repeatedly found to branch with the *Poecilimon*-cluster (Ullrich et al. 2010; Mugleston et al. 2013, 2016; Grzywacz et al. 2014b), which is also supported here for *L. punctatissima*. Interestingly, the other three species, *Leptophyes albobittata* (KOLLAR, 1833), *L. boscii* FIEBER, 1853 and *L. discoidalis* (FRIVALDSZKY, 1868), nested in another subgroup. This split is coherent with differences in general morphology and bioacoustic data (Bey-Bienko 1954; Kleukers et al. 2010; Sevgili 2004); however,



we leave this question open until more *Leptophyes* species are studied. The genus *Andreiniimon* CAPRA, 1937, based on its overall appearance, used to be related to *Leptophyes* (Bey-Bienko 1954), but our study found it genetically clustered with *Metaplastes* RAMME, 1939, *Barbitistes* CHARPENTIER, 1825, and *Ancistrura* UVAROV, 1921. Interestingly, highly modified external male genitalia are a shared trait of *Ancistrura*–*Andreiniimon*–*Barbitistes*–*Metaplastes*, which is coupled to a unique sperm-removal ability in the genus *Metaplastes* (von Helversen and von Helversen 1991; Foraita et al. 2017). Therefore, the modified external male genitalia might be a synapomorphic character for the group and the overall similarity of *Andreiniimon* with *Leptophyes* either results from plesiomorphy or convergent evolution. The genus *Isophya*, with the second highest species-number (Chobanov et al. 2013, 2017; Grzywacz et al. 2014a), has an ancestral position within the Barbitistini.

The full development of wings is the plesiomorphic character state in the Phaneropterinae. Therefore, it was well expected that the European long-winged genera are rather unrelated, separated by long branching axes. This is in complete agreement with the morphologically classified traditional system, where *Acrometopa* FIEBER, 1853, *Ducetia* STÅL, 1874, *Phanoptera* SERVILLE, 1831, and *Tylopsis* FIEBER, 1853 are placed in different tribes (Cigliano et al. 2018). The deep splits between the genera studied here are also supported by a much broader phylogenetic approach (Mugleston et al. 2013, 2016). Interestingly, none of the long-winged genera that currently occur in the Mediterranean region seem to be closely related to the short-winged Odonturini in the Southwest or the short-winged Barbitistini in the Southeast. As concluded by several authors (Braun 2011; Naskrecki and Bazelet 2011; Grzywacz et al. 2014b; Massa 2015), wing size reduction resulting in flightlessness must have occurred multiple times in the Phaneropterinae, which is supported by the recent discovery of the short-winged East African genus *Peronurella* HEMP, 2017, belonging to the tribe Acrometopini (Hemp et al. 2017b). The conclusions of our study are in line with such a notion and support two evolutionary events within the Western Palaearctic and a probable third in South America. The overall number of times this type of concerted evolution has occurred is obviously higher, as worldwide many more short-winged Phaneropterinae genera of unknown tribal affinities occur (Supplementary Table 2). Based on their distinct appearance, we can easily conclude that the short-winged genera do not form a single worldwide group, and instead likely developed regionally on different continents. Phaneropterinae generally lack genital titillators as a shared character (Vahed et al. 2011; Lehmann et al. 2017), but it occurs independently in central and North American taxa of the *Dichopetala* group (Cohn et al. 2014; Rocha-Sánchez et al. 2015; Barrientos-Lozano et al. 2016), the South African genus *Brinckiella* CHOPARD, 1955 (Naskrecki and Bazelet 2009), and the

Asian *Letana inflata* BRUNNER VON WATTENWYL, 1878 (Heller and Liu 2015). However, the convergent development of titillators is linked to mating-related features such as copulation duration (Vahed et al. 2011; Lehmann et al. 2016) or polyandry (Lehmann et al. 2017), but is not coupled to flightlessness. In conclusion, the evolutionary transition to flightlessness seems to be moderately common in bushcrickets and the five events mentioned in the global analysis by Mugleston et al. (2013) are an underestimation.

Brachypterism can be interpreted to be an evolutionary adaptation resulting from a variety of different environmental conditions. At least three conditions could support flightlessness: first, habitat stability is often an explanation, as dispersal becomes less favorable with increasing stability. The chance of ending up at inferior places after emigration may counter its positive effects, such as outbreeding opportunities. Second, the same is true for isolated habitats, where travel by air is either impossible, like in caves, or dangerous as on islands or mountains (Roff 1990; Wagner and Liebherr 1992). Third, whatever the ecological circumstances, an individual is more likely to disperse when the potential benefits exceed the risks and travel costs (Roff 1984, 1990; Wagner and Liebherr 1992).

In line with the general picture for insects (Roff 1990; Guerra 2011), the loss of flight in tropical bushcrickets occurs predominantly at higher altitudes (Braun 2011), either to save metabolic energy at lower temperatures or because long-distance dispersal provides fewer opportunities to colonize new habitats on mountains than in flat landscapes. However, the situation in the Western Palaearctic needs closer examination. Barbitistini genera have an early seasonal occurrence coupled with a rather short and synchronized life history (Lehmann and Lehmann 2006; Lehmann 2012). This might be a leftover from multiple ice-cycles in which the flightless species adapted to local climates. In line with such a scenario, many species nowadays occur on mountains and abundantly thrive in mesophilic meadows. Alternatively, the pattern of a short and early adult season may allow the species to avoid the hot and dry Mediterranean summers. Chobanov et al. (2017) estimated the common ancestor of Barbitistini to have evolved during the so-called Middle Miocene climatic transition, characterized by a global drop in temperatures and dry climates. The loss of flight and fast life cycle may therefore be connected with the necessity to avoid water loss (Chobanov et al. 2017). For example, the species *Poecilimon thessalicus* BRUNNER VON WATTENWYL, 1891 is known to suffer from the early onset of the dry Mediterranean summer, which leads to the populations living on the dryer, eastern mountain slopes to have a smaller body size than those on the wetter, western mountain slopes (Lehmann and Lehmann 2008).

Whatever the evolutionary forces that led to the multiple loss of flight, the reduced dispersal capacities have promoted speciation, as in other insects (Ikeda et al. 2012; Vogler and Timmermans 2012; Sota et al. 2014). The overall diversity in Barbitistini with almost 300 species is an impressive example of rapid speciation (Lehmann 1998) within a restricted temporal (Ullrich et al. 2010; Chobanov et al. 2017) and spatial frame (Heller 1984; Willemse and Heller 1992). Thus, it could be speculated that brachyptery in Barbitistini bushcrickets has contributed to the impressive species number—mainly by allopatric separation events during multiple ice-cycles in an oro-geographic diverse landscape (Lehmann 1998).

## Conclusion

Based on a phylogenetic reconstruction using three nuclear markers, we found strong evidence for multiple flight loss in Phaneropterinae bushcrickets. In the temperate zone of the Western Palaearctic, flightlessness originated twice. In the first group, the Barbitistini, speciation led to an impressive number of mainly allo- and parapatrically distributed species in Southeastern Europe, Anatolia, and the Middle East. The second group, the Odonturini, occurs as a limited number of species in Southwestern Europe and Northern Africa. Interestingly, the closest relatives of the Odonturini are still unknown but may be best searched for in afro- or asi-tropical species, which invaded Europe during a warmer climatic period, while the Barbitistini probably originated from long-winged species found their refugia in the Eastern Mediterranean.

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## References

- Anichini, M., Kuchenreuther, S., & Lehmann, G. U. C. (2017). Allometry of male sound-producing structures indicates sexual selection on wing size and stridulatory teeth density in a bushcricket. *Journal of Zoology*, 301, 271–279.
- Barrientos-Lozano, L., Rocha-Sanchez, A. Y., Zaldivar-Riverón, A., & Correa-Sandoval, A. (2016). Additional new species of the genus *Obolopteryx* Cohn et al. 2014 (Ensifera: Tettigoniidae) from north-eastern Mexico. *Zootaxa*, 4168, 401–452.
- Bey-Bienko, G. Ya. (1954). *Orthoptera. Leaf bush-cricket (Phaneropterinae). Fauna of the USSR*, Vol. II, Sect. 2 (pp 385). USSR: Zoological Institute of the Academy of Sciences. [in Russian, English translation 1965].
- Bowler, D., & Benton, T. (2005). Causes and consequences of animal dispersal strategies: Relating individual behaviour to spatial dynamics. *Biological Reviews*, 80, 205–225.
- Boztepe, Z., Kaya, S., & Ciplak, B. (2013). Integrated systematics of the *Poecilimon luschni* species group (Orthoptera, Tettigoniidae): Radiation as a chain of populations in a small heterogeneous area. *Zoological Journal of the Linnean Society*, 169, 43–69.
- Braun, H. (2010). On the neotropical species described under the genus *Isophya* (Orthoptera, Tettigoniidae, Phaneropterinae). *Zootaxa*, 2444, 58–60.
- Braun, H. (2011). A brief revision of brachypterous Phaneropterinae of the tropical Andes (Orthoptera, Tettigoniidae, Odonturini). *Zootaxa*, 2991, 35–43.
- Brunner von Wattenwyl, C. (1878). *Monographie der Phaneropteriden*. Wien: Brockhaus.
- Brunner von Wattenwyl, C. (1891). Additamenta zur Monographie der Phaneropteriden. *Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien*, 41, 1–196.
- Chobanov, D. P., Grzywacz, B., Iorgu, I., Ciplak, B., Ilieva, M., & Warchałowska-Śliwa, E. (2013). Review of the Balkan *Isophya* (Orthoptera: Phaneropteridae) with particular emphasis on the *Isophya modesta* group and remarks on the systematics of the genus based on morphological and acoustic data. *Zootaxa*, 3658, 1–81.
- Chobanov, D. P., Kaya, S., Grzywacz, B., Warchałowska-Śliwa, E., & Ciplak, B. (2017). The Anatolio-Balkan phylogeographic fault: A snapshot from the genus *Isophya* (Orthoptera, Tettigoniidae). *Zoologica Scripta*, 46, 165–179.
- Cigliano, M. M., Braun, H., Eades, D. C., & Otte, D. (2018). Orthoptera species file. Version 5.0/5.0. <http://Orthoptera.SpeciesFile.org>. Accessed 02 Dec 2018.
- Cohn, T. J., Swanson, D. R., & Fontana, P. (2014). *Dichopetala* and new related north American genera: A study in genitalic similarity in sympatry and genitalic differences in allopatry (Tettigoniidae: Phaneropterinae: Odonturini). *Miscellaneous Publications Museum of Zoology, University of Michigan*, 203, 1–179.
- Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S., Edgecombe, G. D., Macaranas, J., et al. (1998). Histone H3 and U2 snRNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, 46, 419–437.
- Denno, R. E., Roderick, G. K., Peterson, M. A., Huberty, A. F., Dobel, H. G., Eubanks, M. D., et al. (1996). Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers. *Ecological Monographs*, 66, 389–408.
- Dixon, A. F. G., Horth, S., & Kindelmann, P. (1993). Migrations in insects: Cost and strategies. *Journal of Animal Ecology*, 62, 182–190.
- Engel, M. S., Davis, S. R., & Prokop, J. (2013). Insect wings: The evolutionary development of nature's first flyers. In A. Minelli, G. Boxshall, & G. Fusco (Eds.), *Arthropod biology and evolution* (pp. 269–298). Berlin: Springer Berlin Heidelberg.
- Farris, J. S., Källersjö, M., Kluge, A. G., & Bult, C. (1995). Constructing a significance test for incongruence. *Systematic Biology*, 44, 570–572.
- Fong, D. W., Kane, T. C., & Culver, D. C. (1995). Vestigialization and loss of nonfunctional characters. *Annual Review of Ecology and Systematics*, 26, 249–268.
- Foraita, M., Lehfeldt, S., Reinhold, K., & Ramm, S. A. (2017). Strategic investment in sperm removal behaviour in a bushcricket. *Journal of Insect Behavior*, 30, 170–179.

- Grimaldi, D. A., & Engel, M. S. (2005). *Evolution of the insects*. New York: Cambridge University Press.
- Grzywacz, B., Maryńska-Nadachowska, A., Chobanov, D. P., Karamysheva, T., & Warchałowska-Śliwa, E. (2011). Comparative analysis of the location of rDNA in the Palaearctic bushcricket genus *Isophya* (Orthoptera: Tettigoniidae: Phaneropterinae). *European Journal of Entomology*, 108, 509–517.
- Grzywacz, B., Chobanov, D. P., Maryńska-Nadachowska, A., Karamysheva, T. V., Heller, K.-G., & Warchałowska-Śliwa, E. (2014a). A comparative study of genome organization and inferences for the systematics of two large bushcricket genera of the tribe Barbitistini (Orthoptera: Tettigoniidae: Phaneropterinae). *BMC Evolutionary Biology*, 14, 48.
- Grzywacz, B., Heller, K.-G., Lehmann, A. W., Warchałowska-Śliwa, E., & Lehmann, G. U. C. (2014b). Chromosomal diversification in the flightless western Mediterranean bushcricket genus *Odontura* (Orthoptera: Tettigoniidae: Phaneropterinae) inferred from molecular data. *Journal of Zoological Systematics and Evolutionary Research*, 52, 109–118.
- Guerra, P. A. (2011). Evaluating the life-history trade-off between dispersal capability and reproduction in wing dimorphic insects: A meta-analysis. *Biological Reviews*, 86, 813–835.
- Harz, K. (1969). Die Orthopteren Europas I. In W. Junk (Ed.), *Series Entomologica*. Netherland: The Hague.
- Hawltischek, O., Morinière, J., Lehmann, G. U. C., Lehmann, A. W., Kropf, M., Dunz, A., Glaw, F., Detcharoen, M., Schmidt, S., Hausmann, A., Szucsich, N. U., Caetano-Wyler, S. A., & Haszprunar, G. (2017). DNA barcoding of crickets, katydids, and grasshoppers (Orthoptera) from Central Europe with focus on Austria, Germany, and Switzerland. *Molecular Ecology Resources*, 17, 1037–1053.
- Heller, K.-G. (1984). Bioakustik und Phylogenie der Gattung *Poecilimon* (Orthoptera, Tettigoniidae, Phaneropterinae). *Zoologische Jahrbücher für Systematik*, 111, 69–117.
- Heller, K.-G. (1988). *Bioakustik der Europäischen Laubheuschrecken. Ökologie in Forschung und Anwendung 1*. Josef Margraf (pp. 358).
- Heller, K.-G. (1990). Evolution of song pattern in East Mediterranean Phaneropterinae: Constraints by the communication system. In W. J. Bailey & D. C. F. Rentz (Eds.), *The Tettigoniidae. Biology, systematics and evolution* (pp. 130–151). Berlin: Springer-Verlag.
- Heller, K.-G. (1992). Risk shift between males and females in the pair-forming behavior of bushcrickets. *Naturwissenschaften*, 79, 89–91.
- Heller, K.-G. (2006). Song evolution and speciation in bushcrickets. In S. Drosopoulos & M. F. Claridge (Eds.), *Insect sounds and communication, physiology, behaviour, ecology and evolution* (pp. 207–219). USA: CRC Press, Boca Raton.
- Heller, K. G., & Liu, C. (2015). Mating behavior of *Letana inflata*, a duetting phaneropterine bush-cricket species with unusual male genitalic organs (Orthoptera: Tettigoniidae: Phaneropteridae). *Journal of Insect Behavior*, 28, 513–524.
- Heller, K.-G., & von Helversen, D. (1993). Calling behaviour in bushcrickets of the genus *Poecilimon* with differing communication system (Orthoptera: Tettigoniidae, Phaneropteridae). *Journal of Insects Behavior*, 6, 361–377.
- Heller, K.-G., Hemp, C., Ingrisch, S., & Liu, C. (2015). Acoustic communication in Phaneropterinae (Tettigoniidae) - a global review with some new data. *Journal of Orthoptera Research*, 24, 7–18.
- Hemp, C., Heller, K. G., Warchałowska-Śliwa, E., Grzywacz, B., & Hemp, A. (2017a). Review of the east African species of the phaneropterine genus *Parapyrrhia* Brunner von Wattenwyl, 1891 (Insecta: Orthoptera): Secret communication of a forest-bound taxon. *Organisms Diversity & Evolution*, 17, 231–250.
- Hemp, C., Heller, K.-G., Warchałowska-Śliwa, E., Grzywacz, B., & Hemp, A. (2017b). New genera and new species of Acrometopini (Orthoptera: Tettigoniidae Phaneropterinae) from East Africa and a review of all known stridulatory organs, songs and karyotypes of the tribe. *Insect Systematics & Evolution*, online first., 49, 241–298. <https://doi.org/10.1163/1876312X-00002170>.
- Hemp, C., Voje, K. L., Heller, K. G., & Hemp, A. (2009). Biogeography, phylogeny and acoustics of the flightless bush-cricket of the east African genus *Monticolaria* Sjöstedt, 1909, with the description of a new species (Orthoptera: Phaneropterinae). *Zoological Journal of the Linnean Society*, 156, 494–506.
- Hewitt, G. M. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405, 907–913.
- Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359, 183–195.
- Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: Species and community responses to altitude. *Biological Reviews*, 80, 489–513.
- Hovmöller, R., Pape, T., & Kallers, M. (2002). The Palaeoptera problem: Basal Pterygote phylogeny inferred from 18S and 28S rDNA sequences. *Cladistics*, 318, 313–323.
- Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.
- Hunter, A. F. (1995). The ecology and evolution of reduced wings in forest Macrolepidoptera. *Evolutionary Ecology*, 9, 275–287.
- Ikeda, H., Nishikawa, M., & Sota, T. (2012). Loss of flight promotes beetle diversification. *Nature Communications*, 3, 648.
- Jost, M. C., & Shaw, K. L. (2006). Phylogeny of Ensifera (Hexapoda: Orthoptera) using three ribosomal loci, with implications for the evolution of acoustic communication. *Molecular Phylogenetics and Evolution*, 38, 510–530.
- Kaya, S., Boztepe, Z., & Ciplak, B. (2015). Phylogeography of the *Poecilimon luschni* species group (Orthoptera, Tettigoniidae): A radiation strictly correlated with climatic transitions in the Pleistocene. *Zoological Journal of the Linnean Society*, 173, 1–21.
- Kingsolver, J. G., & Koehl, M. A. R. (1994). Selective factors in the evolution of insect wings. *Annual Review of Entomology*, 39, 425–451.
- Kleukers, R. M. J. C., Ode, B., & Fontana, P. (2010). Two new cryptic *Leptophyes* species from southern Italy (Orthoptera: Tettigoniidae). *Zootaxa*, 2506, 26–42.
- Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., Coss, R. G., Donohue, K., & Foster, S. A. (2009). Relaxed selection in the wild. *Trends in Ecology & Evolution*, 24, 487–496.
- Langellotto, G. A., & Denno, R. T. (2001). Benefits of dispersal in patchy environments: Mate location by males of a wing-dimorphic insects. *Ecology*, 82, 1870–1878.
- Lehmann, A.W. (1998). Artbildung, akustische Kommunikation und sexuelle Selektion bei griechischen Laubheuschrecken der *Poecilimon propinquus*-Gruppe (Orthoptera: Phaneropteridae). Dissertation Universität Erlangen-Nürnberg (pp 134).
- Lehmann, G. U. C. (2012). Weighing costs and benefits of mating in bushcrickets (Insecta: Orthoptera: Tettigoniidae), with an emphasis on nuptial gifts, protandry and mate density. *Frontiers in Zoology*, 9, 19.
- Lehmann, G. U. C., & Lehmann, A. W. (2006). Potential lifetime reproductive success of male bushcrickets parasitized by a phonotactic fly. *Animal Behaviour*, 71, 1103–1110.
- Lehmann, G. U. C., & Lehmann, A. W. (2008). Variation in body size between populations of the bushcricket *Poecilimon thessalicus* Brunner von Wattenwyl, 1871 (Orthoptera: Phaneropteridae): An ecological adaptation? *Journal of Orthoptera Research*, 17, 165–169.
- Lehmann, G. U. C., Strauß, J., & Lakes-Harlan, R. (2007). Listening when there is no sexual signalling? - maintenance of hearing in the asexual bushcricket *Poecilimon intermedius*. *Journal of Comparative Physiology A*, 193, 537–545.



- Lehmann, G. U. C., Siozios, S., Bourtzis, K., Reinhold, K., & Lehmann, A. W. (2011). Thelytokous parthenogenesis and the heterogeneous decay of mating behaviours in a bushcricket (Orthopteroidea). *Journal of Zoological Systematics and Evolutionary Research*, 49, 102–109.
- Lehmann, G. U. C., Heller, K.-G., & Mai, R. (2016). Mate guarding as an alternative to male nuptial gift investment in the bushcricket *Letana inflata* (Orthoptera: Tettigoniidae). *Journal of Ethology*, 34, 191–194.
- Lehmann, G. U. C., Gilbert, J. D. J., Vahed, K., & Lehmann, A. W. (2017). Male genital titillators and the intensity of post-copulatory sexual selection across bushcrickets. *Behavioral Ecology*, 28, 1198–1205.
- Lutizoni, F., Wagner, P., Reeb, V., & Zoller, S. (2000). Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violation positional homology. *Systematic Biology*, 49, 628–651.
- Massa, B. (2015). Taxonomy and distribution of some katydids (Orthoptera Tettigoniidae) from tropical Africa. *ZooKeys*, 524, 17–44.
- Mayhew, P. J. (2007). Why are there so many insect species? Perspectives from fossils and phylogenies. *Biological Reviews*, 82, 425–454.
- McCartney, J., Potter, M. A., Robertson, A. W., Telscher, K., Lehmann, G. U. C., Lehmann, A. W., et al. (2008). Understanding nuptial gift size in bush-crickets: An analysis of the genus *Poecilimon* (Tettigoniidae: Orthoptera). *Journal of Orthoptera Research*, 17, 231–242.
- McCartney, J., Lehmann, A. W., & Lehmann, G. U. C. (2010). Lifetime spermatophore investment in natural populations of two closely related bush-cricket species (Orthoptera: Tettigoniidae: Poecilimon). *Behaviour*, 147, 285–298.
- McCartney, J., Kokko, H., Heller, K. G., & Gwynne, D. T. (2012). The evolution of sex differences in mate searching when females benefit: New theory and a comparative test. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 1225–1232.
- Misof, B., Liu, S., Meusemann, K., Peters, R. S., Donath, A., Mayer, C., Frandsen, P. B., Ware, J., Flouri, T., Beutel, R. G., Niehuis, O., Petersen, M., Izquierdo-Carrasco, F., Wappler, T., Rust, J., Aberer, A. J., Aspöck, U., Aspöck, H., Bartel, D., Blanke, A., Berger, S., Böhm, A., Buckley, T. R., Calcott, B., Chen, J., Friedrich, F., Fukui, M., Fujita, M., Greve, C., Grobe, P., Gu, S., Huang, Y., Jermiin, L. S., Kawahara, A. Y., Krogmann, L., Kubiak, M., Lanfear, R., Letsch, H., Li, Y., Li, Z., Li, J., Lu, H., Machida, R., Mashimo, Y., Kapli, P., McKenna, D. D., Meng, G., Nakagaki, Y., Navarrete-Heredia, J. L., Ott, M., Ou, Y., Pass, G., Podsiadlowski, L., Pohl, H., von Reumont, B. M., Schütte, K., Sekiya, K., Shimizu, S., Slipinski, A., Stamatakis, A., Song, W., Su, X., Szucsich, N. U., Tan, M., Tan, X., Tang, M., Tang, J., Timelthaler, G., Tomizuka, S., Trautwein, M., Tong, X., Uchifune, T., Walz, M. G., Wiegmann, B. M., Wilbrandt, J., Wipfler, B., Wong, T. K. F., Wu, Q., Wu, G., Xie, Y., Yang, S., Yang, Q., Yeates, D. K., Yoshizawa, K., Zhang, Q., Zhang, R., Zhang, W., Zhang, Y., Zhao, J., Zhou, C., Zhou, L., Ziesmann, T., Zou, S., Li, Y., Xu, X., Zhang, Y., Yang, H., Wang, J., Wang, J., Kjer, K. M., & Zhou, X. (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science*, 346, 763–767.
- Mitterboeck, T. F., & Adamowicz, S. J. (2013). Flight loss linked to faster molecular evolution in insects. *Proceedings of the National Academy of Sciences of the United States of America*, 280, 1128.
- Mitterboeck, T. F., Liu, S., Adamowicz, S. J., Fu, J., Zhang, R., Song, W., Meusemann, K., & Zhou, X. (2017). Positive and relaxed selection associated with flight evolution and loss in insect transcriptomes. *GigaScience*, 6(10), 1–14.
- Mugleston, J. D., Song, H., & Whiting, M. F. (2013). A century of paraphyly: A molecular phylogeny of katydids (Orthoptera: Tettigoniidae) supports multiple origins of leaf-like wings. *Molecular Phylogenetics and Evolution*, 69, 1120–1134.
- Mugleston, J. D., Naegle, M., Song, H., Bybee, S. M., Ingley, S., Suvorov, A., & Whiting, M. F. (2016). Reinventing the leaf: Multiple origins of leaf-like wings in katydids (Orthoptera: Tettigoniidae). *Invertebrate Systematics*, 30, 335–352.
- Naskrecki, P., & Bazelet, C. S. (2009). A species radiation among south African flightless spring katydids (Orthoptera: Tettigoniidae: Phaneropterinae: *Brinckiella* Chopard). *Zootaxa*, 2056, 46–62.
- Naskrecki, P., & Bazelet, C. S. (2011). A revision of the south African katydid genus *Austrodontura* Fontana & Buzzetti (Orthoptera: Tettigoniidae: Phaneropterinae). *Zootaxa*, 2873, 51–59.
- Nicholson, D. B., Ross, A. J., & Mayhew, P. J. (2014). Fossil evidence for key innovations in the evolution of insect diversity. *Proceedings of the Royal Society of London B: Biological Sciences*, 281, 20141823.
- Nylander, J. A. A. (2004). *MrModeltest v2. Program distributed by the author*. Sweden: Evolutionary Biology Centre, Uppsala University.
- Ragge, D. R. (1980). A review of the African Phaneropterinae with open tympana. *Bulletin of the British Museum of (Natural History). Entomology*, 40, 67–192.
- Rambaut, A., & Drummond, A. J. (2009). Tracer version 1.5 [computer program] <http://beast.bio.ed.ac.uk>.
- Reinhold, K. (1999). Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology*, 13, 217–224.
- Rocha-Sánchez, A. Y., Barrientos-Lozano, L., & Zaldivar-Riverón, A. (2015). Additional new species of the genus *Pterodichopetala* (Phaneropteridae: Phaneropterinae) from northeastern Mexico. *Zootaxa*, 3956, 301–344.
- Roff, D. A. (1984). The cost of being able to fly: A study of wing polymorphism in two species of crickets. *Oecologia*, 63, 30–37.
- Roff, D. A. (1990). The evolution of flightlessness in insects. *Ecological Monographs*, 60, 389–421.
- Roff, D. A. (1994a). The evolution of flightlessness: Is history important? *Evolutionary Ecology*, 8, 639–657.
- Roff, D. A. (1994b). Habitat persistence and the evolution of wing dimorphism in insects. *The American Naturalist*, 144, 772–798.
- Ronquist, F., & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Sevgili, H. (2004). Review of the genus *Leptophyes* of Turkey with the description of a new species (Orthoptera, Phaneropterinae). *Transactions of the American Entomological Society*, 130, 95–112.
- Snaell, N., Tammaru, T., Wahlberg, N., Viidalepp, J., Ruohomäki, K., Savontaus, M. L., & Huoponen, K. (2007). Phylogenetic relationships of the tribe Operophterini (Lepidoptera, Geometridae): A case study of the evolution of female flightlessness. *Biological Journal of the Linnean Society*, 92, 241–252.
- Song, H., Amédégno, C., Cigliano, M. M., Desutter-Grandcolas, L., Heads, S. W., Huang, Y., Otte, D., & Whiting, M. F. (2015). 300 million years of diversification: Elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. *Cladistics*, 31, 621–651.
- Sota, T., Kagata, H., Ando, Y., Utsumi, S., & Osono, T. (2014). Accelerated diversification by spatial and temporal isolation associated with life-history evolution in insects. In *Species diversity and community structure* (pp. 45–61). Tokyo: Springer.
- Steenman, A., Lehmann, A. W., & Lehmann, G. U. C. (2015). Life-history trade-off between macroptery and reproduction in the wing-dimorphic pygmy grasshopper *Tetrix subulata* (Orthoptera: Tettigidae). *Ethology, Ecology and Evolution*, 27, 93–100.
- Strauß, J., Lehmann, G. U. C., Lehmann, A. W., & Lakes-Harlan, R. (2012). Spatial organization of tettigoniid auditory receptors: Insights from neuronal tracing. *Journal of Morphology*, 273, 1280–1290.
- Strauß, J., Lehmann, A. W., & Lehmann, G. U. C. (2014). Sensory evolution of hearing in tettigoniids with differing communication systems. *Journal of Evolutionary Biology*, 27, 200–213.

- Stumpner, A., & Heller, K.-G. (1992). Morphological and physiological differences of the auditory system in three related bushcrickets (Orthoptera: Phaneropteridae: *Poecilimon*). *Physiological Entomology*, 17, 73–80.
- Svenson, G. J., & Whiting, M. F. (2004). Phylogeny of Mantodea based on molecular data: Evolution of a charismatic predator. *Systematic Entomology*, 29, 359–370.
- Swofford, D. L. (2002). *PAUP<sup>\*</sup>. Phylogenetic analysis using parsimony (\*and other methods). Version 4*. Sunderland: Sinauer Associates.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
- Ullrich, B., Reinhold, K., Niehuis, O., & Misof, B. (2010). Secondary structure and phylogenetic analysis of the internal transcribed spacers 1 and 2 of bush crickets (Orthoptera: Tettigoniidae: Barbitistini). *Journal of Zoological Systematics and Evolutionary Research*, 48, 219–228.
- Vahed, K., Lehmann, A. W., Gilbert, J. D. J., & Lehmann, G. U. C. (2011). Increased copulation duration prior to ejaculate transfer is associated with larger spermatophores, and male genital titillators, across bushcricket taxa. *Journal of Evolutionary Biology*, 24, 1960–1968.
- Vogler, A. P., & Timmermans, M. J. T. N. (2012). Speciation: Don't fly and diversity? *Current Biology*, 22, 284–286.
- von Helversen, D., & von Helversen, O. (1991). Premating sperm removal in the bushcricket *Metaplastes ornatus* Ramme 1931 (Orthoptera, Tettigonoidea, Phaneropteridae). *Behavioral Ecology and Sociobiology*, 28, 391–396.
- Wagner, L., & Lieberr, J. K. (1992). Flightlessness in insects. *Trends in Ecology and Evolution*, 7, 216–220.
- Warchałowska-Śliwa, E. (1998). Karyotype characteristics of katydid orthopterans (Ensifera, Tettigoniidae) and remarks on their evolution at different taxonomic levels. *Folia Biologica (Kraków)*, 46, 143–176.
- Warchałowska-Śliwa, E., & Heller, K.-G. (1998). C-banding patterns of some species of Phaneropterinae (Orthoptera, Tettigoniidae) of Europe. *Folia Biologica (Kraków)*, 46, 177–181.
- Warchałowska-Śliwa, E., Heller, K.-G., Maryńska-Nadachowska, A., & Lehmann, A. W. (2000). Chromosome evolution in the genus *Poecilimon* (Orthoptera, Tettigoniidae, Phaneropteridae). *Folia Biologica (Kraków)*, 48, 127–136.
- Warchałowska-Śliwa, E., Chobanov, D., Grzywacz, B., & Maryńska-Nadachowska, A. (2008). Taxonomy of the genus *Isophya* (Orthoptera, Phaneropteridae, Barbitistinae): Comparison of karyological and morphological data. *Folia Biologica (Kraków)*, 56, 227–241.
- Warchałowska-Śliwa, E., Maryńska-Nadachowska, A., Grzywacz, B., Karamysheva, T., Lehmann, A. W., Lehmann, G. U. C., & Heller, K.-G. (2011). Changes in the numbers of chromosomes and sex determination system in bushcrickets of the genus *Odontura* (Orthoptera: Tettigoniidae: Phaneropterinae). *European Journal of Entomology*, 108, 183–195.
- Warchałowska-Śliwa, E., Grzywacz, B., Maryńska-Nadachowska, A., Karamysheva, T., Heller, K.-G., Lehmann, A. W., Lehmann, G. U. C., & Chobanov, D. P. (2013). Molecular and classical chromosomal techniques reveal diversity in bushcricket genera of Barbitistini (Orthoptera). *Genome*, 56, 667–676.
- Weekers, P. H. H., De Jonckheere, J. F., & Dumont, H. J. (2001). Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographic patterns in representatives of the genus *Calopteryx* (Insecta: Odonata) of the West Mediterranean and adjacent west European zone. *Molecular Phylogenetics and Evolution*, 20, 89–99.
- Whiting, M. F., Carpenter, J. C., Wheeler, Q. D., & Wheeler, W. C. (1997). The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, 46, 1–68.
- Willemse, F., & Heller, K.-G. (1992). Notes on systematics of Greek species of *Poecilimon* Fischer, 1853 (Orthoptera: Phaneropterinae). *Tijdschrift voor Entomologie*, 135, 299–315.
- Zera, A. J. (2005). Intermediary metabolism and life history trade-offs: Lipid metabolism in lines of the wing-polymorphic cricket, *Gryllus firmus*, selected for flight capability vs. early age reproduction. *Integrative and Comparative Biology*, 45, 511–524.
- Zera, A. J., & Zhao, Z. (2006). Intermediary metabolism and life-history trade-offs: Differential metabolism of amino acids underlies the dispersal-reproduction trade-off in a wing-polymorphic cricket. *The American Naturalist*, 167, 889–900.