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A Problem of Taxonomic Status of "Banana Cricket" from Culture of the Moscow Zoo Insectarium

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Abstract—The "banana cricket" is one of the preferred laboratory objects used for studying general and applied biological problems. However, the exact identity of this form remains obscure. Correct identification of the insects maintained in culture is vital for the correct prediction of the properties of the object in question and comparative studies. Analysis of acoustic signals showed that the banana cricket from the Moscow Zoo culture did not belong to the *Gryllus assimilis* (F.) group as it was assumed earlier. Analysis of acoustic signals and genitalia revealed similarity between the banana cricket and insects from the culture maintained at the Institute of Evolutionary Physiology and Biochemistry (IEPhB, St. Petersburg), which were supposed to be *Gryllus argentinus* (Sauss.). The calling songs and genitalia of crickets from both cultures differed from those of *G. argentinus*. Thus, the banana cricket and *Gryllus* sp. from the IEPhB culture belong to the same species but the exact identity of that species has not been yet determined.

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The "banana cricket" has been maintained in the culture of Moscow Zoo for more than 20 years and is known under this name to a wide circle of researchers. At present, this cricket is one of the preferred laboratory objects used for studying various biological problems, in particular infectious diseases of insects and their effects on the life cycle of the host (Tokarev et al., 2008). Besides, this cricket is an important food object for many animals kept in zoos (Yasyukevich et al., 2008; Berezin et al., 2008). The banana cricket was brought from East Germany where it had been cultivated since 1977. The culture originated from individuals collected in the savannahs of Ecuador (South America). Until recently, this cricket was considered to belong to the *Gryllus assimilis* (F.) group but its exact species identity has remained open to question.

On the other hand, a culture of crickets brought from France under the name *G. argentinus* (Sauss.) has been maintained since 1997 at I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry (IEPhB) of the Russian Academy of Sciences in St. Petersburg. Its similarity to the banana cricket has been repeatedly discussed by specialists (Knyazev, pers. comm.). Recently, Pinho Martins and Zefa (2011) published a redescription of *G. argentinus*, in which, along with morphological characters, some parameters of the calling songs were described. Therefore, we decided to elucidate the taxonomic position of the banana cricket by comparing the acoustic signals of individuals from both cultures (Moscow Zoo and IEPhB) with those of *G. assimilis* from the culture of McGill University (Montreal, Canada) and those of *G. argentinus* described by Pinho Martins and Zefa (2011).

The calling songs of individuals from the IEPhB culture were recorded earlier but their frequency and amplitude-temporal analysis was not performed (Ozerski and Schestakov, 2009). Since closely related species of the genus *Gryllus* often have a similar morphology, parameters of their acoustic signals are often used in identification together with morphological characters. Species specificity and stability of the structure of acoustic signals in orthopterans allow them to be used as taxonomic characters for establishing the status of close taxa and for identification of morphologically indistinguishable sibling species (Zhantiev, 1981; Popov, 1985; Bukhvalova, 1997; Heller et al., 2006; Tischechkin, 2008; Heller and Korsunovskaya, 2009; Willemse et al., 2009). Differences in acoustic signals usually testify to the species being different, whereas their similarity does not necessarily indicate conspecificity (Ragge and Reynolds, 1998; Heller, 2006; Savitsky, 2007).

Table 1. Parameters of the calling songs of crickets

Parameter	*Gryllus* sp. (banana cricket)			*Gryllus* sp. (IEPhB culture)			*Gryllus assimilis*		
	M	*SD*	*CV*	*M*	*SD*	*CV*	*M*	*SD*	*CV*
Pulse duration, s	0.020	0.004	0.18	0.021	0.006	0.27	0.009	0.001	0.08
Pulse period, s	0.034	0.008	0.23	0.029	0.004	0.14	0.013	0.003	0.21
Chirp period, s	1.859	0.683	0.37	2.108	0.591	0.28	1.127	0.307	0.27
Chirp duration, s	0.060	0.006	0.10	0.065	0.010	0.16	0.115	0.007	0.06
Number of pulses in the chirp, s	1.929	0.187	0.10	1.924	0.143	0.07	8.768	0.961	0.11
Dominant pulse frequency, Hz	4229	422	0.10	4230	81	0.02	3382	393	0.12

Notes: *M* is the mean, *SD* is the standard deviation, *CV* is the coefficient of variation.

Elements of the male genitalia are considered to be the most species-specific morphological characters in Gryllidae. Therefore, together with acoustic signals, we compared the structure of the male genitalia in the banana cricket, *Gryllus* sp. from the IEPhB culture, and *G. argentinus* redescribed from Argentina and Brazil (Pinho Martins and Zefa, 2011).

MATERIALS AND METHODS

Our material included banana crickets from the insectarium of the Moscow Zoo, *Gryllus* sp. (supposedly *G. argentinus*) from IEPhB, and *G. assimilis* from McGill University. Nymphs of *Gryllus* sp. were brought to IEPhB in 1997 from the Museum of Natural History (Paris, France) to which they had been delivered in the same year by French colleagues from an expedition to Ecuador. The culture of *G. assimilis* originated from individuals brought about 20 years before from the south of Florida (US). For the song recordings, the virgin males and females of age of 4–5 days after imaginal molt were used. The last instar nymphs were separated by sex. The calling songs were recorded in solitary males; to record the courtship songs the male was placed in one cage with the female. The temperature during recording was 25–28°C. The songs were recorded with a 1/2″ microphone, a Bruel & Kjaer amplifier, and a specially designed AD card (digitization frequency 100 kHz). Analysis and processing of signals were carried out by means of CoolEdit and TurboLab software. The calling songs were recorded in six males of the banana cricket, four males of *Gryllus* sp. from IEPhB, and eight males of *G. assimilis*. The courtship songs were recorded in seven males of the banana cricket, four males of *Gryllus* sp., and ten males of *G. assimilis*. Six parameters of the calling song and eight parameters of the courtship songs were measured. For each parameter, 10 measurements were made in each signal recorded.

The preparations of genitalia were made by boiling the posterior abdomen of a male in 10% NaOH solution for 30–40 min, after which the material was placed in 70% ethyl alcohol for defatting and preparation of the epiphallic system. The preparations were preserved in glycerol and documented using a binocular microscope equipped with a digital camera. The genital morphology was studied in four males from each culture.

RESULTS

The Acoustic Signals

Detailed parameters of calling songs of the species in question are given in Table 1. The calling songs of the banana cricket and *Gryllus* sp. do not significantly differ in all the six parameters studied (the Mann-Whitney test, $0.39 < p < 0.83$). On the contrary, the calling songs of the banana cricket and *Gryllus* sp. differ significantly from those of *G. assimilis* in all the parameters studied (the Mann-Whitney test, $p < 0.02$). The calling songs of all the males studied consist of chirps of repeated pulses (Fig. 1). Yet, in the songs of the banana cricket (Fig. 1*b*, 1*c*) and *Gryllus* sp. the chirp consists of 2–3 pulses, whereas in the song of *G. assimilis* the chirp includes, on average, 8–9 pulses (Fig. 1*e*, 1*f*). The duration of pulses is, on average, greater in the songs of the banana cricket and *Gryllus* sp. (0.02 s) than in those of *G. assimilis* (0.009 s). The average pulse period is also longer in the banana cricket and *Gryllus* sp. (0.029–0.034 s) than in *G. assimilis* (0.013 s). The chirp period in the banana cricket and *Gryllus* sp. (1.86–2.11 s) surpasses that in *G. assimilis* (1.13 s). The frequency parameters of the calling songs also differ: the domi-

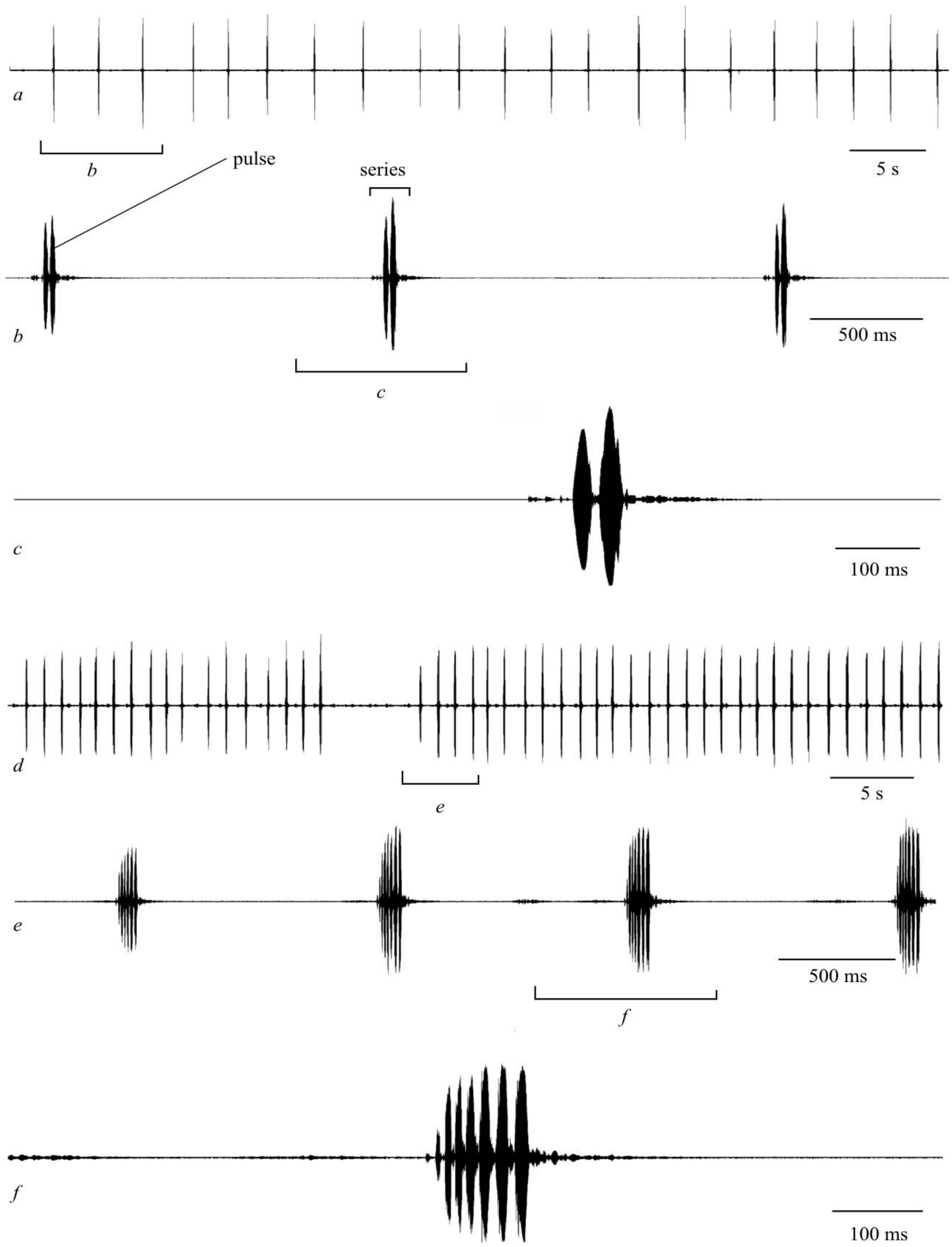

Fig. 1. Oscillograms of calling songs emitted by the banana cricket (*a–c*) and *Gryllus assimilis* (*d–f*).

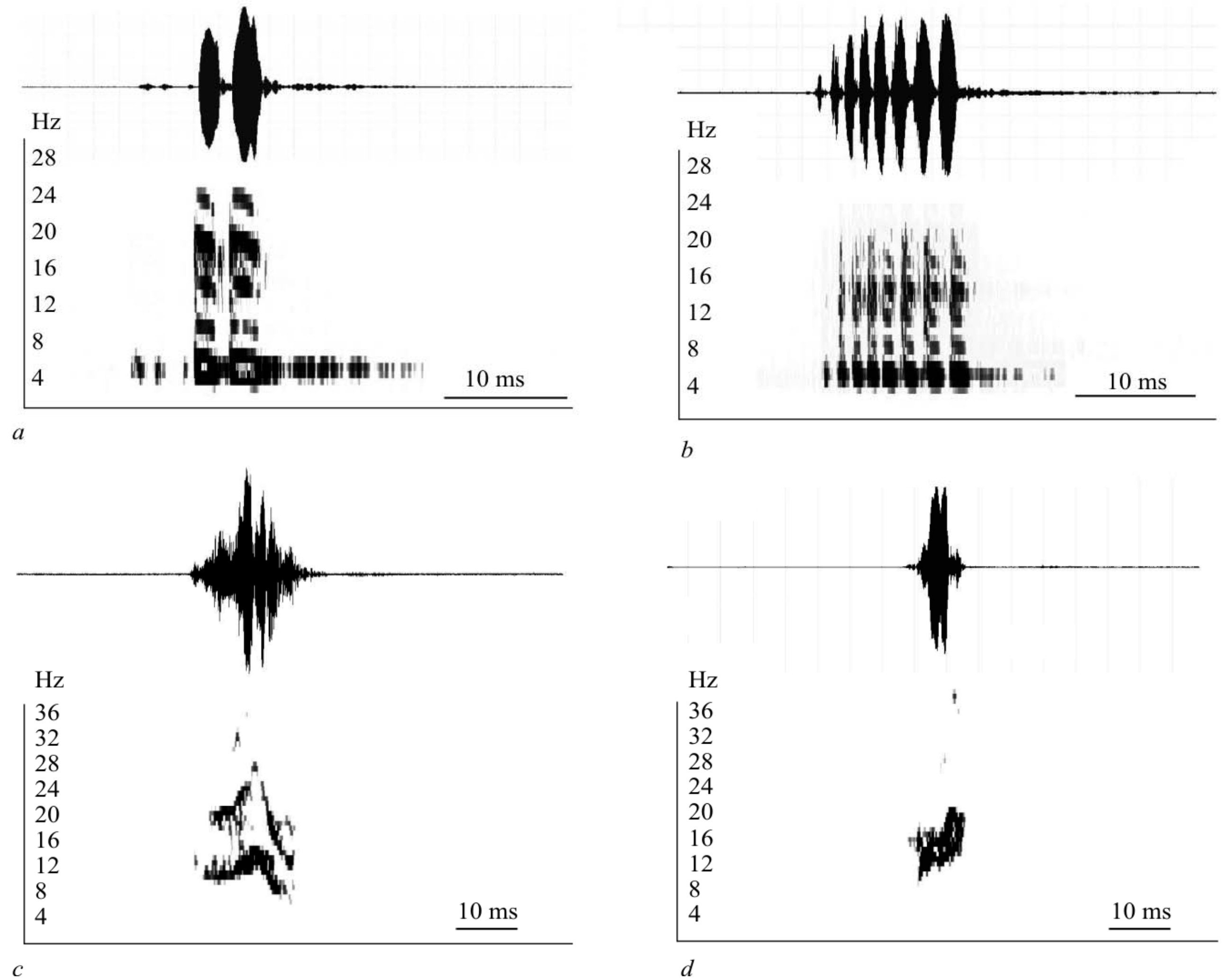

Fig. 2. Oscillograms and sonograms of pulses in the calling song of the banana cricket (*a*) and *Gryllus assimilis* (*b*) and of ticks in the courtship song of the banana cricket (*c*) and *Gryllus assimilis* (*d*). In each case the oscillogram is above, the sonogram is below.

nant signal frequency in the banana cricket and *Gryllus* sp. (4229–4230 Hz) is on average higher than that in *G. assimilis* (3382 Hz). The similarity of frequency characteristics is revealed only in the fact that the sonograms of signals of both species reveal not only the fundamental frequency but also numerous harmonics (Fig. 2*a*, 2*b*).

The courtship song of the banana cricket and *Gryllus* sp. has a more complicated structure than the calling song. It is an alternation of high-amplitude, high-frequency ticks and low-amplitude, low-frequency pulses (Fig. 3). The pulses are continuous sequences usually filling up the whole interval between repeated double ticks (Fig. 3*b*, 3*d*). Another variant is also possible in which low-amplitude pulses in the courtship song are grouped into more or less distinct chirps; however, the boundaries of these chirps are less distinct than in *G. assimilis* (Fig. 4). In the courtship song of *G. assimilis* the pulses are grouped into well distinguishable chirps (Fig. 4*b*, 4*d*). Moreover, the amplitude of pulses in the chirps varies strongly and may reach the level of ticks. In the songs of the banana cricket and *Gryllus* sp. the average duration of ticks is twice as long (0.015 s) as in those of *G. assimilis* (0.009 s) (Table 2). The pulses also differ in duration, the mean value being greater in the banana cricket and *Gryllus* sp. (0.012–0.016 s) than in *G. assimilis* (0.008 s). The songs of these species do not differ by the number of ticks in the phrase, which may vary from one to four; the frequency characteristics of ticks in the songs of all the species are also similar (Table 2). Their dominant frequency varies, on average, from 13 985 to 16 141 Hz. It is remarkable that in all the species, the frequency modulation of ticks is considerably more expressed than in the

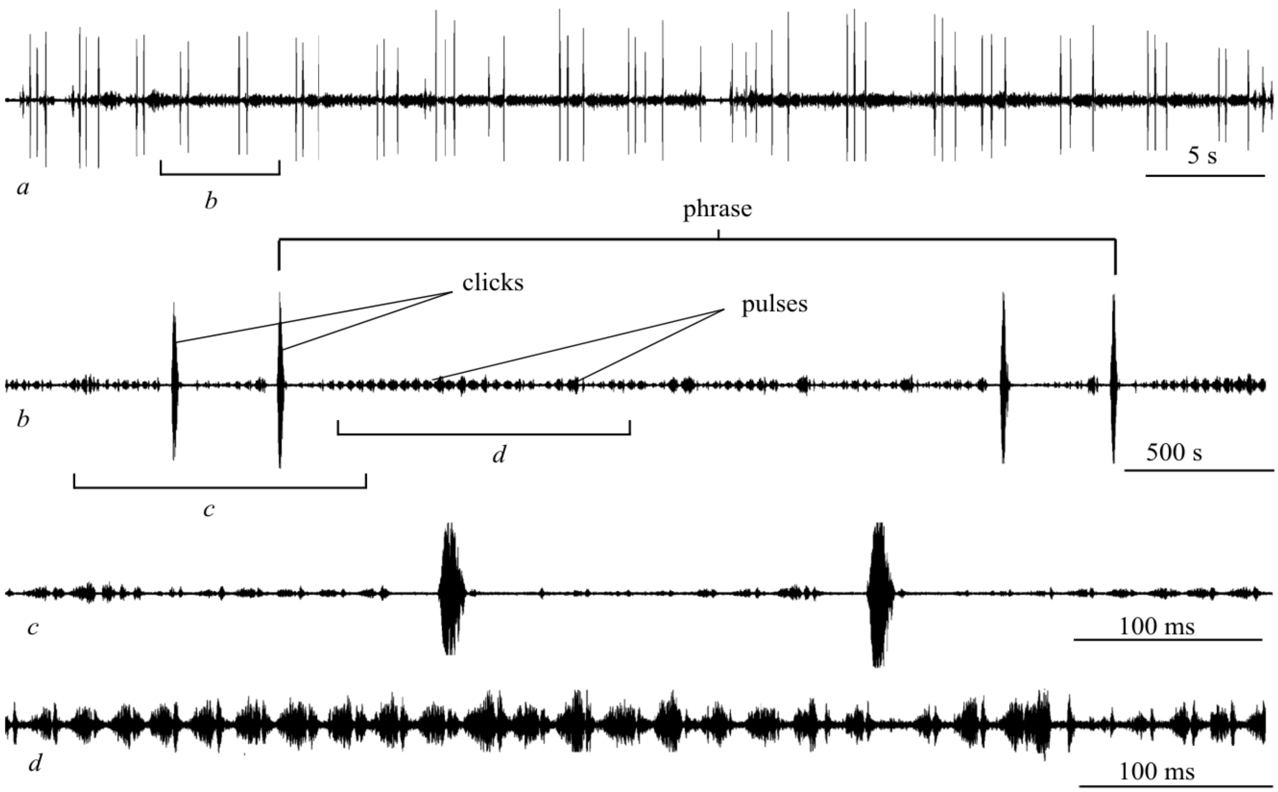

Fig. 3. The courtship song of the banana cricket. Oscillogram (*a*) and its fragments shown at expanded time-scale (*b*–*d*). The time marks are shown under each oscillogram.

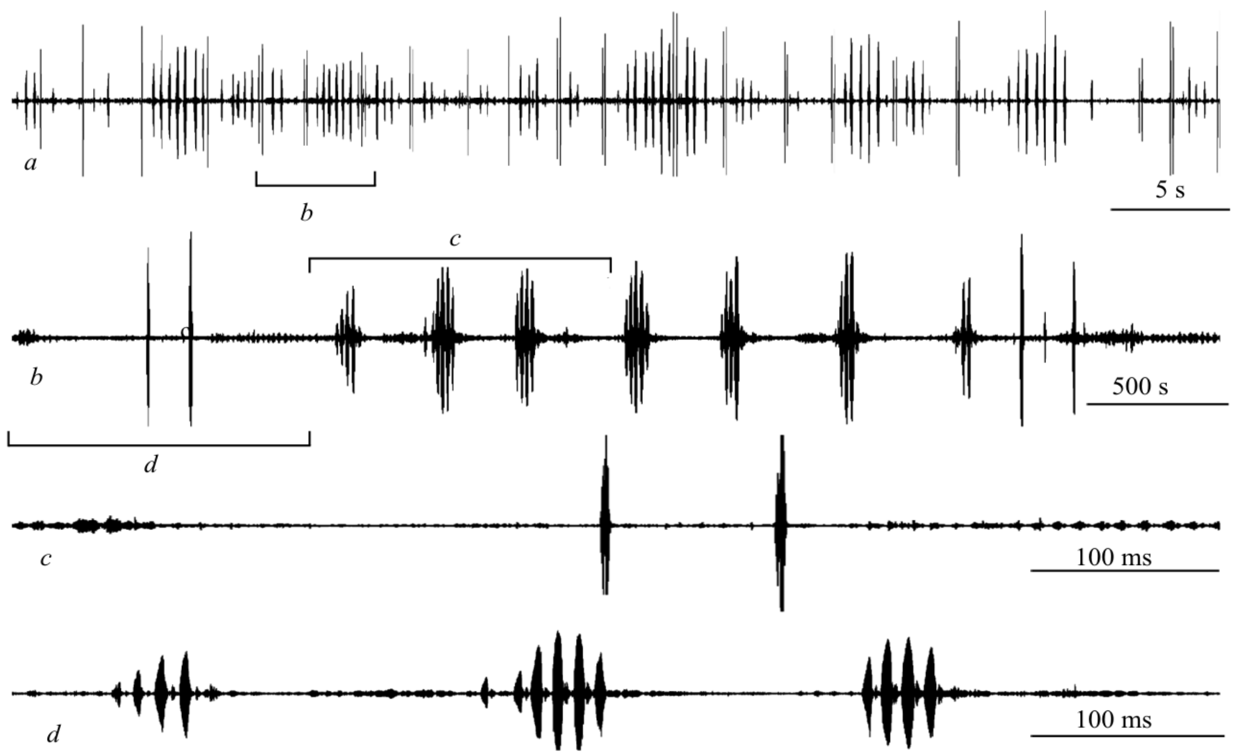

Fig. 4. The courtship song of *Gryllus assimilis*. Oscillogram (*a*) and its fragments shown at expanded time-scale (*b*–*d*). The time marks are shown under each oscillogram.

Table 2. Temporal and frequency parameters of the cricket courtship songs

Parameter	*Gryllus* sp. (banana cricket)			*Gryllus* sp. (IEPhB culture)			*Gryllus assimilis*		
	M	*SD*	*CV*	*M*	*SD*	*CV*	*M*	*SD*	*CV*
Tick duration, s	0.015	0.002	0.15	0.015	0.001	0.06	0.009	0.001	0.09
Duration of pause between ticks, s	0.184	0.044	0.24	0.180	0.050	0.27	0.098	0.018	0.18
Period of phrase repetition, s	1.482	0.404	0.27	2.310	1	0.43	2.380	0.429	0.18
Number of ticks in the phrase	2.123	0.535	0.25	2.490	0.551	0.22	2.018	0.445	0.22
Pulse duration, s	0.012	0.002	0.18	0.016	0.001	0.09	0.008	0.001	0.09
Pulse period, s	0.028	0.005	0.16	0.023	0.002	0.10	0.014	0.001	0.09
Dominant tick frequency, Hz	14610	2459	0.17	13985	507	0.04	16141	1001	0.06
Dominant pulse frequency, Hz	4900	499	0.10	4393	371	0.08	–	–	–

Notes: *M* is the mean, *SD* is the standard deviation, *CV* is the coefficient of variation.

pulses of the calling song (Fig. 2*c*, 2*d*). The dominant pulse frequency in the courtship song of the banana cricket and *Gryllus* sp. is similar and varies, on average, between 4393 and 4900 Hz (Table 2). On the contrary, the dominant pulse frequency in *G. assimilis* varies strongly: it may correspond to the fundamental frequency (3752 Hz) or the second and third harmonic. For this reason, the averaged pulse frequency of *G. assimilis* is not shown in Table 2.

On the whole, it should be emphasized that the courtship songs of the banana cricket and *Gryllus* sp. do not differ significantly in most of the parameters studied (the Mann-Whitney test, $0.13 < p < 1$) except the duration of pulses ($p < 0.02$). On the contrary, the courtship songs of *G. assimilis* differ significantly from those of the banana cricket and *Gryllus* sp. in most parameters ($p < 0.02$), except the number of ticks in the phrase ($0.08 < p < 0.69$). Moreover, there are no significant differences between *G. assimilis* and the banana cricket in the dominant frequency of ticks ($p = 0.06$), as well as between *G. assimilis* and *Gryllus* sp. in the phrase repetition period ($p = 0.77$).

The Structure of the Epiphallic Part of the Male Copulatory Apparatus

The genitalia were studied in four males of the banana cricket and four males of *Gryllus* sp. The most reliable characters are the general structure of the sclerotized parts of the copulatory apparatus and the ratio of the median and lateral lobes of the epiphallus. In males from both cultures the median lobe of the epiphallus is slightly (1.5 times) longer than the lateral ones (Figs. 5*a*, 5*c*). The median lobe narrows smoothly towards the end without any bends and has a pointed tip (Figs. 5*b*, 5*d*).

DISCUSSION

Acoustic analysis has shown that crickets from the cultures of Moscow Zoo and IEPhB do not differ in temporal and frequency parameters of the calling song; at the same time, both cultures differ strongly from *G. assimilis*. Our records of the calling signals of *G. assimilis* from the culture of McGill University are identical with records of this species from Jamaica and the south states of the US (Weissman et al., 2009). On the other hand, comparison of the calling songs of the banana cricket and *Gryllus* sp. with those of *G. argentinus* from Brazil and Argentina (Pinho Martins and Zefa, 2011) reveals a number of differences. In particular, the song of *G. argentinus* has a greater duration of pulses and chirps, a lower pulse rate, and a considerably higher chirp rate than the songs of crickets from both of our cultures. Our records were made at the same temperature that was specified by the Brazilian authors. Although in the song of *G. argentinus* the chirps often consist of two pulses, they are quite frequently preceded by one to three low-amplitude pulses. The latter feature was not observed in the songs of crickets from our cultures.

The courtship songs of the banana cricket and *Gryllus* sp. are similar in most temporal and frequency parameters but differ significantly from those of *G. assimilis*. Unfortunately we cannot compare our records with the literary data because the courtship songs in these species were not studied. Different authors mainly study calling songs; this type of signals

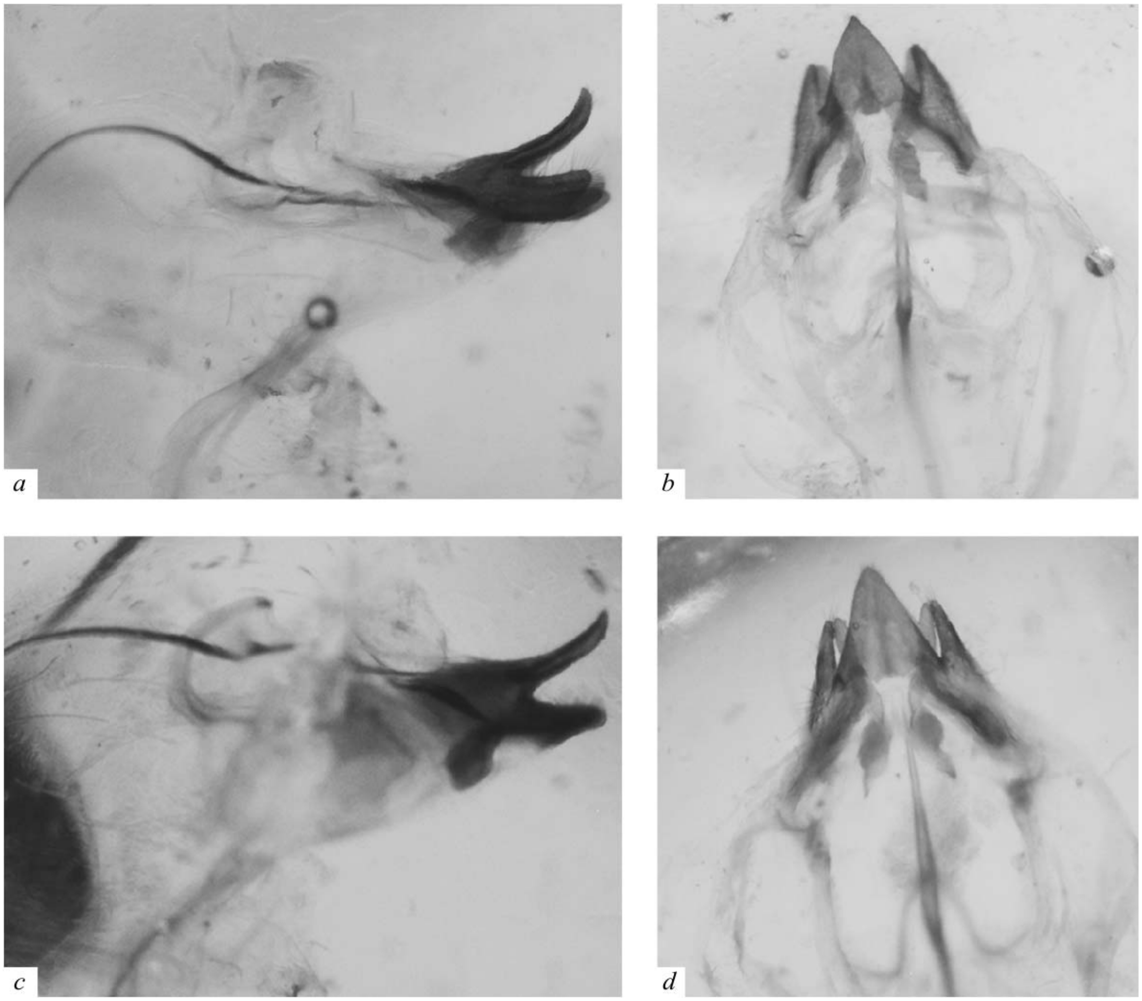

Fig. 5. Male genitalia of the banana cricket (*a*, *b*) and *Gryllus* sp. from the IEPhB culture (*c*, *d*), in lateral (*a*, *c*) and dorsal (*b*, *d*) view.

is considered to be the most stable and therefore the most species-specific because its main function is attracting a conspecific partner from a distance. There is an opinion that courtship songs may be more variable than calling ones since they serve not only for recognition of a conspecific mate but also for assessment of the "quality" of the male (Wagner and Reiser, 2000; Fitzpatrick and Gray, 2001; Vedenina, 2005; Zuk et al., 2008). The courtship songs of some orthopterans, for example grasshoppers, are actively used as a species-specific character (Ragge and Reynolds, 1998; Vedenina and Helversen, 2009). The results of our research indicate that the calling and courtship songs have almost equal number of both stable and highly variable parameters. For instance, in the calling songs the coefficient of variation (Table 1) is relatively small for some parameters (the number of pulses in a chirp, the dominant frequency of pulses) but sufficiently high for others (the pulse and chirp periods). The same can be found in courtship songs (Table 2). For example, such parameters as duration and dominant frequency of ticks are not very variable, whereas the phrase repetition period and the number of ticks in the phrase vary strongly. This once more testifies to the fact that the signals of both types (when present) are important for the species description.

Comparison of the male genitalia of crickets from the cultures of Moscow Zoo and IEPhB has shown them to be similar. On the contrary, the structure of the median lobe of the epiphallus of *G. argentinus* is different from that of crickets from our cultures. In *G. argentinus*, the median lobe is twice as long as the lateral ones (Pinho Martins and Zefa, 2011), whereas in the banana cricket and *Gryllus* sp. the median lobe is only inconsiderably longer than the lateral ones.

CONCLUSION

Similarity of acoustic signals and genital morphology of the banana cricket from the Moscow Zoo culture and *Gryllus* sp. from the culture of IEPhB indicates that these crickets belong to the same taxon. Considerable differences between their acoustic signals and those of *G. assimilis* show that the banana cricket does not belong to this species. The identification of *Gryllus* sp. from the culture of IEPhB as *G. argentinus* should be also considered erroneous since crickets from this culture differ from *G. argentinus* from Argentina and Brazil in their calling songs and genital structure. This fact is rather important because experimental research is carried out on the crickets from the IEPhB culture.

Thus, the taxonomic status of the banana cricket and *Gryllus* sp. from the IEPhB culture remains obscure.

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