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A perfect duet? The acoustic behaviour of *Anaulacomera almadaenis* sp. nov., a species with an unusual chromosome complement, discovered in the footsteps of the explorers Spix and Martius in Brazil

(Orthoptera, Tettigonioidea, Phaneropterinae)

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To find a mate, males and females of phaneropterine bush-crickets engage in duets. The male starts a duet and a female responds if she is prepared to mate. This pattern is exactly followed in the acoustic communication of a species of the genus *Anacaulomera* from the State of Bahia in Brazil which we newly describe in this manuscript. However, the male also acoustically defends the duet. As soon as it hears a female response, it adds an additional component to its own signal to make the localisation of the female difficult for rivals. At the same time the male reduces the interval between its songs for a faster exchange of information about the location of the partner, and reduces the intensity of the song that it can be heard only from nearby. Besides the nearly perfect anti-eavesdropping modifications of the duet the new species is remarkable concerning its karyotype. The meiotic behaviour of chromosomes in the male may indicate the origin of a new type of sex determination mechanism.

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Introduction

In 1891, at the time of the last review of the subfamily Phaneropterinae (Brunner von Wattenwyl 1891), the genus *Anaulacomera* Stål, 1873 was the largest genus of this subfamily containing 40 species, distributed in Central and South America. Now (Cigliano et al. 2018, abbreviated OSFO) it comprises 114 species, but despite this increase it has less species than the East Asian genus *Elimaea* (164 spp. + 7 sspp.) or the Western Paleartic genus *Poecilimon* (136 + 31), which had only 21 and 30 species respectively in 1891. Comparison of the species numbers and their increase suggest that *Anaulacomera* is very species-

rich but poorly studied genus with probably many undescribed species. *Poecilimon*, for example, had attracted the attention of orthopterologists already long time ago with the first review in 1933 (Ramme 1933) and even its subgroups are now subject of revisions (e. g., Chobanov & Heller 2010). There are also many studies on its distribution as well as on bioacoustics, phylogeny and genome organization (e. g., Heller 1984, Ullrich et al. 2010, Ünal 2010, Grzywacz et al. 2014). Using these information, identification of previously unknown forms becomes much faster, allowing an understanding of the distributional patterns of species and species groups as well as phylogenetic studies. For *Anaulacomera*, however, a first key to species groups was published only in 2015 (Cadena-Castañeda 2015). Before that the various species were nearly unidentifiable and studies with comments like "*Anaulacomera* sp. 1–8" (Braun 2002, 2008) had to be published. Up to now neither the male song nor the female response nor any acoustical or ecological factors permitting the coexistence of *Anaulacomera* species are known for any described species of the genus. In Macaulay Library there are 30 recordings of *Anaulacomera* from Trinidad & Tobago made by Walker in 1966 (Walker 1966), but all remained unidentified.

In *Anaulacomera* as well as in *Poecilimon* the structure of the male genitalia presents the most important morphological characteristics for identification, showing a much higher diversity in *Anaulacomera* than in *Poecilimon*. Otherwise the genera differ strongly. *Poecilimon* is micropterous and flightless with many local forms, while all *Anaulacomera* have well developed wings and are good flyers. However, nothing can be said about the exact ranges of the species because of the difficulties with identification.

We came in contact with *Anaulacomera* during a short excursion after the 12th International Congress of Orthopterology, held in October/November 2016 in Ilheus (Bahia, Brazil), when we could stay some days on the nearby Fazenda Almada. To our surprise we discovered later that nearly exactly 200 years earlier (December 1818) the famous naturalists and explorers of Brazil, Johann Baptist von Spix and Carl Friedrich Philipp von Martius, had visited the same place (Martius 1828, p. 681; see also https://de.wikipedia.org/wiki/Johann_Baptist_von_Spix) and met the German settlers who had founded the fazenda. During our stay we found several phaneropterine bush-crickets, among them one adult male and three nymphs which successfully moulted to adults. It turned out that they all belonged to one and the same species of the genus *Anaulacomera*. They are morphologically very characteristic and different from all known species, so we describe them here as new species and as starting point for the study of *Anaulacomera* bioacoustics.

As typical for phaneropterine bush-crickets, females respond acoustically to the male song (Heller et al. 2015). Using this system the male needs to signal only with relatively large intervals, waiting to hear a response. If successful, he should reduce interval length for a fast phonotactic approach. In addition he should make the female response difficult to locate for eavesdropping rivals or conceal it completely. Our species seems to use several of such tactics, not observed or at least not described and quantitatively measured in any other bush-cricket species before.

Material and methods

The animals were held in plastic containers, differing in size depending on the size of the animals, to avoid dehydration in the laboratory and fed with *Taraxacum officinale*, replaced daily.

Measurements. Total body length, lateral aspect, refers to the mid-line length of the insect from fastigium verticis to tip of abdomen including the subgenital plate. In females, the ovipositor is not included in the measurement of the body length. Measurements of the ovipositor are taken laterally in straight line from tip to base not regarding the curvature. To obtain the mass data, living animals and spermatophores were weighed to the nearest 2 mg (balance Tanita Professional Mini 1210-100).

Acoustics. The male calling song was recorded in the laboratory using a digital bat detector (Pettersson D1000X) with a sampling rate of 100 kHz. Duets were recorded in stereo using a Sony ECM-121 microphone (frequency response relatively flat up to 30 kHz according to own tests) and an Uher M645 audio microphone connected to a personal computer through an external soundcard (Transit USB, "M-Audio"; 44.1 kHz or 64 kHz sampling rate). In these experiments male and female were placed separately into two plastic tubes (*Drosophila* tube 28.5 × 95 mm, Biosigma, Cona (VE), Italy) standing side by side, with one microphone placed inside or on top of each vial. Both microphones typically picked up male and female sounds, but with different amplitudes. Duration of echemes and echeme intervals and amplitude data were measured using Canary (http://www.birds.cornell.edu/brp/). Other song measurements and spectrograms were obtained using Amadeus II and Amadeus Pro (Martin Hairer; http://www.hairersoft.com). For frequency analysis additionally the program ZeroCrossing v5 (kprestwi@holycross.edu) was used. Here the singers were caged in gauze cages with microphone fixed at a distance of about 80 cm. Oscillograms of the songs were prepared using Turbolab (Bressner Technology, Germany). All recordings were made at temperatures between 20 and 25 °C. Data are presented as mean ± standard deviation.

Acoustical terminology. Tettigonioids produce their songs by repeated opening and closing movements of their tegmina. The sound resulting during one cycle of movements is called a syllable, often separable in opening and closing hemisyllable (Ragge & Reynolds 1998). Syllable duration: time period measured from the beginning of the pulse to its end (or first impulse to the last); syllable period: time period measured from the beginning of a syllable to the beginning of the next (reciprocal value: syllable repetition rate); echeme: first-order assemblage of syllables; pulse: undivided train of sound waves increasing in amplitude at the beginning and containing many similarly sized wave maxima and minima (cricket-like song structure; example see Fig. 7); impulse: a simple, undivided, transient train of sound waves (here: the damped sound impulse arising as the effect of one tooth of the stridulatory file); latency time: interval between beginning of male (female) song to beginning of female (male) response.

Chromosomal analysis. Two males (CH8308, CH8309) and two females (CH8310, CH8311) were used for chromosomal analyses. Preparations were obtained from testes, ovarioles, and hepatic caeca incubated in hypotonic solution (0.9 % sodium citrate), fixed in ethanol : acetic acid (3 : 1), and squashed in 45 % acetic acid. C-banding was carried out using the version of Sumner (1972), and the silver staining method ($AgNO_3$) for localization of the nucleolus organizer regions (NORs) was performed as previously reported (Warchałowska-Śliwa & Maryańska-Nadachowska 1992). In order to identify GC- and AT-rich regions, the prepared samples were stained with CMA_3 and DAPI, respectively (Schweizer 1976). Chromosomes were studied with a Nikon Eclipse 400 microscope with a CCD DS-U1 camera and NIS-Elements BR2, and the imagines were optimized using Adobe Photoshop. For each individual at least three oogonial/spermatogonial mitotic metaphase and 25 meiotic divisions in male were examined.

Results

The animals we found were identified as members of the genus *Anaulacomera* Stål, 1873 using the keys in Brunner von Wattenwyl (1891) and Cadena-Castañeda (2015). Within the genus, they are possibly similar to the species of the group *alfaroi* according to the size of the cerci as described by Cadena-Castañeda (2012, 2015). However, the cerci are even shorter than in this group where they are said to be slightly longer than the subgenital plate. In shape they differ distinctly from all figured species. Therefore, we describe them as a new species.

Anaulacomera almadaensis Heller sp. nov.

urn:lsid:Orthoptera.speciesfile.org:TaxonName:503430

Material examined and depository. Holotype ♂, allotype ♀ and 2 paratypes, 1♂,1♀. All pinned, original labels "BRAZIL: Bahia, Ilheus, Uruçuca, Fazenda Almada, 95 m, 29 x – 1 xi 2016, leg. K.-G. Heller, M. Volleth, Varja Vedenina". "Holotype Anaulacomera almadaensis" [red handwritten label]. Holo- (CH8308) and allotype (CH8310) in Zoologische Staatssammlung München (ZSM), Germany, Paratypes (CH8309, CH8311) in Collection Heller. One hind leg of CH8308,10-11 separate in pure ethanol in Collection Heller.

Sound files are deposited at OSFO and bio.acusti.ca.

Measurements (in mm; holotype bold). Males. Body length: **23**–24; pronotum length: **5.5**–5.9; pronotum height: 4.0; hind femur: 20–**21**; hind tibiae: **23**–24; tegmina: 34; length of hind wings: 37–**38.5**; tegmina width: **8.5**–9. Females. Body length: 22–23; pronotum length: 5.6; pronotum height: 3.8–4; hind femur: 21; hind tibiae: 24; tegmina: 32–33.5; length of hind wings: 36–38; tegmina width: 7.5–8.5; ovipositor 11–12.

Locality and time. The type locality is situated at Fazenda Almada at about 14.6563° S, 39.1884° W, 26 m a.s.l., as read from Google maps using http://www.mapcoordinates.net/en. For details of the area see http://www.fazenda-almada.com/en/willkommen/. One male was caught as adult (30th October) and sang in the night after the capture, the other moulted to adult more than three weeks later, on 24th November. The two females became adults on 18th and 23th November.

Description

Habitus and colour. Predominantly green, medium-sized bush-crickets (Fig. 1), large for the genus (compared to the data from Brunner von Wattenwyl 1878, 1891). Male stridulatory area in both tegmina brown. Female tegmina only with with-brown spot at articulation. In living specimens of both sexes with a series of about six white spots distributed along the radius.

Male. Head with round eyes, fastigium verticis expanded at tip (see Figs 2b, 3a), half as wide as scapus of antenna, not in contact with fastigium frontis (Fig. 3a); fronto-genal carinae very indistinct; antennal sockets prominent; antennae about as long as tegmina. Pronotum without lateral carinae, slightly longer than high, covered with very fine hairs; prozona hardly separable from metazona, anterior margin straight, posterior margin rounded, with evident lateral excisions where wings are inserted. Prothoracic spiracle narrow, long, reaching

Fig. 1. *Anaulacomera almadaensis* sp. nov., habitus: **a–b,** male; **c–d,** female; **a, c,** alive; **b,** (mirror image); **d,** dried. Scale 5 mm.

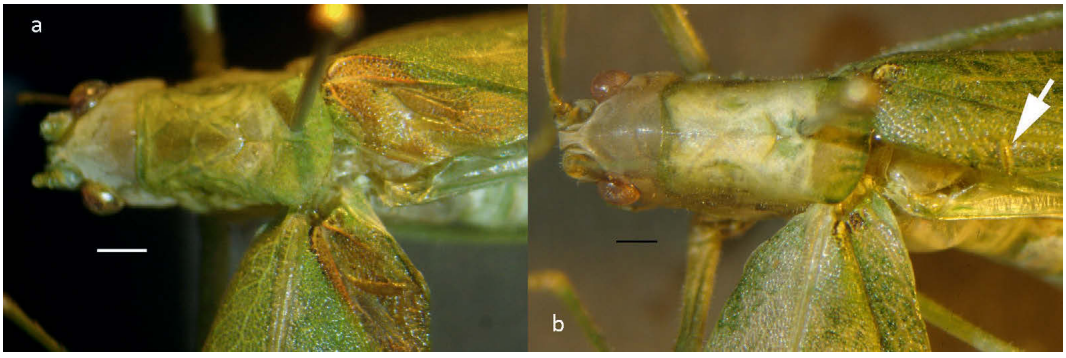

Fig. 2. *Anaulacomera almadaensis* sp. nov., dorsal view of head, pronotum and base of tegmina: **a,** male; **b,** female. Note the single stridulatory vein in the right female tegmen (arrow). Scale 1 mm.

to about two third of pronotal height. Ventral edge of paranota rounded. Tegmina wider than pronotal length. The stridulatory area of the left tegmen brown and green (Fig. 1), with distinct and weakly elevated stridulatory vein (Fig. 2a), of right tegmen with several irregular veins, without glossy mirror (Fig. 2b). The stridulatory vein on the underside of the left tegmen with 57–63 (n = 2) relatively evenly spaced teeth, only at the distal end a little bit denser (Fig. 5a). Hind wings longer than tegmina. Meso- and metasterna with two lobes each, rounded.

Fore coxae armed, fore femora ventrally with a few small spines, fore tibiae ventrally with five spines each on inner and outer edge, rounded, dorsal side rounded, without dorsal spurs. Tympana open on inner and on outer side. Mid femora below with several, mid tibiae with about ten spinules. Hind femora armed ventrally with a few spines distally, genicular lobes without spines. Hind tibiae armed ventrally and dorsally, furrowed on all sides. Hind tibiae longer than femora.

Abdomen. Subgenital plate long, strongly curved upwards, tapering into a weakly incised expanded caudal part with drawn out tips, but without styli (Fig. 4e–f); very large, vertically oriented supraanal plate, which prevents the view on a possibly existing sclerotized titillator. Cerci (Fig. 4a–d) short and stumpy, with a thick basal part carrying an upper and lower terminal branch; upper branch ending in two inward-curved tips, the outer with one sharp spine, the inner with a broad ending; lower part outwardly-curved with two spines, the lower much larger than the upper; connection between both parts concave, opening directed outwards. Cerci densely covered with long hairs.

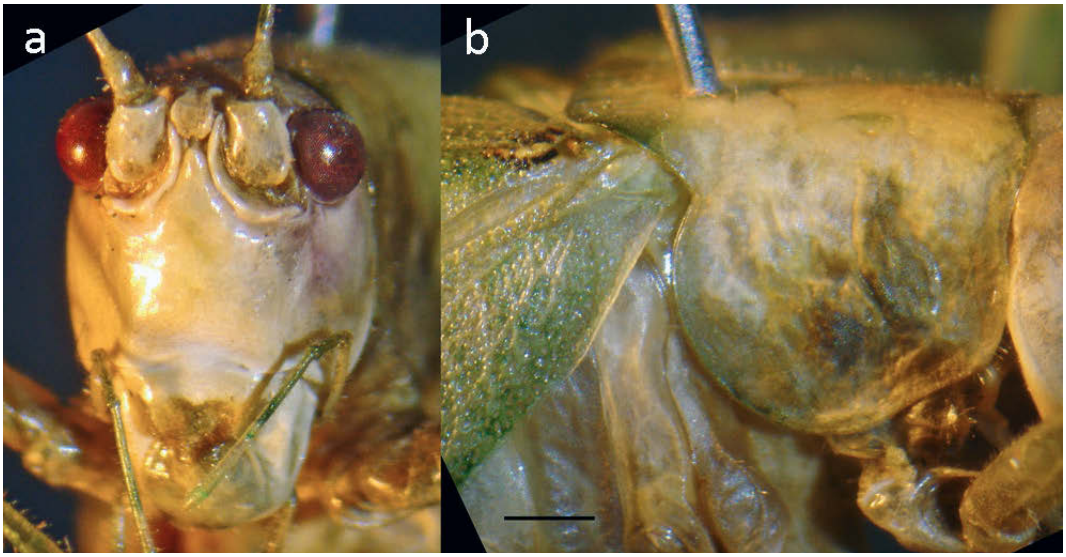

Fig. 3. *Anaulacomera almadaensis* sp. nov., head and pronotum: **a,** head in frontal view; **b,** lateral view of pronotum. Scale 1 mm.

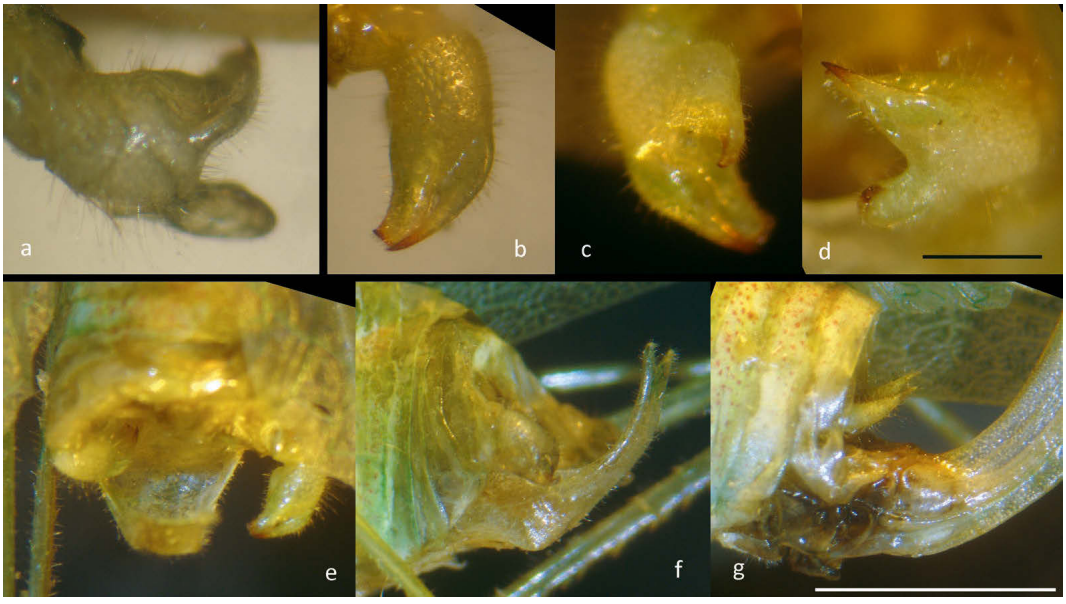

Fig. 4. *Anaulacomera almadaensis* sp. nov., cerci and sub genital plate: **a–d,** male cerci; **a,** latero-posterior view; **b,** view from above; **c,** view from below; **d,** view from behind; scale 1 mm (b–d); **e–f,** male subgenital plate; **e,** dorsal view with cerci (left one near natural resting position, right one opened widely); **f,** lateral view; **g,** female subgenital plate and lateral sclerites, lateral view; scale 5 mm (e–g).

Female. As male, except stridulatory organs and genitalia. Proximal quarter of right tegmen (dorsal area) with one distinct stridulatory vein carrying about 15 teeth (Fig. 5b). Ovipositor long, evenly curved (Fig. 1d). Subgenital plate short and broad, lateral sclerites complicated corresponding to the male cercus shape (Fig. 4g).

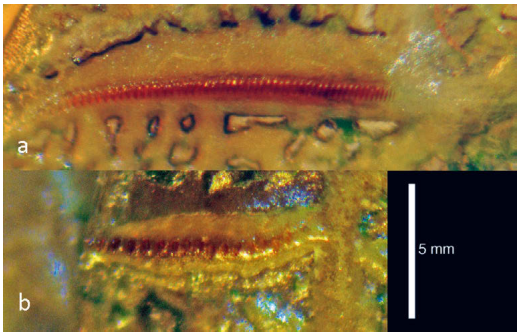

Fig. 5. Stridulatory files in male and female *Anaulacomera almadaensis* sp. nov.: **a,** male stridulatory file on lower side of left tegmen; **b,** female stridulatory file on upper side of right tegmen.

Eggs. Mature eggs were taken from the females after death and preserved dried. They show the flat, ovoid shape, typical for phaneropterines (length 4.70 ± 0.07 mm, width 1.68±0.11 mm, n = 4 (2 per female)).

Diagnosis. *A. almadaensis* differs from the other species of the genus by its short, stumpy cerci ending in an upper and lower branch (Fig. 4a–d). Tips of the cerci are directed inwards while in resting position, but even when opened shorter than subgenital plate.

Derivatio nominis. The name refers to the locality, the fazenda and the river Almada, therefore *almada-ensis*, adjective.

Mating behaviour. One male (body mass 876 mg and 986 mg) copulated with both females (body mass 994 mg, 650 mg) at an interval of 8 days (10.12.2016 and 18.12.2016). They mated at night with a mating duration of ca. 17 minutes (no data for other mating) and transferred spermatophores of 172 and 217 mg (mean of male loss and female gain), thus about 20 % of the male body mass. The spermatophylax was correspondingly large, the ampulla orange.

Acoustics. The calling song of an isolated male consisted of echemes, repeated in intervals of around 15 s (M1, 30.10.2016, T = 25 °C; 16.8 ± 5.4 s; 26.11.2016, T = 20 °C; 11.1 ± 2.2 s; M2, 3.12.2016, T = 23 °C; 17.0 ± 6.2 s; n = 10 each). Each echeme contained 5–7 syllables, produced with a rate of ca. 30 Hz (syllable period 30.7 ± 0.8 ms; 36.0 ± 0.8 ms; 33.3 ± 0.7 ms; as above; Fig. 6a). The syllables were very short, less than 5 ms (3.6 ± 0.5 ms; T = ca. 22 °C, n = 12), typically consisting of one cricket-like pulse (Fig. 7a). The duty cycle can thus be estimated as (6 × 3.6 ms) × 4/60000 ms = 0.1 %.

The females ready to mate reacted to the male song by emitting their own acoustic signals. They always answered after the last syllable of an echeme with a latency of ca. 100 ms (Fig. 6b–c; W1: 103 ± 13 ms, W2; 102 ± 13 ms, range 74–118 ms; T = 22–23 °C, n = 12 each). In one female the response often consisted of one impulse, in the other two or three impulses were observed with an interval of up to 10 ms between the first and the last.

After having heard a female response, the acoustic behaviour of the male changed (Fig. 9). Immediately after the response – within the next 50–150 ms (102 ± 20 ms, range 44–127 ms, n = 25), it produced (an) additional sound(s). This fast reaction happened even after quite long times without female response. After at least 93, 53 and 52 minutes (the three longest available intervals) without female response the male immediately modified its song. It added one to four additional syllables (Fig. 6c). After one interval of usual duration (or even longer than the preceding; Fig. 9b,d) the male produced echemes at a faster rate than before the response. In addition, it modified again the pattern of the echeme and its amplitude. These changes were most obvious when the male had heard one or several responses before (as in Fig. 9b). Often the next echemes had low amplitude, the syllables consisted of several (im)pulses, contained clearly visible opening and closing hemisyllables and, at the time when a female response was to expect, the male produced a series of impulses (Fig. 6d). In situations when the female responded more frequently her response arrived always before these impulses (Fig. 6e). This elongation of the echeme disappeared at first if the female did not continue to respond, but the other modifications could be relatively long lasting (longer than 1 min). If the female responded regularly, occasionally combinations of both echeme modifications (adding an impulse series and syllables) were observed. Concerning loudness, however, it cannot be excluded that the reduction of sound amplitude was at least partly an effect of a changed position of the male relative to the microphone (see Discussion). All these song modifications were observed only in the song of the male caught as adult; in the only 2 h-recording of the younger male at the age of 10 days it behaved as if was deaf – no reaction to female responses at all.

The carrier spectrum of male calling song is relatively narrow-banded with its maximum at 11–12 kHz (Fig. 8). The syllables showed a slight frequency modulation, often upwards in the first syllables. The last syllables of an echeme were mostly flat, but sometimes also downward modulated (rarely also seen in the first syllable) (see Fig. 7b for examples). The spectrum of the soft song of the male, however, is more broad-banded, similar to the spectrum of the female responses. The peak value

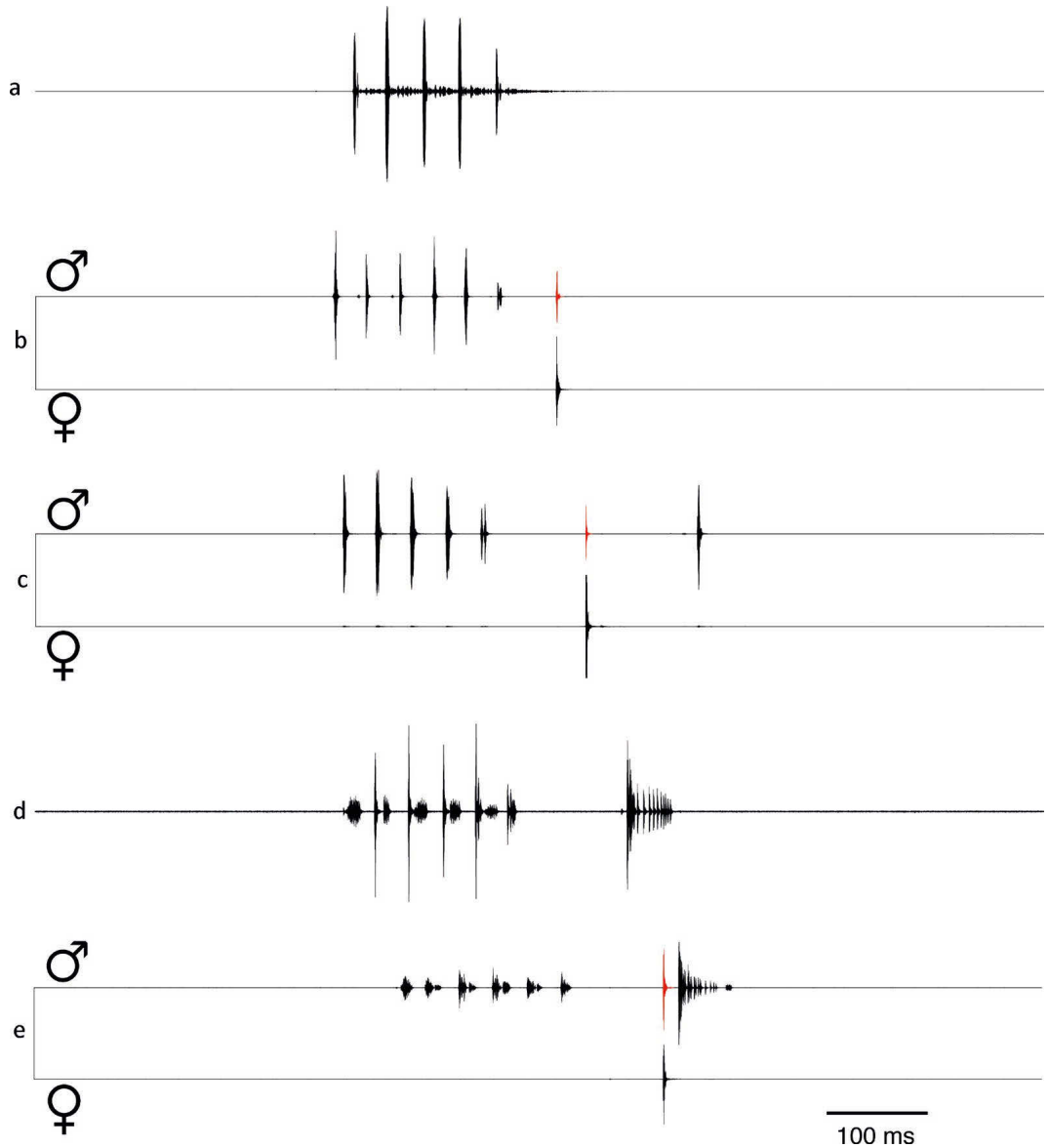

Fig. 6. Time-amplitude pattern of songs of *Anaulacomera almadaensis* sp. nov.: **a,** calling song of isolated male; **b,** male song and female response (rarely observed); **c,** male song, female response and male reaction (nearly always observed); **d,** soft song of the male after he heard a female response; **e,** soft song of the male with female response. Red colour indicates female sounds visible in the male sound track.

of these broad-banded songs is similar to that of the male calling songs (Fig. 8).

Chromosomes. The analysis of mitotic and meiotic chromosomes of *Anaulacomera almadaensis* revealed a diploid chromosome number 2n = 29, X0 and 2n = 30, XX in males and females, respectively (Fig. 10a–b). All autosomes were acrocentric, except of the first pair, and can be divided into two groups: nine large and medium (1–9) and five small ones (10–14), both gradually decreasing in size, which complicates their identification. The sex chromosome (X) was metacentric and the largest element in the karyotype, about twice as long as the first pair of

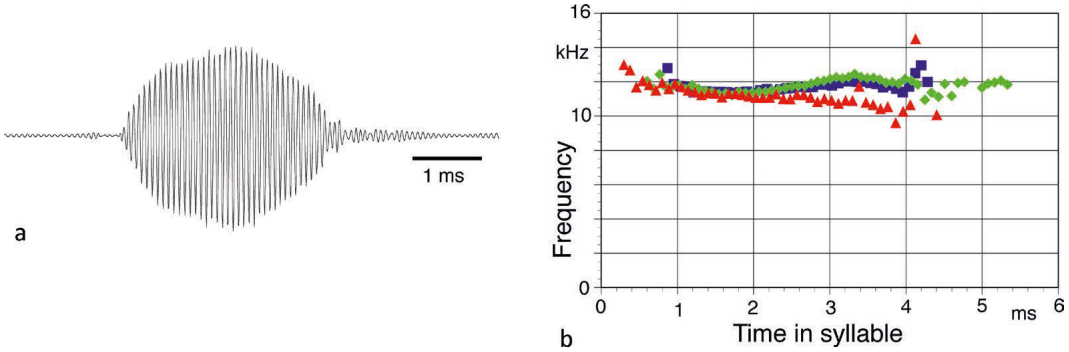

Fig. 7. *Anaulacomera almadaensis* sp. nov., details of male song: **a,** time-amplitude pattern of a syllable of the male song; **b,** cycle-by-cycle frequency of three syllable of one echeme.

autosomes. A heteromorphic long pair (1st), clearly subacrocentric/subtelomeric was found both in females (Fig. 10a) and males (Fig. 10b–c). During spermatogonial metaphase and diplotene/diakinesis a secondary constriction, located interstitially in one arm on the X, was observed (Fig. 10b–c, marked by arrowheads).

Heterochromatic blocks revealed by C-banding were located in the paracentromeric regions of all chromosomes. Thin C-positive regions were generally negative for both DAPI and CMA_3, whilst a C-band in the heteromorphic 5th pair was DAPI-negative and CMA_3-positive. The heterochromatic region located interstitially near the distal ends of the 3rd pair (in males in heteromorphic conditions) was C/DAPI/CMA_3-positive (Fig. 10b–e, marked by asterisks).

The biarmed first pair demonstrating heterochromatic characters is called "megameric" (m). In spermatogonial, oogonial and somatic (cells from hepatic caeca of females) metaphases, a heteromorphism of both C-bands and fluorochrome bands was observed in these chromosomes in terms of the size/strength of bands on homologs. Short arms, differing in size between homologs, were euchromatic, whereas heterochromatin in long arms differs concerning C/DAPI/CMA_3-bands. In one of the homologues of this pair two heterochromatic blocks of unequal size were separated by a short euchromatic region (Fig. 10c–e).

The analysis of meiotic cells in males demonstrates the behaviour of this bivalent which creates a pseudo-trivalent with the X chromosome (marked as m+X). This heterozygous (unequal) bivalent shows strong heterochromatic characteristics (high condensation), from prophase to metaphase II, whereas the X was weakly positive heteropycnotic. In zygotene-pachytene, both unequal bivalents and the X chromosome probably exhibited partial synapsis only at their terminal parts and small interstitial part in one arm of the metacentric X. Thus, a large region remained unpaired forming a loop. Alternatively, they may be connected only in a short part, which would suggest that these chromosomes are not homologous (Fig. 10f). Generally, from early diplotene to early diakinesis, the trivalent showed end-to-end association (probably without chiasma) (Fig. 10g,n,o). Part of the megameric bivalent is probably connected in the secondary constriction of the X (Fig. 10b,c,g). In the late diakinesis and metaphase I, the heteromorphic pair and the X chromosomes were arranged separately (Fig. 10i,p). In anaphase I, unequal chromosomes underwent an equational segregation pattern, whereas the first meiotic division was reductional for the other autosomes (Fig. 10h). As a result, in metaphase II 14 or 15 (with X) chromosomes and rarely 15 or 16 (with X) in both cells with an extra element (e) were observed (Fig. 10j–k). This small euchromatic additional element (e) was often present at different meiotic stages (Fig. 10b,g–i,k,p). At diplotene, it was often seen near that region of the X which was in association with the unequal bivalent (Fig. 10g). Additionally, in some analysed spermatogonial metaphases (6 of 15) 30 or 31 chromosomes (with one or two additional elements) were observed (e.g. Fig. 10b–e). On the other hand, in oogonial and somatic metaphases of females (from hepatic caeca) these element/s were not found. However, such cells were not analysed in males. In anaphase II the megameric "m" chromosomes divided reductionally and segregated to opposite poles (Fig. 10l).

Two active nucleolus organizer regions (NORs) located distally on the long arms of the unequal m bivalent were heterozygous in sizes, and always located near the X chromosome (Fig. 10m–o). NORs coincided with large GC-rich blocks that were intensively stained by CMA_3 and positive and thick

C-bands. Additionally, in some cells small silver dots were observed in the early meiotic prophase on the X, probably a "secondary NOR" associated with the intercalary secondary constriction (not shown).

Discussion

Concerning bioacoustics, the subfamily Phaneropterinae is certainly the most diverse group among ensiferan Orthoptera. This relates to the fact that in most species the females respond acoustically to the male song and establish a duet with the male (for a review, see Heller et al. 2015). Elements of the male song must indicate the female when to respond, and, perhaps even more important, the male should defend the duet against silent, eavesdropping rivals. For this purpose, the males often add self-made female imitations to the song, making the localization of a responding female difficult. Most prominent examples are e. g. *Ducetia antipoda* Heller & Rentz, 2017, where the male song ends in a long series of well-separated impulses (Heller et al. 2017), or *Gonatoxia helleri*, where the male produces special signals which imitate the female signals in carrier frequency (Heller & Hemp 2017). In all these species the female imitations are part of the male song and are emitted independently of the presence of females. *Anaulacomera almadaensis*, however, goes one

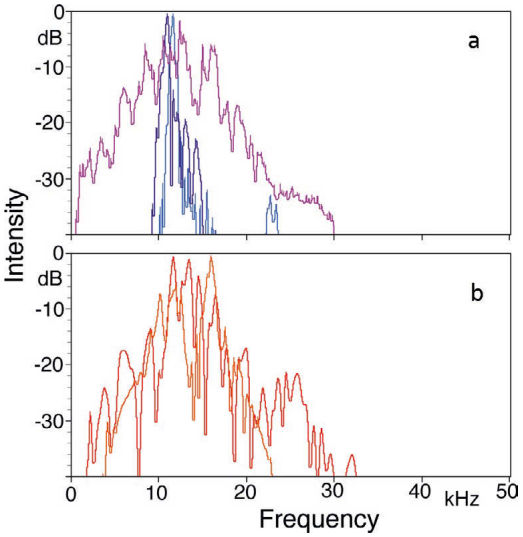

Fig. 8. Power spectra of sounds of *Anaulacomera almadaensis* sp. nov.: **a,** male calling song (blue: two different males) and example of soft song (lila); **b,** female response (red: two different females).

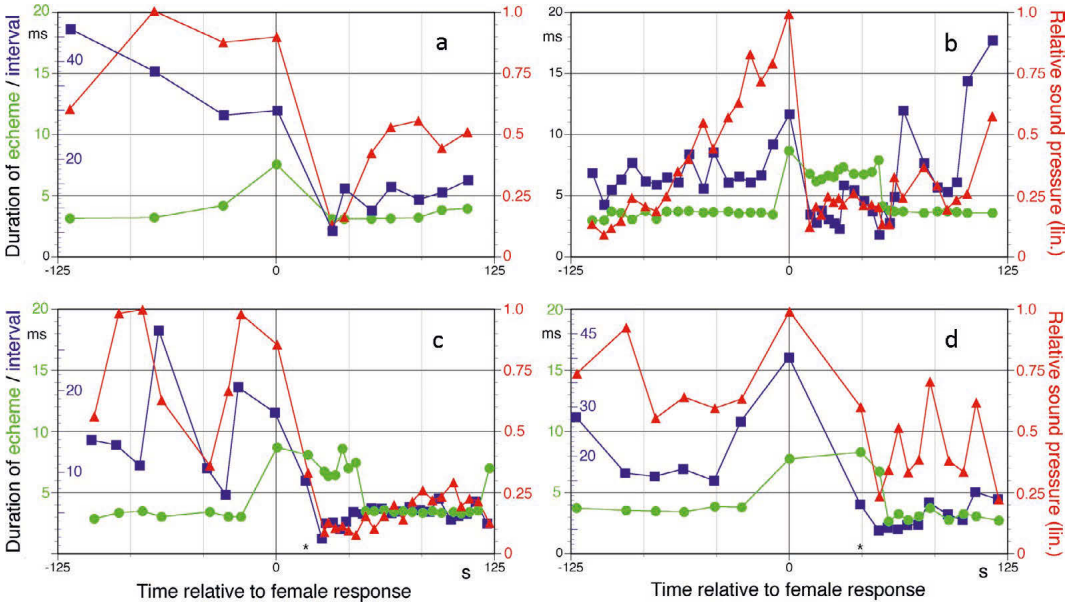

Fig. 9. Examples for changes in male calling behaviour after he had heard a female response: reduction of echeme intervals (blue squares) and sound amplitude (red triangles), increase in duration of echemes (green dots): **a,** reaction to first female response after 40 min of singing; **b,** reaction after having heard a female response shortly before; **c–d,** reaction with two female responses. Blue: interval to following echeme (note different scales in a–d); green: echeme duration; red: relative sound pressure amplitude (linear scale); *, 2nd female response.

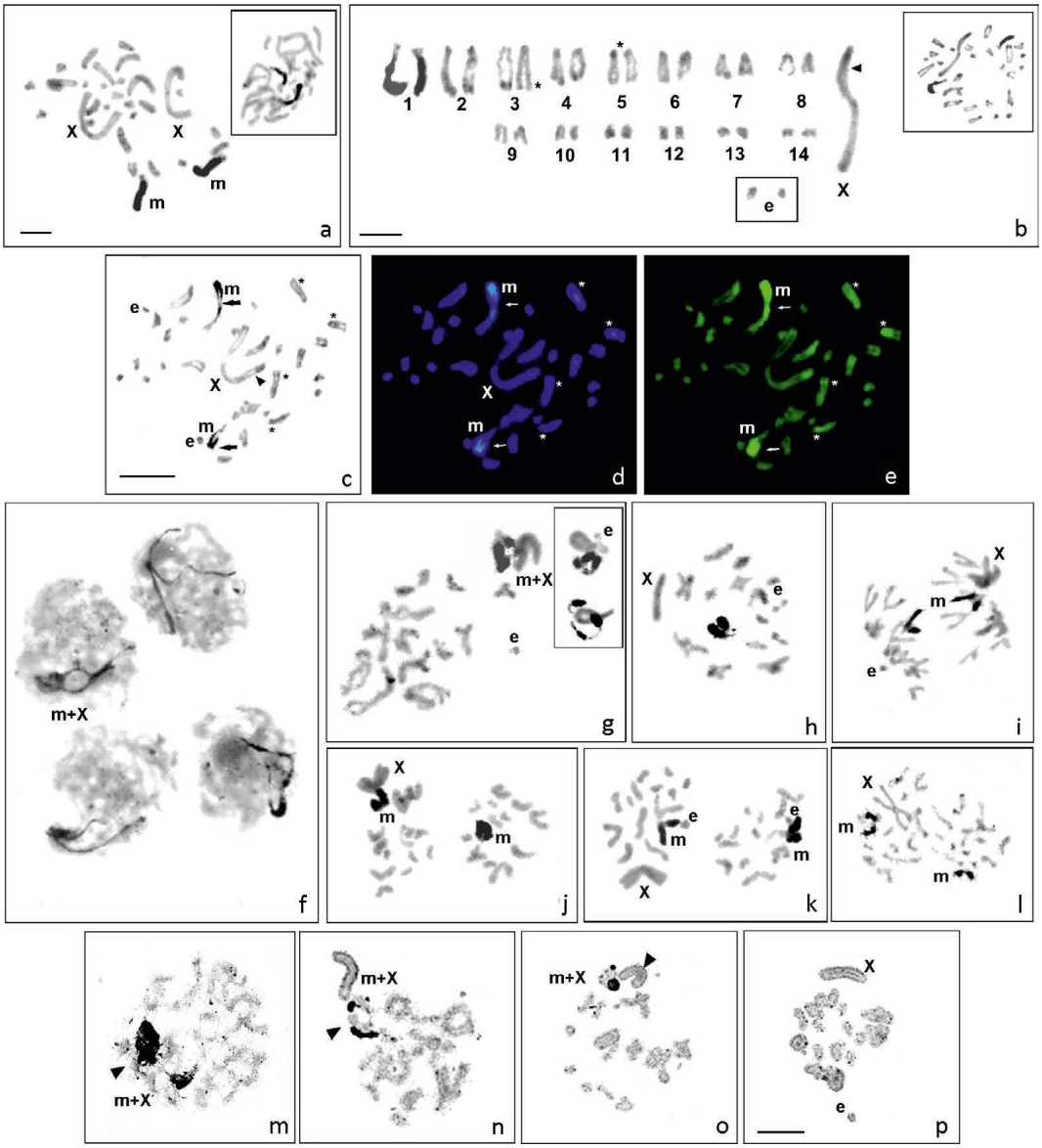

Fig. 10. Examples of C-banded (**a, b, c, f–l**), fluorochrome stained heterochromatin (**d, e**) as well as silver stained (**m–p**) chromosomes of *A. almadaensis* sp. nov.: **a,** oogonial metaphase and somatic metaphase (in the inset) of a female with 30 chromosomes; **b,** karyogram arranged from a spermatogonial metaphase (in the inset) of a male with 29 chromosomes plus two "e" elements; **b, c,** heteromorphic heterochromatic/megameric long chromosomes (probably 1st pair marked in **b**) are indicated with "m" and show clearly short euchromatic arms; arrowheads indicate a secondary constriction in one of arms of X. Thin paracentromeric C-bands (**c**) are negative for DAPI (blue) and CMA_3 (green) bands (**d, e,** respectively); an asterisk marks the heteromorphic paracentromeric region in 5th pair which is DAPI-negative and CMA_3-positive and the heteromorphic 3rd pair with interstitially located C/DAPI/CMA_3-positive region; heterochromatin in long arms of the megameric chromosome differs concerning C/DAPI/CMA_3-bands (arrows); **f–l,** the behaviour of the heteromorphic bivalent which forms a pseudo-trivalent with X chromosomes (marked such m+X) in meiosis: the X chromosome shows weakly positive heteropycnosis, m bivalent is strongly heterochromatic, both from pachytene to metaphase II. In pachytene (**f**) and diplotene/diakinesis (**g, m–o**) chromosomes of the m+X complex form a loop or are connected to each other only in the short part. …

cycle, is well known also from other duetting species. After a female response, the male typically increases its duty cycle by reducing the intervals between its songs. In fact, this variability is considered as the most important advantage of bidirectional communication in Orthoptera (e. g., Heller & Helversen 1993, Helversen et al. 2012). Hartley et al. (1974: p. 387) gave the first figure demonstrating this effect in a bradyporine duetting species. It should be typical for duetting species, but only few examples are known. The second type of song modification concerns loudness. Reducing song amplitude may be similarly common, but data are even rarer. Also the data presented here have problems. In the four examples presented in Figure 9, there is always a clear drop in intensity with the first echeme(s) after the response. However, we cannot exclude the possibility that the male moved rapidly into another position less favourable for the microphone. Since we did not detect any opposite example in all the recordings, we assume that males really reduced the intensity. In theory, the male should use the lowest intensity to elicit a response making it most inconspicuous to predators and rivals. The continuous increase in amplitude after a single female response (Fig. 9b) fits nicely to this hypothesis.

The most unexpected song modifications were the two types of changes in the amplitude pattern. The male always started by adding one or several additional syllables after the female response. These sounds had a latency very similar to that of the female response itself. This is possibly the shortest time for an acousto-motoric reflex (sensu Robinson et al. 1986) in this species. However, it may be too slow to disturb an eavesdropping rival completely. So the other type of modification, an added impulse series, is certainly much more effective. But what is the function of the added syllable? We must assume that it has a special negative effect on rivals – and it is very rarely missing, even after long times without response. Such context-specific changes have not been described in detail for any phaneropterine species, but are mentioned in Spooner's (1968) large descriptive study for two or three species. In *Microcentrum rhombifolium* he refers mainly to the unpublished dissertations of Alexander (1956) and especially of Grove (1959). The latter interpreted "an irregular shuffling sound" – with a possibly similar timing as the impulse series in *A. almadaensis* – already as "to confuse the location of the female" (Spooner 1968). Males of *Scudderia cuneata* and *S. furcata* seem to produce ticks when responsive females are nearby, and subside this ticking after a few minutes when the females are removed (Spooner 1968). This behaviour has similarities with adding the impulse series in *A. almadaensis*, which is also done only for a short time. The increased width of the spectrum of these male signals compared to the typical calling song may be a consequence of the low intensity, but makes the sounds also more similar to the female responses.

A. almadaensis shows all types of song modification presently known to speed up the localisation process and to conceal the duet at the same time from predators and rivals. Therefore we call it a perfect duettist. Single components of this behaviour are certainly widespread among phaneropterines, but even for the most common types (increase of duty cycle and reducing sound amplitude) the data are very limited. This is true, e. g. for increase in duty cycle described only for *Leptophyes punctatissima* on the basis of daily activity measurements (Hartley & Robinson 1976) and for a bradyporine species on hourly rates (Hartley 1993). The combined occurrence of the whole set of modifications may be rarer and not so easy to observe. If a female is older or more eager to mate she will respond continuously to many or even all male songs and the effects of a single answer will be difficult to recognize. It should be noted that nothing is known about modifications of the female signal which also may occur.

As mentioned above, *Anaulacomera* is a huge genus but with very few data on acoustics and none from any identified species. From the figures in Braun (2002) and the recordings by Walker (1966) the presence of short syllables is typical for many species, but not for all (e. g., *Anaulacomera* sp. 3 in Braun 2002). Their spectrum is often narrow-banded (Braun 2002; the recordings of Walker, now available as mp3-audio, cannot be evaluated in this respect). Brunner von Wattenwyl (1878, 1891) had placed *Anaulacomera* together with the East African/Malagasy *Parapyrrhicia* in his group Anaulacomerae (including also the South American *Grammadera* and *Abrodiaeta*), but currently it is placed together with some South American genera in the subtribe Anaulacomerina of Phaneropterini, while *Parapyrrhicia* is separate. Surprisingly, however, the biogeographically very unusual assemblage of Brunner von Wattenwyl is supported by bioacoustics. In both genera the male

◁ … In late diakinesis/metaphase I (**h**) heteromorphic megameric bivalent is not connected with X and during anaphase it undergoes an equational segregation pattern (**i**); as result, in metaphase II there are 14 and 15 chromosomes (**j**) with heteromorphic autosome megameric pair, whereas both chromosomes segregate to opposite poles (**l**). Some elements (e) are seen in different spermatogonial (the lower inset in **b, c–e**) and meiosis stage (**g–i, k, p**). The arrowheads indicate active NORs located in unequal (m) bivalent. X-, sex chromosome. Scale bar 10 µm.

calling songs consist of short, resonant syllables and also the female stridulatory organs are built from few and very conspicuous toothed veins at least in *A. almadaensis* (see Hemp et al. 2017 and above). This female file structure has not been studied systematically in phaneropterines, but the presence of many female stridulatory veins is quite common (e. g., Heller et al. 1997, Hemp et al. 2016), while some *Anaulacomera* females from French Guiana have the same type of veins as seen in *A. almadaensis* (own unpublished observations).

Up to now, the chromosome numbers and morphology of only five species of the subtribe Anaulacomerina were described. *Anaulacomera horti* Piza, 1975, *A. linguata* Piza, 1952, *A. chelata* Brunner von Wattenwyl, 1878, *A.* sp. 1 and *Grammadera clara* Brunner von Wattenwyl, 1878 (described under the synonym *Anaulacomera dimidiata* Piza, 1952) from the State of São Paulo have 30 acrocentric autosomes and a meta-/submetacentric X chromosome in males (2n = 31, FN = 32) with X0/XX sex mechanism (Ferreira 1977, Ferreira & Mesa 2007). In *Anaulacomera brasiliae* Piza, 1977 (from Goiás) the chromosome number is reduced to 2n = 23, FN = 28 (X0) as a result of two independent Robertsonian translocations between autosomes (two metacentric pairs), a pericentric inversion forming a biarmed X chromosome and some tandem fusions/centric fusions followed by pericentric inversions (Ferreira & Mesa 2007).

The chromosome characteristics including male and female karyotypes, meiosis and sex chromosome of *A. almadaensis* show an advanced karyotype evolution. In this species, similar to the above-mentioned species of *Anaulacomera*, (1) a biarmed X chromosome (submeta-/metacentric) arises as result of a pericentric inversion; (2) the ancestral chromosome number is reduced to 2n = 29 (FN = 32) probably as a result of one Robertsonian translocation (as observed in *A. brasiliae*) between long and small pair of chromosomes creating a biarmed heteromorphic, megameric long pair. Such a long chromosome pair considered as megameric was described in other phaneropterines, for example in the genus *Isophya* (e. g., Warchałowska-Śliwa & Bugrov 1998).

In *A. almadaensis* the heteromorphic biarmed pair of chromosomes with distally located active NOR/GC-rich heterochromatin and unequal both heterochromatic and euchromatic arms may have arisen by paracentromeric inversion or pericentric inversion. Both of these aberrations lead to the lack of complete homology in the heteromorphic pair. The behaviour of the trivalent (pseudo-trivalent) observed during meiosis could suggest that some other kind of translocation occurred between the megameric pair of autosomes and the X chromosome. However, the origin and behaviour of this complex is difficult to interpret. The result of these changes is a reduction in chromosome number (2n = 29/30) and occurrence of additional elements which are often connected with the trivalent in meiosis. Thus, different chromosome numbers in meiotic and mitotic stages in the male can be explained by presence of the supernumerary elements (centric/acentric) which are not homologous with autosomes and could be a result of some rearrangements of the karyotype. However, the question remains, why there are no additional elements in the mitosis in females (meiosis was not observed). Possibly this could be explained by the fact that only a small number of good divisions was observed. Among phaneropterine species a pericentric inversion plays an important role in the appearance of biarmed X chromosomes which does not lead to a change in the number of chromosomes (e. g., Warchałowska-Śliwa 1998, Warchałowska-Śliwa et al. 2008, Hemp et al. 2013). Re-structuring the karyotype of *Anaulacomera almadaenis* sp. nov. implies a few more (individual) rearrangements. However, it cannot be excluded that the karyotypic changes consist in the simultaneous occurrence of pericentric inversions in the sex chromosome (formation of biarmed chromosome) and translocation between the megameric pair and the X. On the other hand, based on our observation of the structure and meiotic behaviour of the trivalent (m+X) in the meiosis, it cannot be excluded that these events may indicate the origin of the first stage of a neo-sex system (multiple neo-$X_1X_2Y/X_1X_1X_2X_2$) starting from the X0/XX system. This hypothesis, based on the location of active NOR/s on both autosomal pairs and the X (forming a trivalent), may indicate that the NOR-bearing chromosomes take part in karyotype changes. Cytogenetic analysis of some other tettigoniids, e. g., *Odontura* (Phaneropterinae) or *Spalacomimus* (Hetrodinae) also revealed one or two NORs located only on the sex chromosomes of species with a neo-sex determination system: neo-X (neo-XY) or both neo-X_1 and neo-Y (with neo-X_1X_2Y) (Warchałowska-Śliwa et al. 2011, 2015). An autosome-sex chromosome translocation often lead to a change in the type of sex determination when it causes polymorphism of autosomes (e. g., in grasshoppers, Castillo et al. 2010). However, the heteromorphic pair was observed in oogonial and somatic metaphase of females *A. almadaenis* (present paper), which may contradict the assumption of an initial state of the differentiation process of a neo-sex chromosome system.

Author's contributions

K-GH, MV and VV collected the specimens; K-GH recorded and analysed the songs; EW-S and AM-N analysed the chromosomes; K-GH led the writing.

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References

Alexander, R. D. 1956. A comparative study of sound production in insects with special reference to the singing Orthoptera and Cicadidae of eastern United States. Unpublished Ph.D. dissertation, Ohio State University, Columbus, Ohio.

Braun, H. 2002. Die Laubheuschrecken (Orthoptera, Tettigoniidae) eines Bergregenwaldes in Süd-Ecuador: faunistische, bioakustische and ökologische Untersuchungen. 143 pp., Ph.D. dissertation, Universität Erlangen-Nürnberg.

– – 2008. Orthoptera: Tettigoniidae. Ecotropical Monographs 4: 215–220.

Brunner von Wattenwyl, C. 1878. Monographie der Phaneropteriden. 401 pp., Wien (Brockhaus).

– – 1891. Additamenta zur Monographie der Phaneropteriden. Verhandlungen der k.k. Zoologisch-Botanischen Gesellschaft Wien 41: 1–196.

Cadena-Castañeda, O. J. 2012. The tribe Viadaniini n. trib. (Orthoptera: Tettigoniidae): first contribution to the organization of supra-generic faneropterinos neotropical. Journal of Orthoptera Research 21: 25–43.

– – 2015. The tribe Phaneropterini and additions to the subtribes Anaulacomerina and Pelecynotina n. subtr. (Orthoptera: Tettigoniidae: Phaneropterinae): seventh contribution to the suprageneric organization of the Neotropical phaneropterines. Boletín de la Sociedad Entomológica Aragonesa 57: 53–70.

Castillo, E. R. D., Bidau, C. J. & Marti, D. A. 2010. Sex- and neo-sex chromosomes in Orthoptera: a review. Journal of Orthoptera Research 19: 213–231.

Chobanov, D. & Heller, K. G. 2010. Revision of the *Poecilimon ornatus* group (Orthoptera: Phaneropteridae) with focus on Bulgaria and Macedonia. European Journal of Entomology 107: 647–672.

Cigliano, M. M., Braun, H., Eades, D. C. & Otte, D. 2018. Orthoptera Species File. Version 5.0/5.0. http://Orthoptera.SpeciesFile.org [accessed March 2018].

Ferreira, A. 1977. Cytology of neotropical Phaneropteridae (Orthoptera – Tettigonioidae) [sic]. Genetica 47(2): 81–86.

– – & Mesa, A. 2007. Cytogenetics studies of thirteen Brazilian species of Phaneropterinae (Orthoptera: Tettigonioidea: Tettigoniidae): main evolutive trends based on their karyological traits. Neotropical Entomology 36(4): 503–509.

Grove, D. G. 1959. The natural history of the angular-winged katydid, *Microcentrum rhombifolium*. Unpublished Ph.D. dissertation., Cornell University, Ithaca, N.Y.

Grzywacz, B., Chobanov, D. P., Maryańska-Nadachowska, A., Karamysheva, T. V., Heller, K. G. & Warchałowska-Śliwa, E. 2014. 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.

Hartley, J. C. 1993. Acoustic behaviour and phonotaxis in the duetting ephippigerines *Steropleurus nobrei* and *Steropleurus stali* (Tettigoniidae). Zoological Journal of the Linnean Society 107: 155–167.

– – & Robinson, D. J. 1976. Acoustic behavior of both sexes of the speckled bush cricket *Leptophyes punctatissima*. Physiological Entomology 1: 21–26.

– – , Robinson, D. J. & Warne, A. C. 1974. Female response song in the ephippigerines *Steropleurus stali* and *Platystolus obvius* (Orthoptera, Tettigoniidae). Animal Behaviour 22: 382–389.

Heller, K. G. 1984. Bioakustik und Phylogenie der Gattung *Poecilimon* (Orthoptera, Tettigoniidae, Phaneropterinae). Zoologische Jahrbücher, Systematik 111: 69–117.

– – & Helversen, D. v. 1993. Calling behavior in bushcrickets with differing communication systems (Orthoptera, Tettigonioidea, Phaneropteridae, *Poecilimon*). Journal of Insect Behavior 6: 363–377.

– – & Hemp, C. 2017. Context specific signaling with different frequencies – directed to different receivers? A case study in *Gonatoxia* katydids (Orthoptera, Phaneropteridae). Journal of Insect Behavior 30: 420–431.

– – , Hemp, C., Ingrisch, S. & Liu, C.-X. 2015. Acoustic communication in Phaneropterinae (Tettigonioidea) – a global review with some new data. Journal of Orthoptera Research 24: 7–18.

– – , Ingrisch, S., Liu, C.-X., Shi, F.-M., Hemp, C., Warchalowska-Sliwa, E. & Rentz, D. C. F. 2017. Complex songs and cryptic ethospecies: the case of the *Ducetia japonica* group (Orthoptera: Tettigonioidea: Phaneropteridae: Phaneropterinae). Zoological Journal of the Linnean Society 181: 286–307.

– – , Schul, J. & Ingrisch, S. 1997. Sex-specific differences in song frequency and hearing in some duetting bushcrickets (Orthoptera: Tettigonioidea: Phaneropteridae). ZACS – Zoology Analysis of Complex Systems 100: 110–118.

Helversen, D. v., Helversen, O. v. & Heller, K. G. 2012. When to give up responding acoustically in *Poecilimon* bush-crickets: a clue to population density. Articulata 27(1/2): 57–66.

Hemp, C., Heller, K. G., Warchałowska-Śliwa, E., Grzywacz, B. & Hemp, A. 2013. Biogeography, ecology, acoustics and chromosomes of East African *Eurycorypha* Stål species (Orthoptera, Phaneropterinae) with the description of new species. Organism, Diversity and Evolution 13: 373–395.

– – , Heller, K. G., Warchałowska-Śliwa, E., Grzywacz, B. & Hemp, A. 2017. Review of the East African species of the phaneropterine genus *Parapyrrhicia* Brunner von Wattenwyl, 1891 (Insecta: Orthoptera): secret communication of a forest-bound taxon. Organisms Diversity & Evolution 17: 231–250.

– – , Heller, K. G., Warchałowska-Śliwa, E. & Hemp, A. 2016. Spotted males, uniform females and the lowest chromosome number in tettigoniids recorded: review of the genus *Gonatoxia* Karsch (Orthoptera, Phaneropterinae). Deutsche Entomologische Zeitschrift 63(2): 271–286.

Martius, C. F. P. v. 1828. Reise in Brasilien auf Befehl Sr. Majestät Maximilian Joseph I., Königs von Baiern in den Jahren 1817 bis 1820 gemacht und beschrieben von Johann Baptist von Spix & Carl Friedrich Philipp von Martius. Zweiter Teil. pp. 415–884, München (Lindauer).

Schweizer, D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma 58: 307–324.

Spooner, J. D. 1968. Pair-forming acoustic systems of phaneropterine katydids (Orthoptera, Tettigoniidae). Animal Behaviour 16: 197–212.

Sumner, S. G. 1972. A simple technique for demonstrating centromere heterochromatin. Experimental Cell Research 75: 304–306.

Ragge, D. & Reynolds, W. J. 1998. Songs of the grasshoppers and crickets of Western Europe. 591 pp., Colchester, Essex (Harley Books).

Ramme, W. 1933. Revision der Phaneropterinen-Gattung *Poecilimon* Fisch. (Orth. Tettigon). Mitteilungen aus dem Zoologischen Museum in Berlin 19: 497–575.

Robinson, D. J., Rheinlaender, J. & Hartley, J. C. 1986. Temporal parameters of male female sound communication in *Leptophyes punctatissima*. Physiological Entomology 11: 317–324.

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.

Ünal, M. 2010. Phaneropterinae (Orthoptera: Tettigoniidae) from Turkey and the Middle East II. Transactions of the American Entomological Society 136: 125–183.

Walker, T. J. 1966. Macaulay Library, Cornell Lab of Ornithology. http://macaulaylibrary.org/search?&asset_format_id=1000&collection_type_id=1&layout=1&taxon=Anaulacomera&taxon_id=10558455&taxon_rank_id=62&sort=21 [accessed 9 April 2018].

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.

– – & Bugrov, A. G. 1998. Karyotypes and C-banding patterns of some Phaneropterinae katydids (Orthoptera, Tettigonioidea) with special attention to a post-reductional division of the neo-X and the neo-Y sex chromosomes in *Isophya hemiptera*. Folia Biologica (Kraków) 46: 47–54.

– – & Maryańska-Nadachowska, A. 1992. Karyotypes, C-bands, NORs location in spermatogenesis of *Isophya brevipennis* Brunner (Orthoptera: Phaneropteridae). Caryologia 45: 83–89.

– – , Chobanov, D., Grzywacz, B. & Maryańska-Nadachowska, A. 2008. Taxonomy of the genus *Isophya* (Orthoptera, Phaneropteridae, Barbitistinae): Comparison of karyological and morphological data. Folia Biologica (Kraków) 56: 227–241.

– – , Grzywacz, B., Maryańska-Nadachowska, A., Hemp, C. & Hemp, A. 2015. Different steps in the evolution of neo-sex chromosomes in two East African *Spalacomimus* species (Orthoptera: Tettigoniidae: Hetrodinae). European Journal of Entomology 112 (1): 1–10.

– – , Maryańska-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.

Note added in press. *A. almadaensis* may be grouped in the subgenus *Bovicercora* Gorochov, 2020 (Gorochov, A. V. 2020. Systematics of the American katydids (Orthoptera: Tettigoniidae). Communication 9. Proceedings of the Zoological Institute of the Russian Academy of Sciences 324 (2): 187–220).