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## Morphology, songs and genetics identify two new cicada species from Morocco: *Tettigettalna afroamissa* sp. nov. and *Berberigetta dimelodica* gen. nov. & sp. nov. (Hemiptera: Cicadettini)

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### Abstract

Morocco has been the subject of very few expeditions on the last century with the objective of studying small cicadas. In the summer of 2014 an expedition was carried out to Morocco to update our knowledge with acoustic recordings and genetic data of these poorly known species. We describe here two new small-sized cicadas that could not be directly assigned to any species of North African cicadas: *Tettigettalna afroamissa* sp. nov. and *Berberigetta dimelodica* gen. nov. & sp. nov. In respect to *T. afroamissa* it is the first species of the genus to be found outside Europe and we frame this taxon within the evolutionary history of the genus. Acoustic analysis of this species allows us to confidently separate *T. afroamissa* from its congeners. With *B. dimelodica*, a small species showing a remarkable calling song characterized by an abrupt frequency modulation, a new genus had to be erected. Bayesian inference and maximum likelihood phylogenetic analyses with DNA-barcode sequences of Cytochrome C Oxidase 1 support the monophyly of both species, their distinctness and revealed genetic structure within *B. dimelodica*. Alongside the descriptions we also provide GPS coordinates of collection points, distributions and habitat preferences.

**Key words:** Cicada, new genus, new species, Morocco

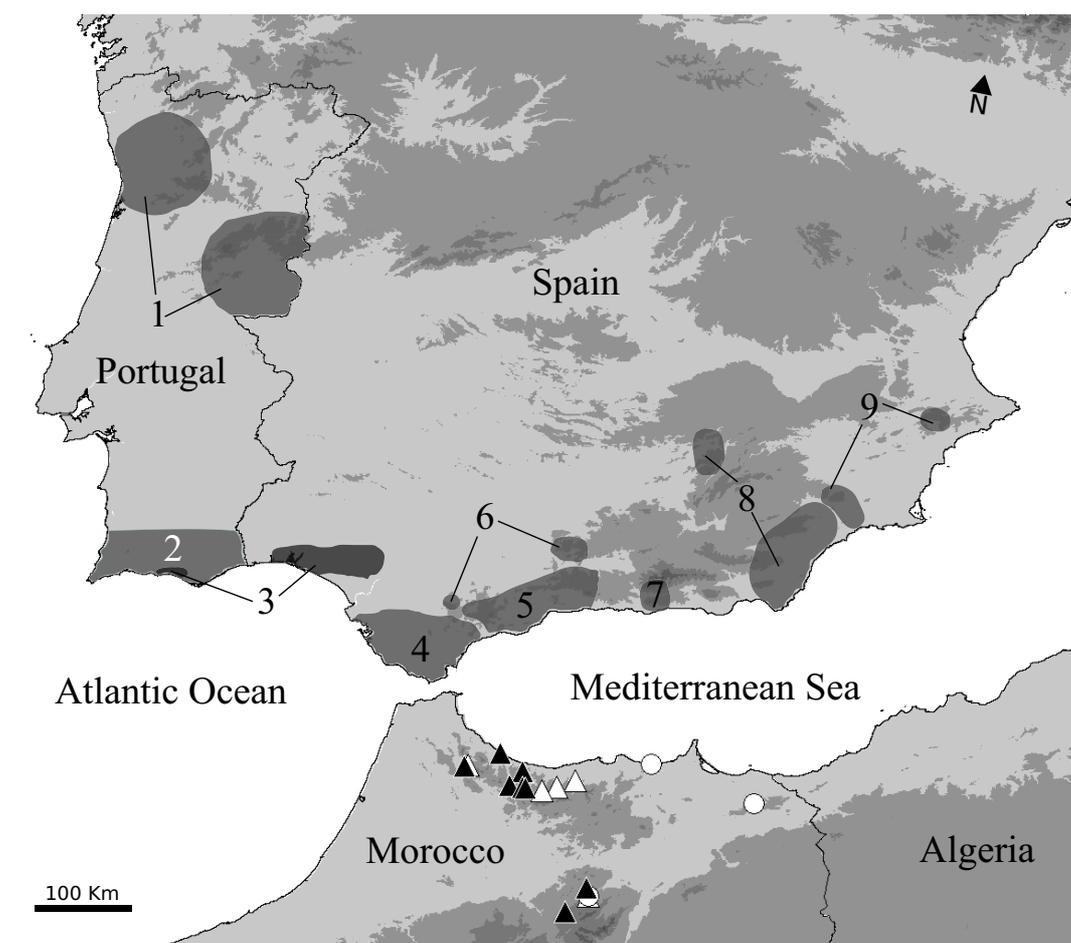
### Introduction

Cicadas (Hemiptera: Cicadoidea) are a successful insect group with a unique sound production system and thousands of species worldwide (Sanborn 2014). Males produce species-specific acoustic signals, mainly to attract females for pairing and reproduction. These signals have influence in reproductive isolation and thus can be used as important taxonomic characters (Claridge 1985; Boulard 2006; Quartau & Simões 2006; Simões & Quartau 2006), enabling taxonomists to confidently diagnose a specimen even when belonging to cryptic species (Simões *et al.* 2000; Sueur & Puissant 2007; Mendes *et al.* 2014; Hertach *et al.* 2015).

As for a wide range of biological groups, the Mediterranean basin was recently confirmed as a hotspot for cicada diversity. There, the Iberian Peninsula is particularly relevant, and recent studies on the group have unveiled new species and provided novel contributions in distribution and ecology (Puissant & Sueur 2010; Simões *et al.* 2013; Nunes *et al.* 2014a). However, the underlying idea is that our knowledge is far from complete, particularly in North Africa, where despite an initial boost in species' description and collection of samples in the past century, little has been investigated—or published—during the last decade. In fact, specimens from the Maghreb countries of Morocco, Algeria and Tunisia are available in several museum collections and represent a rather large number of cicada species (Villiers 1943; Boulard 1980, 1981, 1987). Regrettably, associated with this invaluable data is neither ecology nor the recordings of specific acoustic signals produced by the males, as these descriptions were based almost exclusively on external morphology. Cryptic species complexes, such as *Cicadetta brevipennis* s. l. or *Tettigettalna* are extremely difficult to distinguish this way (Mendes *et al.* 2014; Hertach *et al.* 2016). Therefore, in

order to truly understand this biodiversity hotspot, and other relevant biological data, such as genetics, multivariate morphometric analyses, habitat preferences, distribution range, emergence periods or phenology should be assessed.

In particular, the genetic data coupled with behavioural sound analysis may provide a recommended approach for a more accurate and thorough species description and delimitation in cicadas. This is still missing for many cicadas, namely from North Africa, compromising comparative studies with those from other regions. On what concerns molecular genetics, sequence data is highly desirable in modern taxonomy, as these enable clarification of the taxonomic status of closely-related taxa, such as in the recognition of sibling species, and in addition offering useful phylogenetic information (Hebert *et al.* 2004). More recently, DNA barcoding (Hebert *et al.* 2003) and massive sequencing of large amounts of specimens have fostered a renewal of taxonomic procedures and applications. This is particularly relevant for groups with several, very similar species, as trained specialists are currently in high demand but in short supply.



**FIGURE 1.** Distribution map of the genus *Tettigettalna* with approximate distribution areas extracted from bibliography. The distribution of *T. argentata* is not shown as it is widespread across the Iberian Peninsula. Collection points in Morocco of *T. afroamissa* (white triangle) and *B. dimelodica* (white circle). Black triangles indicate sites where *T. afroamissa* was heard but not collected. Distributions' code: 1—*T. estrellae*; 2—*T. josei*; 3—*T. mariaae*; 4—*T. armandi*; 5—*T. defauti*; 6—*T. aneabi*; 7—*T. helianthemii helianthemii*; 8—*T. h. galantei*; 9—*T. boulandi*. Scale bar indicates 100 km.

A paradigmatic case within cicadas is the European genus *Tettigettalna* Puissant, 2010. Using the current concept of the genus, it is known to comprise several, usually parapatric, species. This genus shows a pattern of increased diversity in the southern area of the Iberian Peninsula (Figure 1), with many narrow endemics bordering the coastline with the Mediterranean sea (Puissant & Sueur 2010; Simões *et al.* 2013, 2014; Nunes *et al.* 2014b) but a widespread member reaching Slovenia to the east (*Tettigettalna argentata* (Olivier, 1790)). The current knowledge on the distribution boundaries of *Tettigettalna* spp. is far from being properly known, and extensive

field surveys for these cicadas are still needed. Given this southern increased species diversity in the genus, its presence in North Africa had long been expected but not yet investigated.

Fieldwork towards a first screening of cicada biodiversity in the northern part of Morocco (Rif and Middle Atlas mountains) was carried out during the summer of 2014. Among the several Cicadettini collected and recorded, there was a medium-sized species phenotypically similar to the European *T. argentata* (Olivier, 1790), singing on holm-oaks and tall shrubs. In the understory there was sometimes a smaller species, mostly singing among middle-sized shrubs. Further analysis of both entities revealed they belong to two undescribed species, namely the first African member of the genus *Tettigettalna*, *Tettigettalna afroamissa* **sp. nov.**, and a second one, belonging to the new genus *Berberigetta* **gen. nov.**, *i.e.*, *dimelodica* **sp. nov.** Descriptions of both species are here provided and are based on distinctive morphological, bioacoustic and genetic information.

## Materials and methods

Collection of specimens was performed by hand or sweeping net and GPS data was assigned to each capture site. Acoustic data was recorded whenever possible with a CANON EOS 70D camera with an upper frequency limit of over 20 KHz. Distance of the insect to the camera varied between close recordings to up to 0.5–1 m of total distance.

Specimens were photographed or filmed and respective habitats were characterized *in loco*. In the lab, each specimen was assigned a tracking number, pinned and assigned to a morphotype. For most specimens, a front leg was removed and preserved in alcohol for posterior genetic analysis. Acoustic recordings and specimens are stored at the Department of Animal Biology of the Faculty of Sciences, University of Lisbon, Portugal.

**Morphology** Morphological terminology follows Moulds (2005) and higher systematics follows Sanborn (2014). Both species here described belong to the family Cicadidae Latreille, 1802: subfamily Cicadettinae Buckton, 1889 and tribe Cicadettini Buckton, 1889.

Body, pygophore and aedeagus measurement images were taken on a Zeiss SteREO Lumar V.12 coupled to a TIS DFK 5MPixel camera with IC Capture v.2.1 and calibrated with a 0.01 mm Olympus micrometer. Wing measurements were obtained using photographs taken on a CANON EOS 450D. Each measurement was performed on a single image. Images were calibrated and measured on FIJI (Schindelin *et al.* 2012). Measurement codes and procedure explanation are described on Table 1 and S4. Male genitalia were extracted and placed on a heated 0.1M KOH solution for removal of soft tissues and clarification. Pygophore and aedeagus were conserved on Kaiser gelatin.

**Sound** Acoustic analysis was performed on AviSoft SAS (Specht 2004). Calling songs were initially trimmed to remove bad quality sections of the recordings and a time domain filter (FIR) was applied with a high pass of 4 kHz for the calling song of *T. afroamissa* and of 2.5 kHz for *B. dimelodica* to remove background noise. A frequency domain transformation was also applied at frequencies ranging 15.59–15.80 kHz to remove electromagnetic interference.

For *T. afroamissa* **sp. nov.**, spectrograms were generated with a FFT length of 512, Hamming type window and 50% temporal overlap. Echemes were labeled with a single automatic threshold and temporal and frequency based variables were generated as described in Pinto-Juma *et al.* 2005. For *B. dimelodica* **gen. & sp. nov.**, due to song peculiarities, an additional Hamming type window with FFT length= 128 was generated.

Discrete values are shown as median  $\pm$  SD and continuous values as average  $\pm$  SD followed by (minimum–maximum, total number of observations).

**Genetics** For the genetic analysis, whole-genome DNA was isolated from a front leg of each specimen with the DNeasy Blood & Tissue Kit (Qiagen). Primers LepF and LepR (Hajibabaei *et al.* 2006) were used to obtain 648 bp of the 5' region of the cytochrome C oxidase I (COI) mitochondrial gene (the 'barcode' region), using the same PCR conditions as Nunes *et al.* (2014a). PCR products were purified with SureClean (Bioline) and sequencing was carried out by Macrogen Europe. Sequences were first corrected in Sequencher 4.0.5 (Gene Codes Co.), then aligned with MAFFT 7.273 (Katoh & Standley 2013) and visually inspected in BioEdit 7.0.9.0 (Hall 1999) and trimmed to the final, same length of 581 bp. The alignment has no gaps or stop codons. Sequences were deposited in GenBank (accession numbers KX582146 to KX582168, see Table 2).

**TABLE 1.** List and description of the 23 morphological variables analyzed in *T. afroamissa* and *B. dimelodica*, described with codes and abbreviations (Abbr.).

Body region	Code	Abbr.	Description
Head and thorax	1	TL	Total length measured from tip of the head to end of the wings in resting position
	2	HL	Head length measured from the front to the end of the head measured by the dorsal median line
	3	HW	Maximum head width measured between exterior eye margins
	4	EO	Eye-ocellum distance between the margin of a compound eye and the margin of the nearest ocellum
	5	OO	Greatest distance between the two dorsal ocelli
	6	LrL	Labrum length measured between the margin of the anteclypeus to the end of the labrum
	7	LiL	Labium length distance between end of labrum and tip of labium
	8	VW	Vertex width measured with the smallest interocular distance
	9	FR	Front length measured along the dorsal median line
	10	PC	Postclypeus length measured along the median line
	11	PL	Pronotum length
	12	PW	Pronotal width measured at the maximum width of pronotal collar
	13	ML	Mesonotum length measured along dorsal midline until end of scutellum
Abdomen	14	OP	Greatest width of operculum as exemplified on Image 1.
	15	LS	Sternite VII length measured along ventral midline
	16	TyL	Tymbal length as exemplified on Image 1.
	17	TyW	Tymbal width as exemplified on Image 1.
Legs	18	PF	Profemur length measured along median line
Wings	19	FwL	Forewing length measured from intersection of costal vein and CuP+1A vein until apex of wing.
	20	FwW	Forewing width measured from intersection of R+Sc vein and node until intersection of CuP+1A vein and CuA <sub>2</sub> vein.
	21	BCL	Basal cell length measured from intersection of costal vein and CuP+1A vein until beginning of M+CuA vein
	22	MCuA	Length of M+CuA vein
	23	RCL	Radial cell length measured from beginning of M+CuA until intersection of R+Sc vein and node.

Genetic distances (Kimura-2-parameter and p-distances) were obtained with Mega 6 (Tamura *et al.* 2013). Sequences generated for this study were aligned with sequences available in GenBank from Mediterranean species published by Nunes *et al.* (2014a) and Simões *et al.* (2014) from genera *Tettigettalna* Puissant 2010, *Tettigettacula* Puissant, 2010; *Tympanistalna* Boulard, 1982 and *Cicada* Linnaeus, 1758, (see Table S1 for accession numbers). For comparative purposes, specimens from two additional Mediterranean genera were also sequenced: *Hilaphura varipes* (Waltl, 1837) and *Euryphara contentei* Boulard, 1982.

The complete matrix with 58 taxa was converted from fasta to nexus with Concatenator 1.1.0 (Pina-Martins & Paulo 2008). A Bayesian phylogenetic tree was generated by MrBayes 3.2.1 (Ronquist *et al.* 2012). The best model of sequence evolution (HKY+ G) was selected under the corrected Akaike information criterion (AICc), as implemented in MrModeltest 2.3 (Nylander *et al.* 2004). The Metropolis-coupled Markov chain Monte Carlo analysis was carried out with four chains. The posterior probabilities for each node were generated from 10<sup>8</sup> generations, sampling at every 100th iteration. The burn-in was set to the first 25% trees, and the remaining trees were used to generate a consensus tree by the 50% majority rule. For maximum likelihood analysis, we used RaxML (Stamatakis 2014) with a GTRCAT model and ran with 10 000 generations. *Cicada barbara* (Stål, 1866) and *Cicada orni* L. 1758, two species belonging to tribe Cicadini and occurring both in the Iberian Peninsula and Morocco, were set as outgroup taxa for Bayesian and ML analyses.

**TABLE 2.** Description of the collection sites and NCBI accession numbers for COI DNA barcoding of the paratypical series of *T. afroamissa* and *B. dimelodica*. Bold sample IDs indicate the type specimens. Collectors name code: EM—E. Marabuto; VN—VL Nunes; TL—T. Laurentino.

Species	Sample ID	Sex	Population	Locality	Coll.	GPS coordinates	GenBank Accession n.
<i>T. afroamissa</i>	SP18_3779	♂	Rif Mountains	Chefchaouane	EM	35° 11' 2.53" N 5° 13' 25.93" W	(1)
	SP18_3780	♀	Rif Mountains	Chefchaouane	EM	35° 11' 2.53" N 5° 13' 25.93" W	(1)
	SP18_3781	♂	Rif Mountains	Chefchaouane	EM	35° 11' 2.53" N 5° 13' 25.93" W	KX582158
	SP18_3782	♂	Rif Mountains	Chefchaouane	EM	35° 11' 2.53" N 5° 13' 25.93" W	KX582159
	SP18_3783	♂	Rif Mountains	Chefchaouane	EM	35° 11' 2.53" N 5° 13' 25.93" W	KX582160
	SP18_3786	♀	Middle Atlas	Afouzar	EM	33° 52' 16.73" N 4° 1' 42.75" W	KX582161
	SP18_3805	♀	East Rif	Bni Hadifa	EM	35° 01' 48" N 4° 9' 51.85" W	(1)
	SP18_3806	♂	East Rif	Bni Hadifa	EM	35° 01' 48" N 4° 9' 51.85" W	KX582162
	SP18_3807	♂	East Rif	Bni Hadifa	VN	35° 01' 48" N 4° 9' 51.85" W	KX582163
	SP18_3808	♂	East Rif	Bni Hadifa	VN	35° 01' 48" N 4° 9' 51.85" W	KX582164
	SP18_3813	♂	East Rif	Targuist	EM	34° 57' 54.58" N 4° 20' 38.73" W	KX582165
	SP18_3814	♂	East Rif	Tizi Tchen	EM	34° 55' 44.18" N 4° 29' 31.87" W	KX582166
	SP18_3815	♂	East Rif	Tizi Tchen	EM	34° 55' 44.18" N 4° 29' 31.87" W	KX582167
	<i>B. dimelodica</i>	SP19_3787	♀	Middle Atlas	Afouzar	VN	33° 52' 16.73" N 4° 1' 42.75" W
SP19_3788		♂	Middle Atlas	Afouzar	VN	33° 52' 16.73" N 4° 1' 42.75" W	(1)
SP19_3789		♂	Middle Atlas	Afouzar	VN	33° 52' 16.73" N 4° 1' 42.75" W	(1)
SP19_3790		♂	Middle Atlas	Afouzar	EM	33° 52' 16.73" N 4° 1' 42.75" W	KX582146
SP19_3791		♂	Middle Atlas	Afouzar	EM	33° 52' 16.73" N 4° 1' 42.75" W	KX582147
SP19_3792		♂	Middle Atlas	Afouzar	EM	33° 52' 16.73" N 4° 1' 42.75" W	KX582148
SP19_3793		♂	Middle Atlas	Afouzar	TL	33° 52' 16.73" N 4° 1' 42.75" W	KX582149
SP19_3794		♂	Berkane	Berbers	VN	34° 47' 59.1" N 2° 23' 59.5" W	(1)

.....continued on the next page

**TABLE 2.** (Continued)

Species	Sample ID	Sex	Population	Locality	Coll.	GPS coordinates	GenBank Accession n.
	SP19_3795	♂	Berkane	Berbers	VN	34° 47' 59.1" N 2° 23' 59.5" W	(1)
	SP19_3796	♂	Berkane	Berbers	VN	34° 47' 59.1" N 2° 23' 59.5" W	KX582150
	SP19_3797	♂	Berkane	Berbers	VN	34° 47' 59.1" N 2° 23' 59.5" W	KX582151
	SP19_3798	♂	Berkane	Berbers	TL	34° 47' 59.1" N 2° 23' 59.5" W	KX582152
	SP19_3799	♂	Berkane	Berbers	EM	34° 47' 59.1" N 2° 23' 59.5" W	KX582153
	SP19_3803	♂	El Hoceima	Assihel	VN	35° 11' 15.86"N 3° 24' 38.93" W	KX582154

(1) These specimens were not sequenced in order to preserve their morphology for collection purposes.

## Results

### *Tettigettalna Puissant 2010*

Originally described and diagnosed by Puissant & Sueur (2010), encompasses nine European species: *T. argentata* (Olivier, 1790), *T. aneabi* (Boulard, 2000), *T. armandi* Puissant, 2010, *T. boulandi* Puissant, 2010, *T. defauti* Puissant, 2010, *T. estrellae* (Boulard, 1982), *T. helianthemis* (Rambur, 1840), *T. josei* (Boulard, 1982) and *T. mariae* (Quartau & Boulard, 1995). Only *T. argentata* is widespread, reaching, France, Italy, Switzerland and Slovenia to the east. The remaining are (rather) narrow Iberian endemics (see Figure 1).

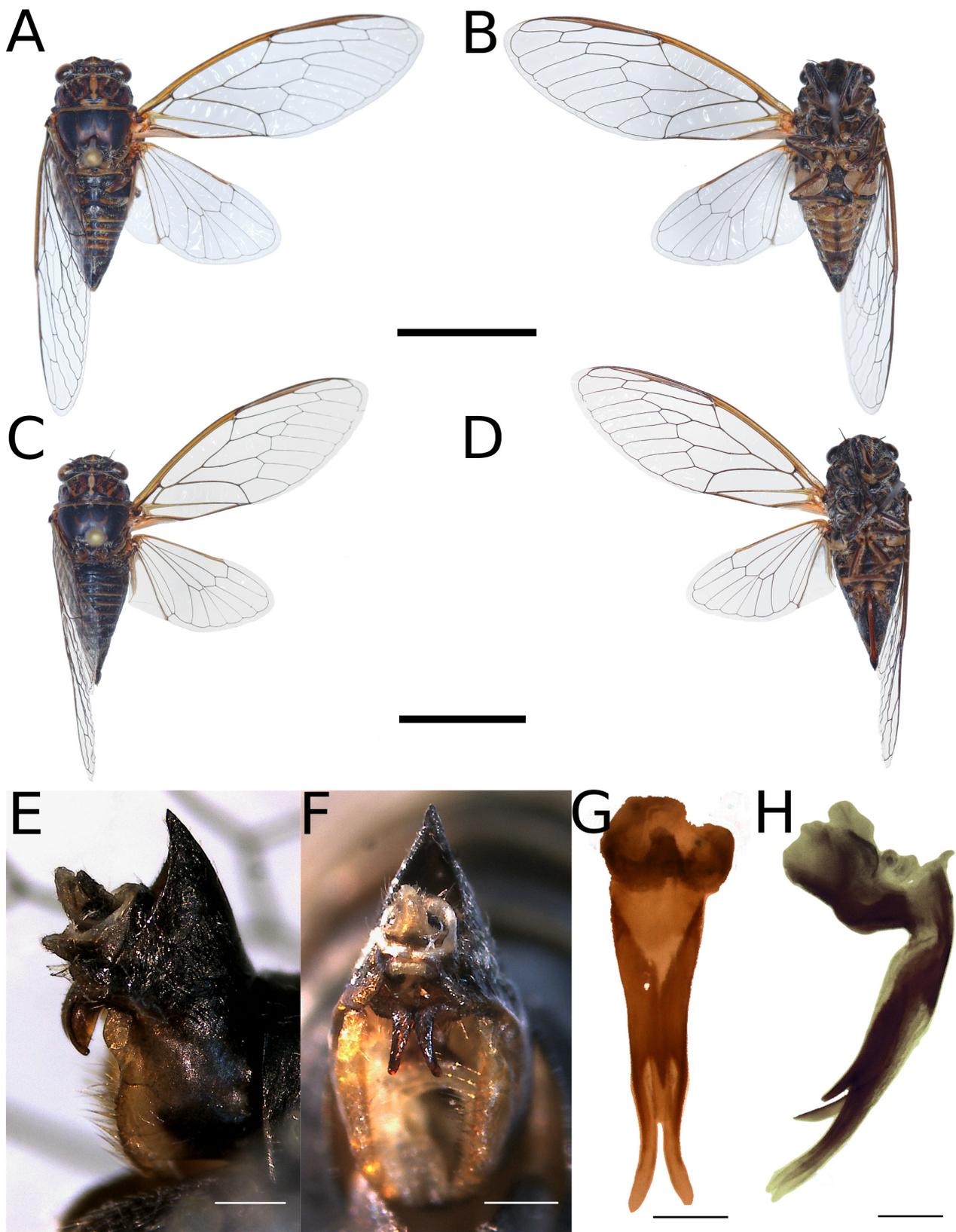
### *Tettigettalna afroamissa* sp. nov. Costa, Nunes, Marabuto, Mendes & Simões

**Material examined** Paratypical series consist of 13 specimens (ten males and three females). Designated holotype is SP18\_3779 (♂) and female paratype is SP18\_3780 (♀). See Table 2 for additional information on the paratypical series, specimen IDs, collection sites and GPS data. See Figure 2 for images of male holotype, female paratype and for details of the male genitalia.

#### Male morphology

**Head** Head slightly less broad than pronotum; Supra-antennal plates nearly meeting the eye and produced into a pointed lobe; Postclypeus rounded to subquadrate in frontal view, rounded between top and sides in lateral view, transversely grooved towards distal ends; Rostrum brown, reaching the center of mid-trochanters (in rest). Antennae dark-brown, 7-segmented. Dorsal surface of head brown with front bearing a yellowish stripe extending to outer borders; Yellowish stripe at beginning of epicranial suture extending to pronotum. Eyes brown, three red ocelli. Postclypeus dark brown, with apical yellowish-brown spot extending to frons, grooves light-brown or yellowish. Supra-antennal plates dark-brown and yellowish-brown towards distal ends. Gena and lorum brown to dark-brown covered in long white pilosity. Anteclypeus brown to dark-brown with a lighter brownish fascia surrounding a central dark-brown spot.

**Thorax** Pronotal collar slightly larger than head width, widened, sloping laterally and evenly rounded dorsally. Pronotal tooth present mid-laterally. Scutellum wider than long. Epimeral lobe not reaching operculum. Submedian sigillae well defined. Metanotum partly visible at dorsal midline not expanded over tymbals. Pronotum with an olive-green arrow shaped stripe at dorsal midline bordered with dark-brown in fresh specimens (in preserved specimens this fades away to light brown). Remainder of pronotum brown, with dark-brown markings bearing yellowish borders. Mesonotum on overall brown, with a lighter “crown-like” marking, lateral margins of mesonotum yellowish. Scutellum brown, with a longitudinal dark-brown fascia at midline expanding towards the ends, reaching metanotum. Sides of scutellum with a dense pilosity on lateral-anterior ends with a fading gradient



**FIGURE 2.** Body and male genitalia morphology of *Tettigettalna afroamissa*. A,B—Designated male holotype of *T. afroamissa* in dorsal and ventral views, respectively. Scale bar equals 10 mm; C, D—Designated female paratype of *T. afroamissa* in dorsal and ventral views, respectively. Scale bar equals 10 mm; E, F—Male paratype’s pygophore in in lateral and posterior views, respectively. Scale bar equals 500  $\mu$ m. Photos taken on dry specimens; G, H—Aedeagus in upper and lateral views, respectively. Scale bars equal 200  $\mu$ m. Photos taken of material preserved in Kaiser gelatin.

of dark-brown to yellowish towards the posterior end with defined, longitudinal, slightly transverse grooves. Metanotum brown, with a dark-brown patch at dorsal midline. Ventral side of thorax brown.

**Legs** Profemur with three to four dark-brown erect spines. Primary spine clearly separated. Metatibiae with three to four long fine spurs on inner side, and two smaller spurs on outer side with finely dispersed white pilosity. Apex of metatibia surrounded by smaller numerous brown spurs. Tarsal formula: 3-3-3. Legs generally brown in colour. Coxae and trochanters yellowish with a central dark-brown stripe, better defined on the hind legs. Femurs and tibiae brown with two dark-brown longitudinal fasciae. Profemurs with a swollen dark brown fascia surrounded by two yellowish/ light brown stripes, varying somewhat among individuals. Dark-brown border along the spines. Tarsi dark-brown on dorsal side, brown on ventral side. Protarsi darker in colour.

**Wings** Forewing and hindwing with eight and six apical cells, respectively. Ulnar cell 3 angled towards radial cell; Forewing costa parallel-sided to radial cell; Pterostigma present. CuA vein weakly bowed; M+CuA meeting at basal cell with stems fused. Vein RA<sub>1</sub> aligned closely with subcostal for its length. CuA<sub>1</sub> divided by a crossvein with shorter proximal part. CuP and 1A unfused at their bases. Veins C and R+Sc close together. Outer forewing margin developed for its total length. Hindwing first cubital cell width at distal end much greater than second cubital cell. Hindwing anal lobe broad with 3A vein long and strongly curved at distal end. Hindwing RP and M veins fused at their base. Larger forewing proximal veins yellowish with smaller apical veins brown, same for hindwing. Forewing basal membrane yellow. Hindwing plaga yellow.

**Opercula** More or less confluent with distal margin of tympanal cavity, well developed towards abdominal midline with sharply rounded apices facing midline. Opercula extending but not reaching posterior border of StII. Opercula distally yellow, dark-brown at base. Meracanthus triangular, following same colour pattern as opercula.

**Tymbals** Tymbals lacking a tymbal cover. Five ribs, four of which arising from top of a large basal dome, covering about half the tymbal width, and expanding in width towards the posterior side. Fifth rib as an extension of basal dome more or less defined, varying between specimens. First and second anterior ribs, slender, with a transverse break at about halfway of basal dome. Tymbal plate light-grey, ribs and basal dome brownish-grey.

**Abdomen** Abdomen with somewhat scattered white pilosity. T1 uniformly dark-brown; T2 uniformly dark-brown with a transversal stripe, slightly pointed towards posterior end of abdomen on each side; T3 to T7 dark-brown anteriorly becoming lighter on posterior side; T8 dark-brown. StI mainly dark-brown, yellow posterior margin; StII mainly dark-brown, with yellow lateral borders. StIII to StVI light brown, with a brown spot at midline, forming a well-defined stripe. StVII large, brown, as long as or slightly longer than StVIII; StVIII brown, densely covered in pilosity. Epipleurites brown with yellow posterior border.

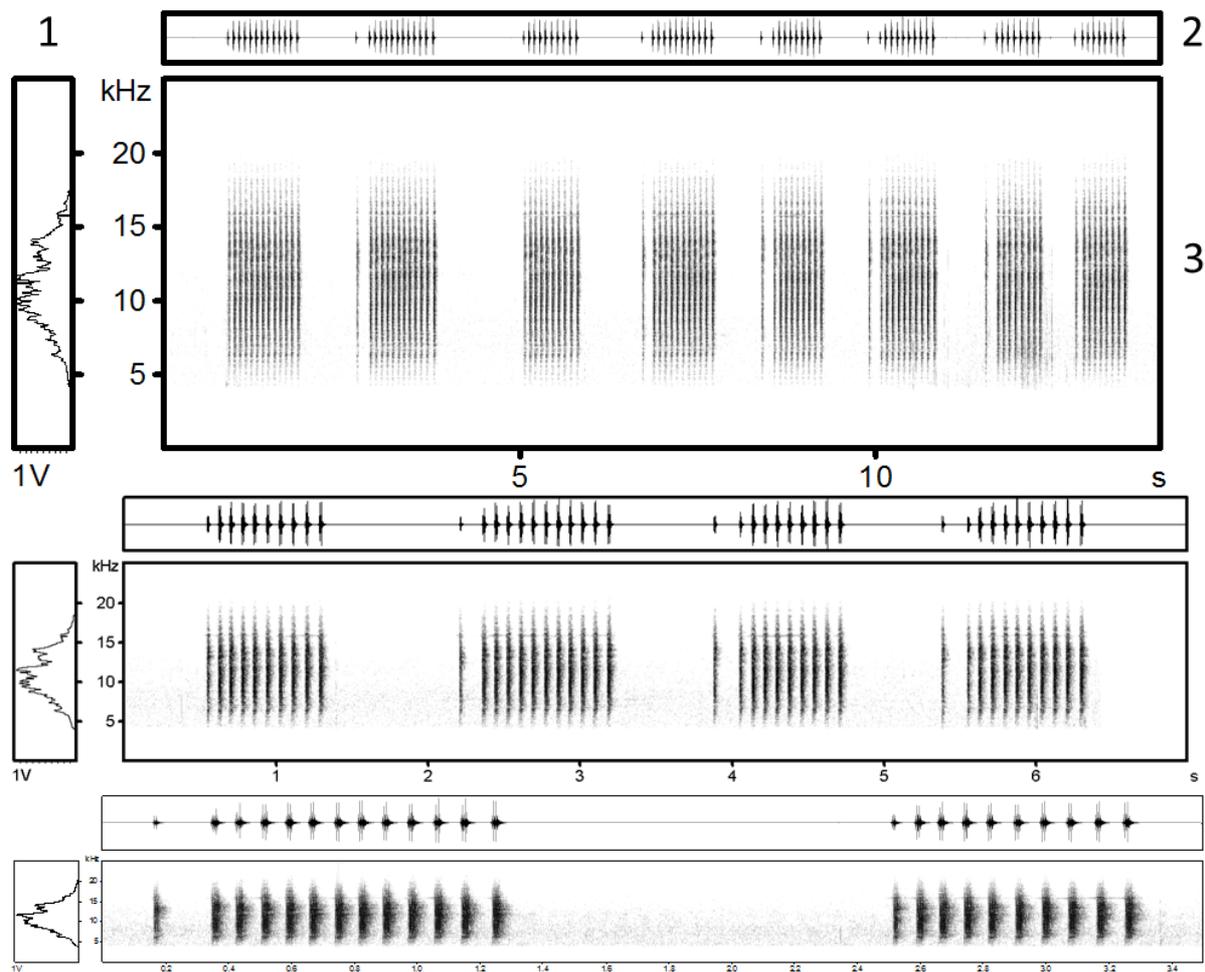
**Genitalia** (Figures 2E to 2H) Pygophore dark-brown on dorsal surface and brown on lateral sides. Pygophore distal shoulder not developed. Pygophore inner tooth absent. Upper lobe flat and moderately developed, distant from dorsal beak with a sharply rounded tip; Basal lobe present, moderately developed and rounded in lateral view. Dorsal beak present and part of chitinized pygophore. Claspers dark-brown, medium-sized, closely aligned ending on a rounded, sharp tip. Uncus brown, duck-bill shaped, small and flat, not dominant. Uncus lateral lobes absent. Aedeagus basal plate, in lateral view, with an undulated ventral surface skewed towards the proximal end; In ventral view, apically broad with a small constriction mid-ventrally expanding afterwards with a midgroove between two longitudinally expanded lobes; Basal portion of basal plate directed forwards and away from thecal shaft; Basal plate ventral rib not apparent; Basal plate attached with a functional membranous “hinge”. Theca, in lateral view, curved into a gentle arc; Thecal pseudoparamers present, dorsal of theca, originating closer to theca than its base; Endothecal ventral support present; Thecal aperture upper diagonal in lateral view.

**Female morphology** Females overall slightly darker than males. Pronotal posterior border light-brown. Mesonotal “crown-like” mark much more faded and smaller than males. Scutellum light-brown. Meso- and metatarsi lighter in colour, light-brown turning brown towards claws. Opercula almost reaching posterior border of StII but much smaller. T1 and T2 totally dark-brown. Abdominal ventral midline fascia dark-brown very well defined. StVII yellowish and split, with a light-brown groove on each side. Stigma dorsal beak dark-brown. Ovipositor brown with dark-brown tip.

**Body measurements for *T. afroamissa* males (n=10)** Total length:  $27.17 \pm 1.25$  mm; Pronotal length:  $2.79 \pm 0.13$  mm; Mesonotal length:  $4.35 \pm 0.26$  mm; Forewing length:  $21.26 \pm 0.97$  mm; M+CuA length:  $1.26 \pm 0.19$  mm. Female and additional body measurements can be found on Table 3.

**Bioacoustics** The male acoustic signals here described are based on the analysis of the calling song of six males recorded at T= 38–40 °C (see Figure 3). The typical calling song is composed by the repetition of a phrase

subdivided into two parts: A—a first single, short echeme and B—a longer group composed of  $9 \pm 7.461$  echemes (6–50,  $n=124$ ) and the interval between parts A and B has a duration of  $155 \pm 53$  ms (112–539 ms;  $n=99$ ). In 23.6% of the phrases part A was absent. We also report a single calling song with a continuous phrase without any apparent pauses.



**FIGURE 3.** *Tettigetta afroamissa* nov. sp. calling song profile with successive ampliation of recorded phrases. Mean frequency spectrum (1), oscillogram (2) and spectrogram (3). Calling song recorded on Afouzar, Middle Atlas, Morocco at 39–40°C.

Peak frequency of all calling songs is at  $11.72 \pm 0.79$  kHz, maximum and minimum frequencies are  $18.45 \pm 1.74$  kHz and  $4.14 \pm 0.44$  kHz, respectively.

Additional temporal and frequency-based variables are indicated in Table 4. Because of the similarities in frequencies of parts A and B, these were grouped in the same analysis.

**Diagnosis** *T. afroamissa* is morphologically similar to all other *Tettigetta* spp. but presents some peculiarities, allowing for its ready separation from its closest relatives. With an average total body length of 27 mm, it seems to be the genus' largest species (Mendes *et al.* 2014, Simões *et al.* 2014, Puissant & Sueur 2010). *T. afroamissa* shows unique colour traits: all examined specimens have a black stripe running across the entire length of the ventral surface of the abdomen and an olive-green arrow-shaped stripe in the pronotum midline, which, upon death, fades over time to a paler shade of green in dry specimens (see image S5 for a live male bearing the typical olive-green stripe on the pronotum).

**TABLE 3.** Descriptive statistics of morphological variables performed on samples of *T. afroamissa* and *B. dimelodica*. Body measurements are presented as average  $\pm$  SD in mm.

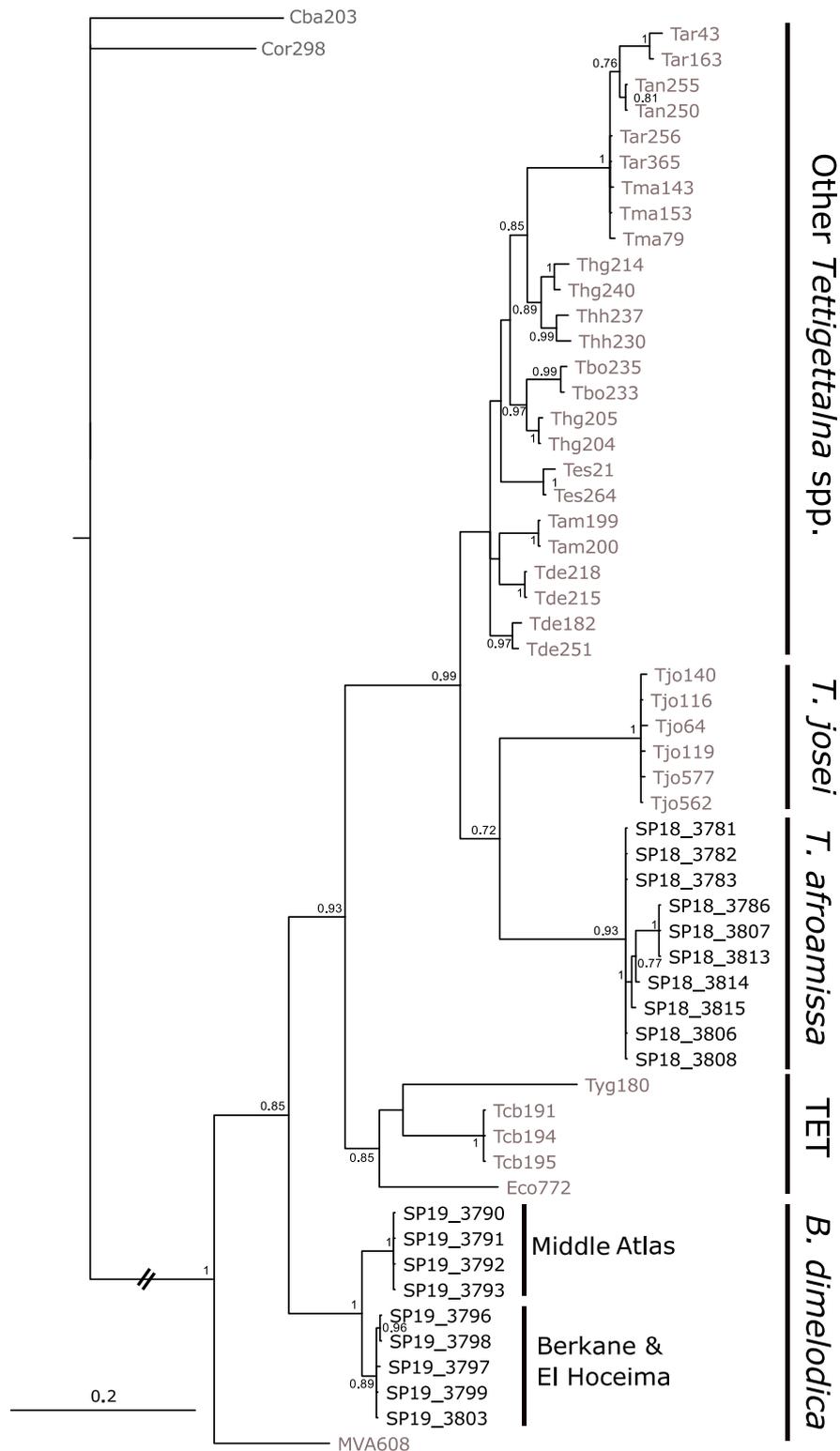
Body region	Code	<i>T. afroamissa</i>						<i>B. dimelodica</i>			
		Male (n=10)			Female (n=3)			Male (n=13)			Female (n=1)
		Mean $\pm$ SD	Min-Max	Mean $\pm$ SD	Min-Max	Mean $\pm$ SD	Min-Max	Mean $\pm$ SD	Min-Max	Min-Max	
Head and thorax	TL	27.31 $\pm$ 1.11	25.93–29.21	25.84 $\pm$ 1.16	24.72–27.03	16.99 $\pm$ 0.78	15.59–18.30			17.30	
	HL	2.05 $\pm$ 0.12	1.86–2.20	1.98 $\pm$ 0.06	1.93–2.02	1.47 $\pm$ 0.11	1.24–1.61			-	
	HW	5.97 $\pm$ 0.21	5.72–6.29	5.64 $\pm$ 0.32	5.41–5.86	3.86 $\pm$ 0.15	3.56–4.13			-	
	EO	0.77 $\pm$ 0.04	0.71–0.84	0.76 $\pm$ 0.03	0.73–0.78	0.54 $\pm$ 0.05	0.46–0.61			-	
	OO	1.37 $\pm$ 0.05	1.3–1.46	1.34 $\pm$ 0.18	1.22–1.47	0.89 $\pm$ 0.04	0.81–0.96			-	
	LrL	1.15 $\pm$ 0.11	1.01–1.37	1.05 $\pm$ 0.16	0.94–1.16	0.85 $\pm$ 0.08	0.76–1.04			-	
	LiL	2.92 $\pm$ 0.14	2.66–3.10	2.62 $\pm$ 0.07	2.57–2.67	1.97 $\pm$ 0.11	1.70–2.15			-	
	VW	2.91 $\pm$ 0.12	2.71–3.10	2.82 $\pm$ 0.25	2.64–2.99	1.91 $\pm$ 0.12	1.68–2.06			-	
	FR	0.62 $\pm$ 0.05	0.55–0.69	0.6 $\pm$ 0.03	0.59–0.62	0.40 $\pm$ 0.06	0.31–0.52			-	
	PC	2.36 $\pm$ 0.11	2.23–2.50	2.28 $\pm$ 0.01	2.28–2.29	1.54 $\pm$ 0.09	1.36–1.66			-	
	PL	2.82 $\pm$ 0.09	2.67–2.95	2.66 $\pm$ 0.25	2.48–2.83	1.74 $\pm$ 0.15	1.46–1.98			-	
	PW	6.71 $\pm$ 0.37	6.20–7.34	6.31 $\pm$ 0.43	6.01–6.62	4.31 $\pm$ 0.26	3.65–4.58			-	
	ML	4.40 $\pm$ 0.19	4.15–4.68	4.12 $\pm$ 0.52	3.76–4.49	2.67 $\pm$ 0.11	2.43–2.85			-	
Abdomen	OP	3.92 $\pm$ 0.17	3.64–4.17	1.76 $\pm$ 0.22	1.60–1.91	2.55 $\pm$ 0.16	2.09–2.73			-	
	LS	1.62 $\pm$ 0.09	1.52–1.77	-	-	1.32 $\pm$ 0.13	1.03–1.49			-	
	TyL	1.53 $\pm$ 0.06	1.43–1.64	-	-	0.99 $\pm$ 0.07	0.90–1.14			-	
	TyW	2.84 $\pm$ 0.06	2.76–2.95	-	-	1.93 $\pm$ 0.10	1.67–2.06			-	
Legs	PF	3.20 $\pm$ 0.11	3.05–3.36	3.08 $\pm$ 0.06	3.04–3.13	2.10 $\pm$ 0.15	1.78–2.26			-	
Wings	FwL	21.37 $\pm$ 0.85	20.16–22.84	20.28 $\pm$ 0.91	19.42–21.23	13.39 $\pm$ 0.54	12.42–14.27			13.59	
	FwW	7.50 $\pm$ 0.27	7.13–7.88	7.11 $\pm$ 0.48	6.67–7.62	5.37 $\pm$ 0.84	4.87–8.10			5.10	
	BCL	1.85 $\pm$ 0.14	1.68–2.06	1.72 $\pm$ 0.10	1.62–1.81	1.23 $\pm$ 0.11	1.09–1.42			1.24	
	McuA	1.26 $\pm$ 0.18	0.92–1.48	1.34 $\pm$ 0.21	1.10–1.50	1.21 $\pm$ 0.18	0.94–1.41			1.22	
	RCL	8.53 $\pm$ 0.36	7.93–9.16	8.32 $\pm$ 0.39	7.95–8.72	6.12 $\pm$ 0.36	5.20–6.59			6.24	

**TABLE 4.** Time and frequency based parameters of the analyzed phrases of *T. afroamissa*. Frequency variables values are presented in kHz.

<i>T. afroamissa</i>	Phrase	Part A						Part B					
		Mean±SD	Min-Max	n	Mean±SD	Min-Max	n	Mean±SD	Min-Max	n	Mean±SD	Min-Max	n
<b>Time variables</b>													
Duration (ms)	726 ± 582	314–3749	124	10 ± 4.5	5–27	97	720 ± 580	309–3733	124				
Echeme duration (ms)	-	-	-	Same as above			20.97 ± 8.26	5–43	1364				
Echeme rate (echeme.s <sup>-1</sup> )	-	-	-	-	-	-	16.21 ± 1.73	10.88–19.42	1364				
Interval (ms)	326 ± 116	186–906	94	-	-	-	51.20 ± 7.07	26–63	1340				
<b>Frequency variables</b>													
Mean ± SD	11.72 ± 0.79	4.14 ± 0.44	18.45 ± 1.74	14.30 ± 1.87	9.93 ± 0.56	11.50 ± 0.48	12.82 ± 0.45	2.89 ± 0.55					
Min-Max	7.21–14.25	3.93–8.81	15.46–23.81	7.68–19.87	7.59–10.96	10.03–12.75	11.25–14.34	1.41–4.69					

**TABLE 5.** Mean pairwise genetic distances (%) between the taxa considered for phylogenetic analysis: P-distances in the upper diagonal and Kimura 2-parameter distances in the lower diagonal. Highlighted values in bold belong to genus *Tettigetta*.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1. <i>Cicada barbara</i>		12.0	18.6	19.6	18.6	18.9	20.0	19.5	19.6	19.4	19.5	19.3	18.4	19.4	19.1	19.4	19.9	20.2
2. <i>Cicada orni</i>	13.3		17.2	19.3	19.3	19.1	20.0	19.7	19.5	19.4	20.6	18.4	19.3	19.2	18.9	18.7	19.0	18.7
3. <i>Hilaphura varipes</i>	21.5	19.6		11.4	12.4	11.2	11.7	12.4	12.3	12.0	12.0	11.2	10.9	11.3	11.4	10.9	12.9	9.8
4. <i>Eurphara contentei</i>	22.9	22.4	12.5		9.6	8.1	10.8	10.7	10.6	10.3	9.7	10.0	9.2	9.0	9.6	9.4	11.4	9.8
5. <i>Tympanistalna gastrica</i>	21.5	22.4	13.8	10.4		9.8	12.4	13.5	13.2	13.3	12.7	11.4	11.4	11.6	12.4	11.3	12.4	11.5
6. <i>Tettigetacula baenai</i>	21.9	22.2	12.3	8.7	10.7		10.4	10.6	10.6	10.3	9.6	11.0	9.0	8.6	9.0	8.9	11.5	9.7
7. <i>Tettigetamna estrelae</i>	23.4	23.3	12.9	11.7	13.7	11.3		7.7	7.4	7.8	5.3	9.2	5.8	5.1	5.4	4.8	9.5	11.8
8. <i>Tettigetamna argentata</i>	22.7	23.0	13.7	11.7	15.0	11.6	8.3		1.7	1.9	7.4	9.6	6.3	6.6	6.4	7.3	10.4	11.1
9. <i>Tettigetamna mariaae</i>	22.8	22.7	13.7	11.6	14.7	11.6	8.0	1.8		1.2	7.1	9.4	6.1	6.2	6.1	6.9	10.8	10.7
10. <i>Tettigetamna aneabi</i>	22.7	22.6	13.3	11.2	14.8	11.2	8.4	1.9	1.2		7.3	8.8	5.9	6.7	6.0	7.2	10.4	10.0
11. <i>Tettigetamna boulandi</i>	22.8	24.2	13.2	10.5	14.0	10.3	5.6	7.9	7.6	7.8		9.2	5.6	4.3	5.4	4.9	9.8	11.6
12. <i>Tettigetamna josei</i>	22.5	21.1	12.2	10.7	12.5	11.9	10.0	10.4	10.2	9.5	10.0		8.0	8.7	7.9	8.5	9.6	10.0
13. <i>T. helianthemi helianthemi</i>	21.3	22.4	11.9	9.9	12.5	9.7	6.1	6.7	6.4	6.3	5.9	8.5		3.4	5.2	5.2	9.2	10.5
14. <i>T. helianthemi galantei</i>	22.6	22.3	12.4	9.6	12.7	9.2	5.3	7.0	6.6	7.1	4.5	9.4	3.6		5.0	4.3	9.0	11.0
15. <i>Tettigetamna armandi</i>	22.2	21.9	12.5	10.4	13.7	9.6	5.7	6.8	6.5	6.4	5.7	8.5	5.5	5.2		3.7	9.0	10.4
16. <i>Tettigetamna defanti</i>	22.7	21.6	11.9	10.1	12.3	9.5	5.0	7.8	7.3	7.7	5.2	9.1	5.5	4.5	3.9		8.3	10.9
17. <i>Tettigetamna afroamissa</i>	23.4	22.0	14.3	12.5	13.6	12.6	10.3	11.5	11.9	11.5	10.7	10.4	10.0	9.8	9.8	8.9		11.9
18. <i>Berberigetta dimelodica</i>	23.8	21.6	10.6	10.6	12.7	10.5	13.0	12.1	11.6	10.9	12.8	10.8	11.4	12.0	11.3	11.8	13.1	



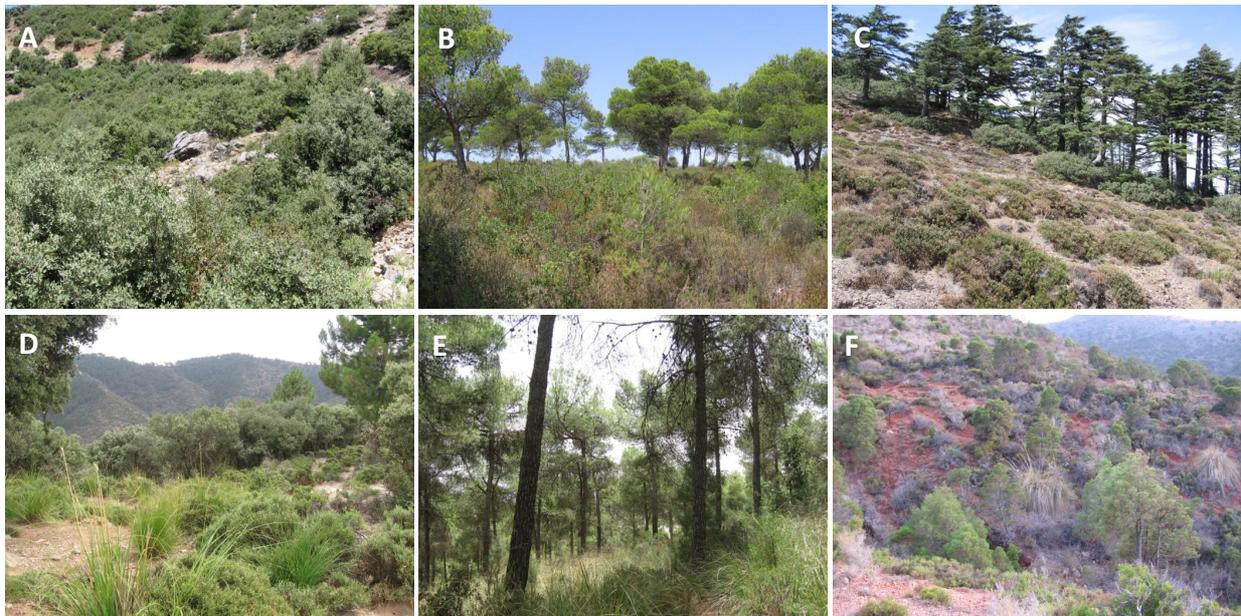
**FIGURE 4.** Bayesian inference phylogenetic tree of Cytochrome C oxidase subunit I mitochondrial DNA of *T. afroamissa* and *B. dimelodica* with other previous published taxa. Posterior probabilities are shown next to branch nodes. TET stands for *Tettigetallna*—*Euryphara*—*Tympanistalna* clade. Scale bar represents the number of estimated changes per branch length. *C. barbara* (Cba203) and *C. orni* (Cor298) were set as an outgroup. *T. afroamissa* and *B. dimelodica* taxa IDs are detailed on Table 2. Additional taxa details are included on supplementary information Table S1. Root was truncated with double dash totalling 0.6 changes per branch length.

Acoustic analysis enables easy and accurate identification of all *Tettigetta* species. *T. afroamissa* is no exception. Its calling song is structurally different from all other *Tettigetta* spp., although reminiscent of *T. argentata* (Olivier, 1790) and *T. boulandi* Puissant, 2010.

The song of *T. afroamissa* can be distinguished from *T. argentata* for it has higher echeme rate ( $t= 16.21 \pm 1.73$  echemes. $s^{-1}$  vs  $t= 12.82 \pm 1.49$  echemes. $s^{-1}$ ) and a shorter inter-echeme interval ( $t= 51.20 \pm 7.07$  ms vs  $t= 71.00 \pm 13.00$  ms) (Mendes *et al.* 2014).

*T. boulandi* has a typical calling song with a short echeme ( $t= 200 \pm 110$  ms) followed by a long echeme ( $t= 2.17 \pm 0.30$  s), whereas in *T. afroamissa* this initial echeme is even shorter ( $t= 10 \pm 4.5$  ms), followed by a succession of very short echemes ( $t=720 \pm 580$  ms), instead of a single one. Inter-phrase interval is also much shorter for *T. afroamissa* ( $t= 326 \pm 116$  ms) than for *T. boulandi* ( $t= 3270 \pm 680$  ms). For additional time and frequency measurements regarding *T. boulandi* see Puissant & Sueur (2010).

**DNA barcoding** Males from all sampled locations were sequenced for COI. Four haplotypes were recovered in a total of 10 sequences. The dataset includes one non-synonymous mutation and a total of 14 polymorphic sites, corresponding to a nucleotide diversity of  $\pi=0.1075$ . All *T. afroamissa* sequences grouped in a fully supported monophyletic clade (Figure 4) and intraspecific pairwise distances (K2P) varied from 0.5 to 2.1 %. This clade clusters with remaining *Tettigetta* spp. in an unresolved polytomy. Mean genetic distances among *T. afroamissa* and all other species of the genus are shown in Table 5, and vary from 8.9% (with *T. defaulti*) to 11.9 % (with *T. mariae*). Thus, the genetic distance associated with the fragment of COI used here, the “barcode gap”, is high enough to be used for DNA barcoding of *T. afroamissa*.



**FIGURE 5.** Habitats of *T. afroamissa* (A-D) and *B. dimelodica* (D-F) in Morocco: Rif mountains near Chefchaouane (A), Bni Hadifa (B) and Taferka (C); Middle Atlas near Taza (D); Berkane (E) and El Hoceima (F). Specimens were captured in all locations but C (see supplementary Table, S2). Photos by VL Nunes.

**Habitat** (Figure 5) An arboreal species, inhabiting open Mediterranean-type woodland and tall scrubland. This species has been scored singing mainly on holm-oak trees (*Quercus rotundifolia*) and bushes such as *Pistacia lentiscus* and *Cistus* spp. but locally, in the Rif, it was found on pine trees (*Pinus* spp.) (Figure 5B), *Abies pinsapo* var. *marocana* and *Cedrus atlantica* (Figure 5C) and almond trees (*Prunus dulcis*).

**Distribution** Northern Morocco, along the Rif Mountains and nearby Mediterranean coastline between Tetuan and Al Hoceima. Also found in the northern parts of the Middle Atlas, near Taza (Figure 1). Not found near Ceuta or Tangier.

**Etymology** Specific epithet formed by combining the suffix *afro* (pertaining to Africa) and the prefix *amissa*, feminine of the latin *amissus*, meaning “having been lost” or “let go”. Literal translation would be “cicada (of the genus *Tettigettna*) left / lost in Africa” as this new species is the only *Tettigettna* spp. known so far to occur in Africa, the remaining being European.

### ***Berberigettna* nov. gen. Costa, Nunes, Marabuto, Mendes & Simões**

**Diagnosis** This genus can be readily distinguished from other morphologically similar genera by the analysis of the male genitalia. The type species has a very large tube-like aedeagus with two pseudoparamers fused until three quarters of total thecal length, ending in a sharp-tip and about of the same length as the endotheca (see Figure 6F). Therefore, it can be distinguished from the similar genus *Tettigettnacula* (type species: *T. baenai* (Boulard, 2000)) for the latter has two unfused thick pseudoparamers arising dorsally from base of the theca, and separate from the endotheca (Puissant & Sueur 2010). *Berberigettna* differs from *Cicadetta* Kolenati, 1857 (type species: *Cicadetta montana* (Scopoli, 1772)) in aedeagus morphology: *C. montana* shows a similarly long aedeagus, yet the pseudoparamers are exceedingly long and partly unfused, surpassing the distal end of theca by about half its length (Moulds 2012).

**Type species** *Berberigettna dimelodica* designed by monotypy.

**Etymology** Name formed by combining the suffix *Berber* (pertaining to the Maghrebian Roman region, Barbaria, and the prevailing ethnic group in northern Maghreb) and the prefix *-gettna*, an arbitrary combination of letters associated with small cicada species, as in *Tettigettna*.

### ***Berberigettna dimelodica* sp. nov. Costa, Nunes, Marabuto, Mendes & Simões**

**Material examined** Paratypical series consists of a total of 14 specimens (13 males and one female). Designated holotype is SP19\_3795 (♂), and female paratype is SP19\_3787 (♀). See Table 2 for additional information on paratypical series, specimen IDs, collection sites and GPS data. See Figure 6 for images on male holotype, female paratype (see supplementary image, S6 for live specimens) and details of the male genitalia.

#### **Male morphology**

**Head** Supra-antennal plate produced into a pointed lobe; Supra-antennal plate nearly meeting the eye. Postclypeus subquadrate to round in front view; Postclypeus transversely grooved towards distal ends. Rostrum brown, reaching the center of mid-trochanters when in resting position. Antennae brown, 7-segmented. Postclypeus dark brown, with apical yellowish-brown spot, grooves light-brown or yellowish; Anteclypeus yellowish with a brown central spot. Gena and lorum brown to light-brown covered with white long pilosity. Supra-antennal plates light brown distally near the eye, becoming dark-brown towards midline. Three red ocelli. Eyes light-brown. Dorsal surface of head dark-brown, supraocular border brown, with yellowish stripe on epicranial suture.

**Thorax** Pronotal collar broad, slightly greater than eye width; Pronotal lateral development ampliate, sloping in lateral view, evenly rounded in dorsal view. Pronotal mid-lateral tooth absent. Scutellum wider than long. Epimeral lobe not reaching operculum. Metanotum partly visible at dorsal midline, not expanded over tymbals. Pronotum brown with a dark-brown stripe along dorsal midline, ending posteriorly in dark-brown spot. Mesonotum with two yellowish fasciae bordering between parapsidal suture and submedian sigillae prolonging to anterior arms of scutellum; Mesonotal lateral dorsal margins yellowish. Central area of scutellum brown with yellowish arms. Metanotum yellowish, brown at dorsal midline.

**Legs** Profemur with a large primary erect spine plus two smaller secondary spines dark-brown/ brownish in colour, some individuals with a much smaller fourth spine. Meracanthus triangular. Tarsal formula 3-3-3. General brown to yellowish in colour. Metatibiae with four long fine reddish spurs on inner side and two smaller reddish spurs on outer side. Coxae yellowish, with a central dark-brown stripe, becoming gradually browner and less yellowish towards metacoxae. Trochanters brown. Meso and metafemurs yellowish with dark-brown to brownish stripes. Tarsi and tibia light-brown.

**Wings** Forewing with eight apical and four subapical cells. Ulnar cell 3 angled to radial cell. Costal vein parallel-sided to node. Pterostigma present becoming darker towards distal end. CuA weakly bowed. M and

CuA meeting at basal cell with stems completely fused. RA<sub>1</sub> slightly diverging from subcostal at subapical region before crossvein. C and R+Sc close together. CuP and 1A non-fused at their bases. Forewing outer margin developed for its total length. Membrane hyaline. Hindwing vein 2A with an infuscation running alongside total length of vein. First cubital cell width at distal end much greater than second cubital cell. Anal lobe broad, with vein 3A bowed at distal end. Larger forewing proximal veins yellowish with smaller apical veins brown, same vein colour pattern for hindwing. Costal vein yellowish. Basal membrane and plaga yellowish.

**Opercula** More or less confluent with distal margin of tympanal cavity, well developed towards abdominal midline with sharply rounded apices facing midline. General opercula colour yellowish becoming brown at the base. Meracanthus following the same colour pattern as opercula.

**Tymbals** Tymbal covers absent. Four to five ribs, broadening apically, three of which arising from anterior proximal part of a large basal dome covering over half total length of tymbal. First anterior rib is slender, with a break at about a third of its length. Fourth rib arising from anterior distal side of basal dome more or less evident amongst individuals. Some specimens present a fifth less defined rib arising from posterior distal end of the basal dome, transversal to fourth rib and converging in a sharp end. Tymbal ribs and basal dome brownish-grey; tymbal plate light-grey.

**Abdomen** Tergites T2 and T3 much enlarged accounting for about a third of total abdominal length. StVIII greater in length than StVII. T1 and T2 dark-brown; T4–7 dark-brown on dorsal midline, sides red and covered in fine silvery pubescence; T8 dark-brown on dorsal midline, sides yellowish. Sternite I brown; StII yellowish with a brown patch on elevated central area; StIII–VIII yellowish. Epipleurites yellowish.

**Genitalia** (Figure 6C to 6F) Pygophore distal shoulder not developed; Pygophore inner tooth absent; Upper lobe present, small and rounded, distant from dorsal beak; Basal lobe small to moderately developed ending in a sharp, rounded tip, in lateral view. Dorsal beak well developed, sharp and part of chitinized pygophore. Ventrobasal pocket absent. Claspers small-medium sized, hooked slightly outwards on distal end, rounded tip. Uncus duck-bill shaped, small and flat, not dominant and retractable within pygophore; Uncus lateral lobes absent. Aedeagal basal plate, undulated in lateral view, weakly depressed on dorsal midline; Basal plate apically broad, flat and rounded in ventral view, with a medial small sharp-tipped lobe on both sides, followed by a tube-like constriction leading to theca, gradually narrowing, slight medial lateral depression; Basal plate bearing a ripple-like pattern in dorsal view. Basal portion directed forwards away from thecal shaft; Ventral rib not apparent; Basal plate completely fused to theca without mobility. Theca very long and J-shaped in lateral view. Thecal pseudoparamers lateral of theca, dorsally fused until two thirds of theca length, very flat, as long as endotheca, ending on an upward pointed, sharp tip; Ventral support absent. Pygophore dorsal surface light-brown to yellow. Claspers dark-brown. Uncus brown.

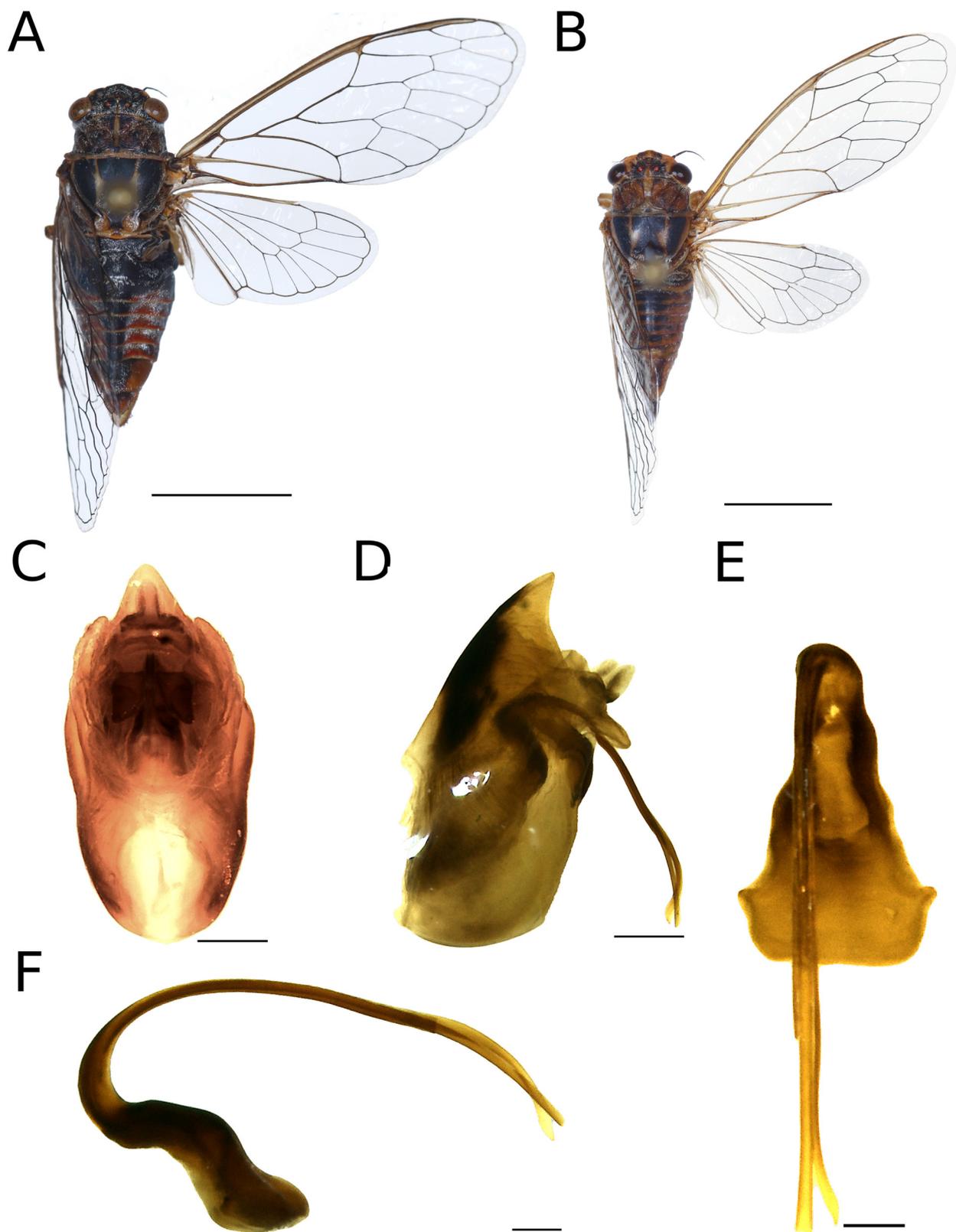
**Female morphology** Only one female known so far (see supplementary image, S6 for the live specimen). Generally lighter in colour than male. Postclypeus yellowish with brown grooves, genae and lora light brown; Legs generally light brown; Dorsal surface of head light-brown with brown patterns; thorax and scutellum light-brown. Abdomen light brown laterally, with a lighter brown on dorsal midline.

**Body measurements for 13 males of *B. dimelodica*** Total length:  $16.99 \pm 0.78$  mm; Pronotal length:  $1.74 \pm 0.15$  mm; Mesonotal length:  $2.67 \pm 0.11$  mm; Forewing length:  $13.39 \pm 0.54$  mm; M+CuA length:  $1.21 \pm 0.18$  mm. Female and additional body measurements can be found on Table 3.

**Bioacoustics** The calling song here described is based on the analysis of recordings of three males singing at T=39–40 °C. A typical phrase is structured into four sequential parts (Figure 7): A, a single echeme; B, a series of  $16 \pm 2.60$  echemes (10–21, n=52) in rapid succession; C, a group of  $8 \pm 3.68$  echemes (5–18, n= 53) ending on D, a single, long echeme. In 21.15% and 9.61% of the phrases part A and part D are missing, respectively.

Calling song frequency-based analysis revealed an interesting frequency modulation in part B. Peak frequency for parts A, C and D is  $13.88 \pm 0.79$  kHz, with maximum frequency of  $20.65 \pm 0.54$  kHz. During part B there is an abrupt reduction of the frequency with a peak frequency of  $7.91 \pm 1.62$  kHz, yet, maintaining the maximum frequency at  $21.62 \pm 1.11$  kHz.

For additional time and frequency variables consult Table 6. Note that, due to frequency modulation in part B, it was separated from parts A, C and D in our analysis.



**FIGURE 6.** Body and male genitalia morphology of *Berberigetta dimelodica*. A—Designated male holotype of *B. dimelodica*. Scale bar equals 10 mm; B—Designated female paratype of *B. dimelodica*. Scale bar equals 10 mm; C, D—Male paratypes' pygophore overview in posterior and lateral views, respectively. Scale bars equal 500  $\mu\text{m}$ . E, F—Aedeagus in upper and lateral views, respectively. Scale bars equal 200  $\mu\text{m}$ . Pygophore and aedeagus photos were taken of material preserved in Kaiser gelatin. Note that the tip of the left pseudoparamer is broken.

**TABLE 6.** Time and frequency based parameters of the analyzed phrases of *B. dimelodica*. In the frequency analysis, part B of the calling song was separated from parts A, C and D due to significant frequency downshift in part B. Frequency variables values are presented in kHz.

<i>B. dimelodica</i>	Phrase			Part A			Part B		
	Mean±SD	Min–Max	n	Mean±SD	Min–Max	n	Mean±SD	Min–Max	n
Time variables	Mean±SD	Min–Max	n	Mean±SD	Min–Max	n	Mean±SD	Min–Max	n
Duration (ms)	2218 ± 559	1357–3448	52	30 ± 10	15–56	47	335 ± 52	212–411	52
Echeme duration (ms)	-	-	-	Same as above			2.14 ± 1.06	0.8–7	849
Echeme rate (echeme.s <sup>-1</sup> )	-	-	-	-	-	-	49.16 ± 6.08	36.08–72.67	52
Interval (ms)	259 ± 82	195–614	49	-	-	-	19.55 ± 5.31	2.8–55	797
	Part C			Part D					
Time variables	Mean±SD	Min–Max	n	Mean±SD	Min–Max	n			
Duration (ms)	1364 ± 679	632–2992	53	252.29 ± 79.23	97–430	41			
Echeme duration (ms)	49.2 ± 20.6	5–253	487	Same as above					
Echeme rate (echeme.s <sup>-1</sup> )	7.10 ± 1.04	3.34–10.32	53	-	-	-			
Interval (ms)	108.83 ± 22.24	34–260	435	-	-	-			

continued.

Frequency variables		Peak frequency	Min frequency	Max frequency	Bandwidth
Part ACD	Mean ± SD	13.88 ± 0.79	4.65 ± 0.96	20.65 ± 0.54	15.94 ± 1.31
	Min–Max	11.50–15.50	1.96–6.00	18.70–22.40	12.80–19.78
Part B	Mean ± SD	7.91 ± 1.62	4.39 ± 1.01	21.62 ± 1.11	17.14 ± 1.59
	Min–Max	5.60–16.50	0.30–5.80	13.92–23.40	9.04–22.80
Frequency variables		Quartile 25	Quartile 50	Quartile 75	Quartile (75%–25%)
Part ACD	Mean ± SD	11.91 ± 0.22	13.48 ± 0.27	14.89 ± 0.32	2.98 ± 0.26
	Min–Max	10.70–12.50	12.28–14.40	13.40–15.80	1.87–4.10
Part B	Mean ± SD	7.54 ± 0.61	9.57 ± 0.79	11.61 ± 1.26	4.07 ± 0.93
	Min–Max	6.30–12.00	7.50–13.50	9.70–17.50	2.70–8.20

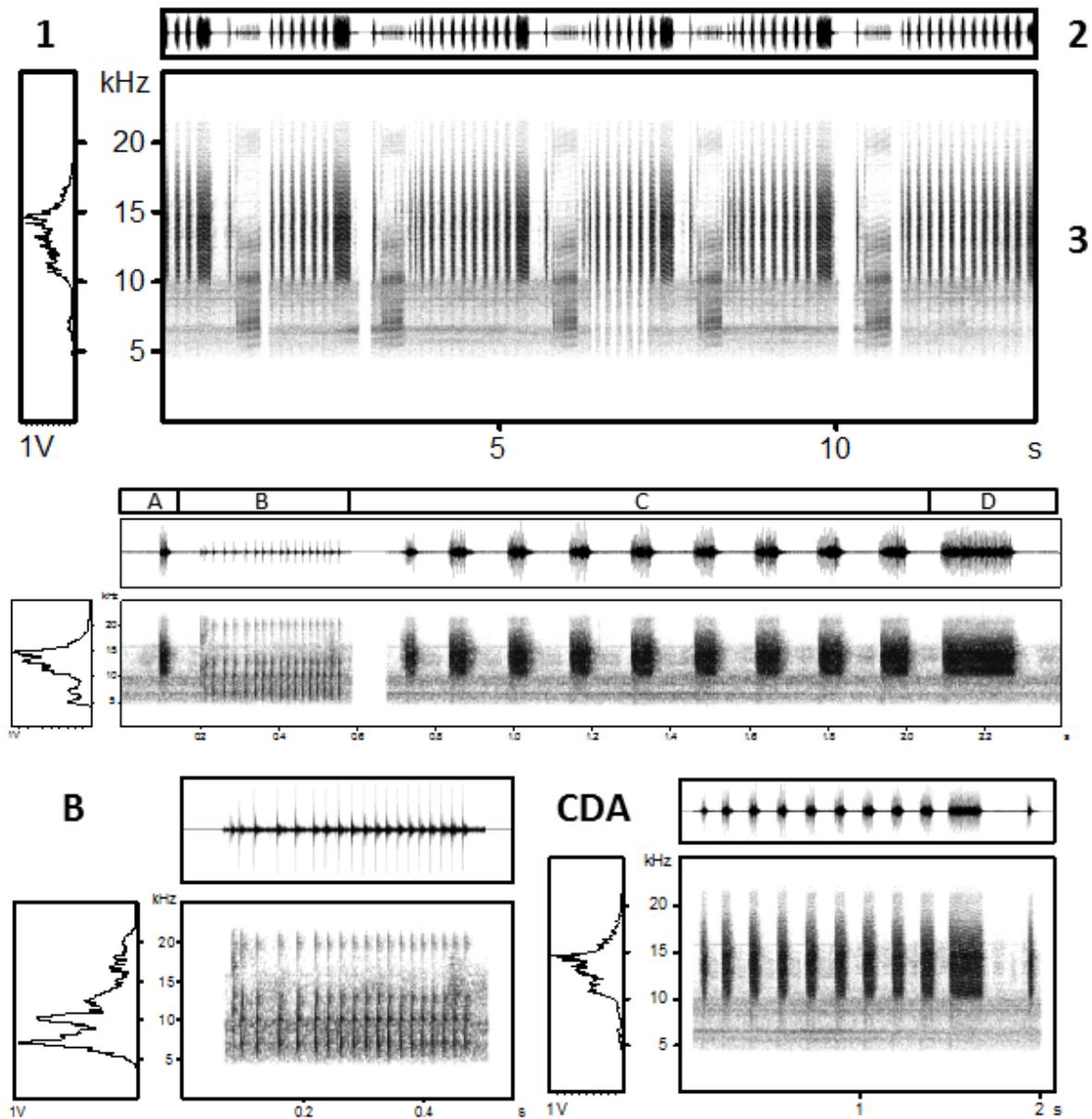
**DNA barcoding** Four haplotypes were recovered among the COI sequences of nine males of *B. dimelodica* sp. nov., with a nucleotide diversity of  $\pi = 0.0164$ . Sequences were clustered into two well supported sister clades (Figure 4) diverging by 2.9 % (K2P distance). These clades are, according to our currently knowledge, geographically segregated. Among the 18 segregating sites observed, 16 are fixed for each clade, being two of them non-synonymous mutations. Mean interspecific genetic distances for *B. dimelodica* are presented in Table 5. The new species is clearly distinguishable within the Cicadettini (*Tettigettna*, *Tettigettnacula*, *Tympanistalna*, *Euryphara* and *Hilaphura*), with mean pairwise genetic distances >10%. The COI fragment is therefore apparently proficient for DNA barcoding of *B. dimelodica*, though the genetic structure reported here must be taken into account.

**Distribution** (Figure 1) Morocco, in the northern parts of Middle Atlas Mountains, near Taza and along the eastern Rif mountains (Al Hoceima), eastward to Berkane (Beni-Snassen Mountains), as the extreme western foot of the Tellian Atlas Mountains. On biogeographical grounds it is possible that this species is also in western Algeria.

**Habitat** (Figure 5) Open scrubland or light xerothermophilous woodland dominated by holm-oak (*Quercus rotundifolia*) in the northern Middle Atlas or mixed pinewoods of *Pinus halepensis* and *Tetraclinis articulata* with a rich understory of *Pistacia lentiscus*, *Chamaerops humilis*, *Rosmarinus officinalis* and *Stipa* spp. Males sing mainly perched on these shrubs, and sometimes on the lower branches of trees (< 3 m height).

**Etymology** Specific epithet *dimelodica* arises from the dual sound production during the calling song of this species, meaning “two melodies”. It consists of two distinct sound patterns, with the second part severely

downshifted in frequency and resembling a human-produced unvoiced linguolabial trill, often referred as “Blowing a raspberry”.



**FIGURE 7.** *Berberigetta dimelodica* calling song profile. Mean frequency spectrum (1), oscillogram (2) and spectrogram (3). Letters A, B, C and D refer to the structural divisions found in a typical phrase. Individualized analysis of part B and parts C, D and A (sequentially) are displayed in the bottom graphs. Calling song recorded on Middle Atlas, Afouzar at 38–40°C.

## Discussion

The two new species described in this paper based on acoustic, morphological and genetic data, used a more comprehensive species concept according to the contemporaneous perspective on species delimitation (De Queiroz 2007, 2016; Hausdorf 2011). For cicadas in general, the male calling song is thought to act as a pre-zygotic barrier which leads to specific-mate recognition and pairing (Paterson 1985), allowing for a reproductive, sometimes semipermeable, separation broadly considered as one of the early stages of species differentiation (Mayr 1963; Nosil 2008).

The placement of *T. afroamissa* **sp. nov.** under *Tettigettna* is supported by aedeagus morphology (Figs. 3D and 3E), size, behaviour and genetic distance. *Tettigettna* spp. are all morphologically similar but are confidently distinguished through the analysis of their calling songs (Puissant & Sueur 2010). While most *Tettigettna* species have small distribution ranges in the Iberian Peninsula, *T. argentata* is an outlier, spreading elsewhere in SW Europe (Puissant & Sueur 2010; Nunes *et al.* 2014b). Despite the limited knowledge on the distribution limits of *T. afroamissa*, the species apparently shows a broad distribution range in Northern Morocco and bears some COI genetic variation, but unlike *T. argentata* (Nunes *et al.* 2014a), it constitutes a monophyletic clade, with no evidence of geographically structured genetic differentiation.

Although the use of the 5' end of the COI gene as DNA barcode has been proven relatively inefficient in the unambiguous identification of European *Tettigettna* spp. (Nunes *et al.* 2014a), this was not the case for *T. afroamissa*. Mean pairwise distance between *T. afroamissa* and all other *Tettigettna* is > 9%, which is well beyond commonly used thresholds for species differentiation with this marker (Hebert *et al.* 2004; Wiemers & Fiedler 2007; Linares *et al.* 2009).

Both phylogenetic trees obtained by Bayesian Inference and Maximum Likelihood (Figure 4 and S3, respectively) agree on the branch topology of the most recent taxa within *Tettigettna*, but such cannot be said about the deeper-level relationships. The new species found in Morocco appears basally segregated in the genus, alongside *T. josei*. Asserting which is the basal taxon will need the inclusion of slower-evolution, nuclear genes. A recent work by Marshall *et al.* 2015, includes a dated global phylogeny from the tribe Cicadettini with mitochondrial and nuclear genes, placing *Tettigettna* very far from all other European genera included in our analyses (*Tettigettna*, *Euryphara*, *Tympanistalna*, *Hilaphura* and *Cicada*). Conversely, it is interesting to note that *Tettigettna* forms a well-defined clade with American, continental Asia, Philippines and Micronesian species. The discovery of the first species of *Tettigettna* out of Europe is an important step towards understanding the place and time of origin of this genus, its evolution and diversification. Further phylogenetic analyses are thus required, with the inclusion of additional genetic data and divergence time estimates.

*Berberigettna* had to be erected as a new genus to accommodate a new species found so far only in Morocco. The type species, *B. dimelodica*, can be readily separated from other closely related genera (*Cicadetta*, *Tettigettna*) with a set of characters, which include genital morphology and a deep genetic divergence. However, the acoustic behaviour of this species turns up as the most striking feature. The very particular calling song shows a downshift in frequency (about 43% reduction) in part B of the phrase. Frequency shifts inside a phrase have also been reported for Dundubinii and Platyleurini cicadas of Southern Asia, amongst others, such as *Meimuna tavoyana* (Distant, 1888), *Purana metallica* Duffels & Schouten, 2007, *Maua albigutta* (Walker, 1857) and *Kalabita operculata* Moulton, 1923 (Gogala 1995; Gogala & Trilar 2004; Gogala *et al.* 2004; Trilar 2006; cf. *P. metallica* as *P. aff. tigrina*).

Some European Cicadettini also reveal some degree of frequency modulation within a phrase, namely *Pagiphora aschei* Kartal, 1978, *P. annulata* (Brullé, 1832), *Euboecana castaneivaga* Gogala *et al.*, 2011 and *H. varipes* (calling songs and spectrograms available at [www.cicadasong.eu](http://www.cicadasong.eu)) but neither as pronounced nor with an abrupt downshift as seen in *B. dimelodica*. Video recordings of a calling male (see video in appendix, S7, credits to E. Marabuto) reveal that during the downshifted portion of the phrase, the male will slightly raise and tighten its abdomen probably with the help of longitudinal ventral muscles, in a similar fashion as *M. albigutta* (Gogala *et al.* 2004), a species with portions of a phrase with abrupt downshifts in frequency. Although it is difficult to uncouple the effect of tympanal gap, opercula and abdominal muscles have in the production and frequency regulation in cicadas, further studies are still needed to better understand the general mechanisms of frequency modulation in cicadas.

Finally, phylogenetic analysis of *B. dimelodica* revealed evidence of population structure. Populations from Berkane and Middle Atlas were recovered as genetically divergent (2.9%) and well resolved sister clades, suggesting two isolated distribution areas. Further fieldwork is required to confirm if they can be separated into different taxa, despite their seemingly alike calling songs. As Berkane is located near the international Morocco-Algeria border, the presence of *B. dimelodica* in this latter country cannot be dismissed.

The two new species here presented confirm the need for more data and effort to properly assess and update our knowledge of biodiversity and evolution of the rich cicadofauna of North Africa. Thus, taking into consideration that the Western Mediterranean area encompasses important biogeographical barriers and each part has been differentially affected by climate changes in the recent geological past, understanding the role of the

Maghreb as a reservoir of biodiversity in general (Schmitt 2007, Husemann *et al.* 2014), or referring to cicadas in particular, is of the utmost importance.

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**S1.** Additional taxa sampling included in our phylogenetic analysis including collection points and GenBank accession numbers.

Taxon	Sample ID	Country	Location	GPS coordinates	GenBank accession n.	Source
<i>Cicada barbara</i>	Cba203	Spain	Sierra Nevada, Lanjarón	36°54'57.78"N; 3°30'14.4"W	KC807317	Nunes <i>et al.</i> , 2014
<i>Cicada orni</i>	Cor298	Portugal	Serra d' Aires e Candeeiros	39°27'17.6"N 8°45'07.8"W	KC807318	Nunes <i>et al.</i> , 2014
<i>Euryphara contentei</i>	Eco772	Portugal	Beringel	38°3'19.5"N; 7°59'50.28"W	KX582155	This paper
<i>Euryphara contentei</i>	Eco827	Portugal	Beringel	38°3'19.5"N; 7°59'50.28"W	KX582156	This paper
<i>Euryphara contentei</i>	Eco828	Portugal	Beringel	38°3'19.5"N; 7°59'50.28"W	KX582157	This paper
<i>Hilaphura varipes</i>	MVA608	Spain	Sierra Nevada, Pinos Genil	37°8'15.5"N; 3°28'34"W	KX582168	This paper
<i>Tettigettalna armandi</i>	Tam199	Spain	near Gibraltar	36°11'17.7"N; 5°21'33.6"W	KC807277	Nunes <i>et al.</i> , 2014
<i>Tettigettalna armandi</i>	Tam200	Spain	near Gibraltar	36°11'17.7"N; 5°21'33.6"W	KC807278	Nunes <i>et al.</i> , 2014
<i>Tettigettalna aneabi</i>	Tan250	Spain	Zagra	37°16'59.82"N; 4°14'4.02"W	KC807301	Nunes <i>et al.</i> , 2014
<i>Tettigettalna aneabi</i>	Tan255	Spain	Zagra	37°16'59.82"N; 4°14'4.02"W	KC807299	Nunes <i>et al.</i> , 2014
<i>Tettigettalna argentata</i>	Tar163	France	Narbonne	43°9'16.92"N; 2°57'49.14"W	KC807234	Nunes <i>et al.</i> , 2014
<i>Tettigettalna argentata</i>	Tar256	Spain	Espiel	38°11'3.72"N; 5°1'36.12"W	KC807232	Nunes <i>et al.</i> , 2014
<i>Tettigettalna argentata</i>	Tar365	Spain	Ayamonte	37°16'3.3"N; 7°20'32.28"W	KC807246	Nunes <i>et al.</i> , 2014
<i>Tettigettalna argentata</i>	Tar43	Portugal	Braga	41°34'54.48"N; 8°19'14.1"W	KC807229	Nunes <i>et al.</i> , 2014
<i>Tettigettalna bouleardei</i>	Tbo233	Spain	Campico de los López, Murcia	37°34'57"N; 1°34'16.5"W	KC807276	Nunes <i>et al.</i> , 2014
<i>Tettigettalna bouleardei</i>	Tbo235	Spain	Campico de los López, Murcia	37°34'57"N; 1°34'16.5"W	KC807275	Nunes <i>et al.</i> , 2014
<i>Tettigettacula baenai</i>	Tcb191	Spain	Grazalema	36°45'24.18"N; 5°24'6.3"W	KC807311	Nunes <i>et al.</i> , 2014
<i>Tettigettacula baenai</i>	Tcb194	Spain	Grazalema	36°45'24.18"N; 5°24'6.3"W	KC807312	Nunes <i>et al.</i> , 2014
<i>Tettigettacula baenai</i>	Tcb195	Spain	Grazalema	36°45'39.18"N; 5°22'57.6"W	KC807313	Nunes <i>et al.</i> , 2014
<i>Tettigettalna defauti</i>	Tde182	Spain	Puerto del Viento, Ronda	36°47'13.32"N; 5°3'11.88"W	KC807305	Nunes <i>et al.</i> , 2014
<i>Tettigettalna defauti</i>	Tde183	Spain	Puerto del Viento, Ronda	36°47'13.32"N; 5°3'11.88"W	KC807307	Nunes <i>et al.</i> , 2014

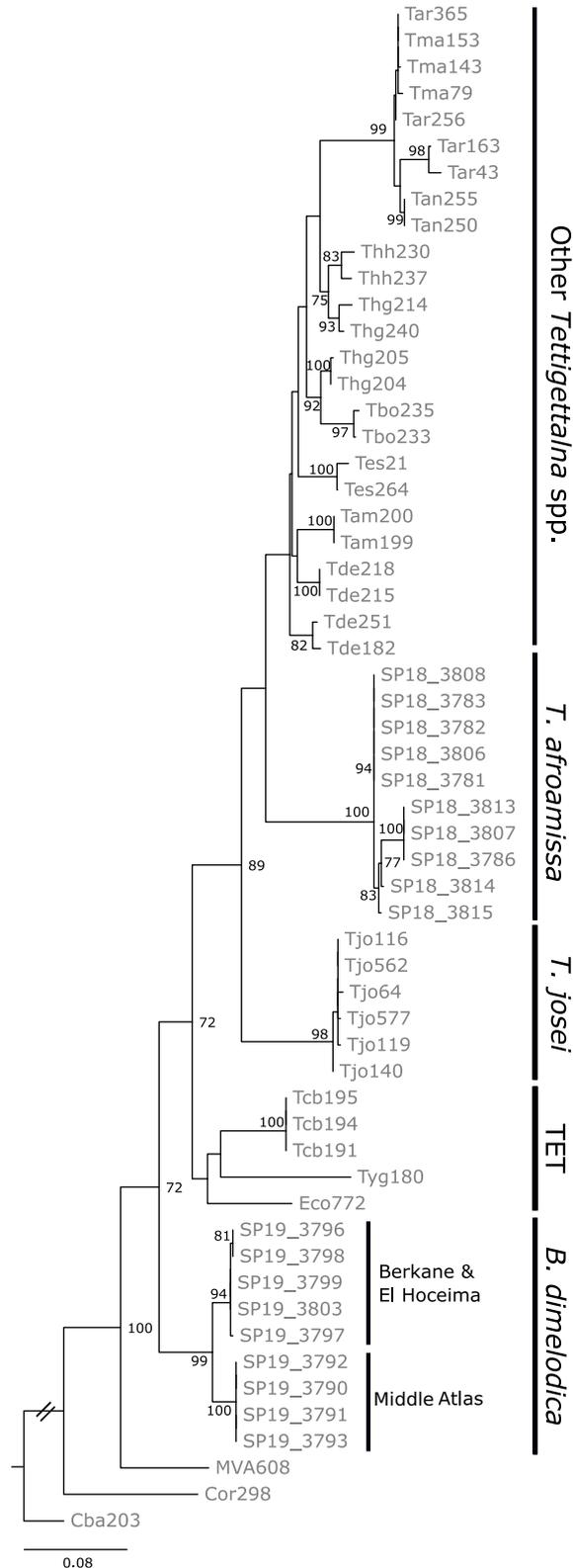
.....continued on the next page

## S1. (Continued)

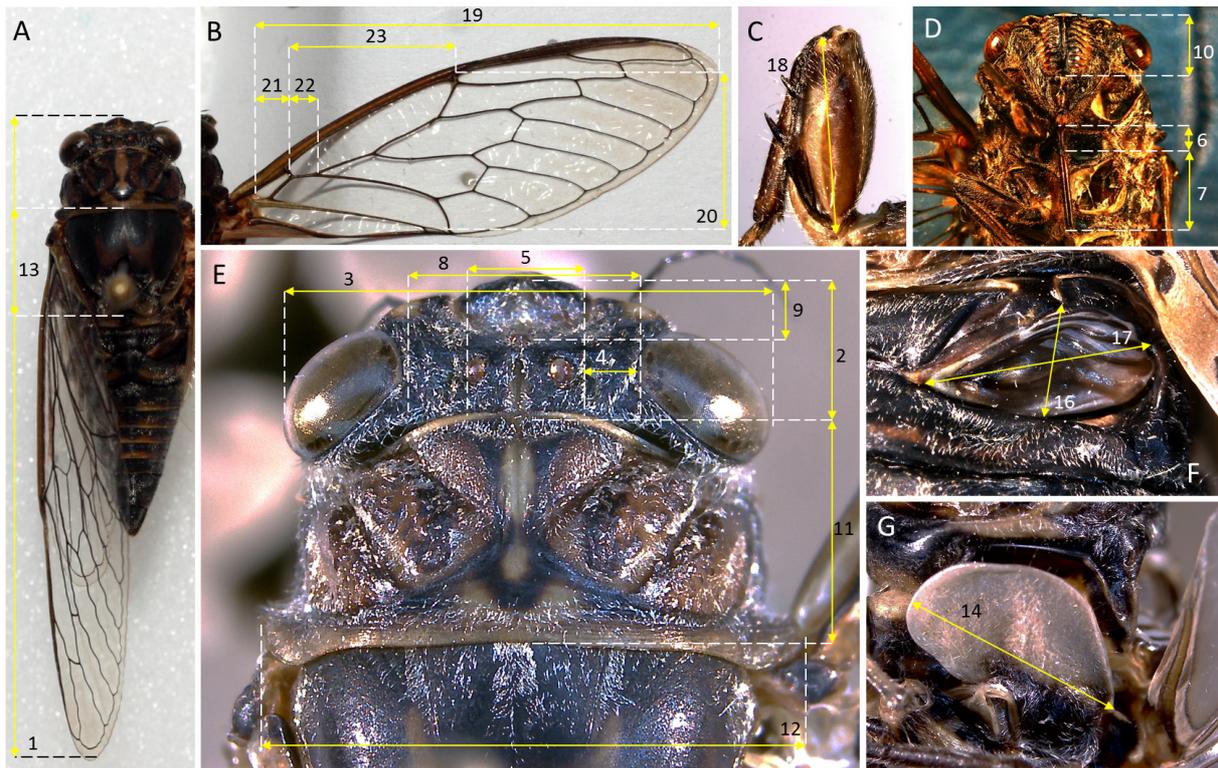
Taxon	Sample ID	Country	Location	GPS coordinates	GenBank accession n.	Source
<i>Tettigettaha defaulti</i>	Tde185	Spain	Puerto del Viento, Ronda	36°47'13.32"N; 5°3'11.88"W	KC807309	Nunes <i>et al.</i> , 2014
<i>Tettigettaha defaulti</i>	Tde188	Spain	Puerto del Viento, Ronda	36°47'13.32"N; 5°3'11.88"W	KC807308	Nunes <i>et al.</i> , 2014
<i>Tettigettaha estrellae</i>	Tes21	Portugal	Braga	41°34'54.48"N; 8°19'14.1"W	KC807263	Nunes <i>et al.</i> , 2014
<i>Tettigettaha estrellae</i>	Tes264	Portugal	Serra da Estrela	40°21'17.76"N; 7°26'24.6"W	KC807265	Nunes <i>et al.</i> , 2014
<i>Tettigettaha helianthemii galantiei</i>	Thg204	Spain	Lanjarón, Sierra Nevada	36°54'57.78"N; 3°30'14.4"W	KC807281	Nunes <i>et al.</i> , 2014
<i>Tettigettaha helianthemii galantiei</i>	Thg205	Spain	Lanjarón, Sierra Nevada	36°54'57.78"N; 3°30'14.4"W	KC807280	Nunes <i>et al.</i> , 2014
<i>Tettigettaha helianthemii galantiei</i>	Thg214	Spain	Capileira, Sierra Nevada	36°57'47.88"N; 3°20'26.52"W	KC807286	Nunes <i>et al.</i> , 2014
<i>Tettigettaha helianthemii galantiei</i>	Thg240	Spain	Laroles, Sierra Nevada	37°2'57.06"N; 3°1'0.9"W	KC807287	Nunes <i>et al.</i> , 2014
<i>Tettigettaha helianthemii helianthemii</i>	Thh230	Spain	Cabo de Gata	36°50'18.3"N; 2°17'35.58"W	KC807297	Nunes <i>et al.</i> , 2014
<i>Tettigettaha helianthemii helianthemii</i>	Thh237	Spain	Vera	37°12'48.06"N; 1°53'58.68"W	KC807293	Nunes <i>et al.</i> , 2014
<i>Tettigettaha josei</i>	Tjo116	Portugal	Lagoa, Algarve	37°8'9.36"N; 8°23'4.2"W	KC807271	Nunes <i>et al.</i> , 2014
<i>Tettigettaha josei</i>	Tjo119	Portugal	Budens	37°4'45.2"N; 8°50'11.6"W	KF977491	Simões <i>et al.</i> , 2014
<i>Tettigettaha josei</i>	Tjo140	Portugal	Castro Marim, Algarve	37°11'10.92"N; 7°29'2.1"W	KC807269	Nunes <i>et al.</i> , 2014
<i>Tettigettaha josei</i>	Tjo562	Spain	Cartaya	37°15'38.4"N; 7°7'43.5"W	KF977504	Simões <i>et al.</i> , 2014
<i>Tettigettaha josei</i>	Tjo577	Spain	Cartaya	37°14'3.7"N; 7°3'56.8"W	KF977505	Simões <i>et al.</i> , 2014
<i>Tettigettaha josei</i>	Tjo64	Portugal	Vale Judeu, Algarve	37°7'39.78"N; 8°5'36.06"W	KC807274	Nunes <i>et al.</i> , 2014
<i>Tettigettaha mariae</i>	Tma143	Portugal	Vale do Lobo, Algarve	37°3'41.1"N; 8°3'39.12"W	KC807253	Nunes <i>et al.</i> , 2014
<i>Tettigettaha mariae</i>	Tma153	Portugal	Vale do Lobo, Algarve	37°3'41.1"N; 8°3'39.12"W	KC807257	Nunes <i>et al.</i> , 2014
<i>Tettigettaha mariae</i>	Tma79	Portugal	Vale Judeu, Algarve	37°6'20.88"N; 8°5'42.66"W	KC807256	Nunes <i>et al.</i> , 2014
<i>Tympanistalna gastrica</i>	Tyg180	Portugal	Sesimbra	38°27'4.5"N; 9°5'27.9"W	KC807314	Nunes <i>et al.</i> , 2014

S2. GPS coordinates and annotated populations where *T. afroamissa* was heard but not collected.

Population	GPS Coordinates	Date	Habitat notes
Chefchaouane	35° 10' 29.34" N 5° 15' 28.93" W	17-07-2014	<i>Quercus rotundifolia</i> , <i>Pinus</i> sp., <i>Abies</i> sp., <i>Cistus</i> spp., <i>Juniperus</i> sp.
Rif	35° 17' 53.50" N 4° 53' 53.60" W	19-07-2014	Near the seashore, dominated by small shrubs.
	35° 6' 55.68" N 4° 40' 45.13" W	19-07-2014	<i>Prunus dulcis</i> orchard, arid habitat.
	34° 59' 6.76" N 4° 48' 35.15" W	19-07-2014	Dominated by <i>Quercus canariensis</i> .
	34° 57' 38.06" N 4° 40' 48.76" W	19-07-2014	<i>Q. rotundifolia</i> , <i>Cupressus</i> sp. and small shrubs.
	34° 57' 32.05" N 4° 39' 2.75" W	19-07-2014	Dominated by <i>Cupressus</i> sp.
Taza	33° 57' 23.00" N 4° 3' 5.00" W	17-07-2014	Mainly <i>Q. rotundifolia</i> and some <i>Pinus</i> sp.
	33° 43' 16.50" N 4° 15' 38.8" W	16-07-2014	<i>Q. rotundifolia</i> and various shrubs.



**S3.** Maximum likelihood phylogenetic tree obtained with Cytochrome C oxidase subunit I mitochondrial DNA of *T. afroamissa* and *B. dimelodica* and with other previous published taxa. Bootstrap values are shown next to branch nodes. TET stands for *Tettigettacula*—*Euryphara*—*Tympanistalna* clade. Scale bar represents the number of estimated changes per branch length. *C. barbara* (Cba203) and *C. orni* (Cor298) were set as an outgroup. *T. afroamissa* and *B. dimelodica* taxa IDs are detailed on Table 2. Additional taxa details are included on Table S1. Root was truncated with double dash totalling 0.35 changes per branch length.



**S4.** Illustration of the 23 variables of external morphology described on Table 1 (codes used are the same as in Table 1). All images are from parotypical series of *T. afroamissa*. A—Dorsal view; B—Right wing view; C—Right profemur; D—Head and thorax ventral view; E—Head and thorax dorsal view; F—Right tymbal; G—Left operculum.



**S5.** Image of a *T. afroamissa* sp. nov. live male. Notice the olive-green stripe in the pronotum. Images by Eduardo Marabuto.



S6. Image of a live male (left) and a female (right) of *Berberigetta dimelodica* **sp. nov.** Images by Eduardo Marabuto.

S7. Video recording of a male *Berberigetta dimelodica* calling. Note the abdomen tightens during part B of the phrase, resounding as “blowing a raspberry”.