SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Cryptic Species of *Gryllus* in the Light of Bioacoustic (Orthoptera: Gryllidae)

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Espécies Crípticas de *Gryllus* à Luz da Bioacústica (Gryllidae: Orthoptera)

RESUMO - O gênero *Gryllus* destaca-se com um grande número de espécies descritas e representa um dos mais complexos gêneros na sistemática dos Orthoptera, caracterizado por um conjunto de espécies cosmopolitas e crípticas. Este trabalho compara os sons emitidos por espécimens de *Gryllus* coletados no campus da UNESP em Rio Claro (SP), bem como a morfologia e morfometria das *pars stridens*, com o objetivo de aplicar os resultados no reconhecimento de possíveis espécies crípticas e contribuir na discussão de processos de especiação. Três grupos de grilos foram discriminados por diferenças nas *pars stridens* e sons de chamado, caracterizados por diferentes ritmos, freqüências e composição das notas, indicando assim, a presença de três espécies no local analisado, morfologicamente pouco distintas. Diante dos resultados, sugere-se a utilização das características da *pars stridens* e do som de chamado como caracteres diagnósticos imprescindíveis na taxonomia dos *Gryllus*.

PALAVRAS-CHAVE: Insecta, bioacústica, *pars stridens*

ABSTRACT - The *Gryllus* genus represents one the most complex in the Orthoptera systematic, characterized by a set of cosmopolite and cryptic species, many of them already described. This study compared the songs emitted by *Gryllus* specimens collected on the UNESP campus in Rio Claro (SP) and the *pars stridens* morphology and morphometry, to use the results to recognize possible cryptic species and contribute to the discussion of speciation processes. Three groups of crickets were discriminated by differences in the *pars stridens* and calling songs, characterized by different rhythms, frequencies and note composition, that indicated the presence of three species in the location analyzed, that presented few morphological differences. It is suggested from the results that the use of the *pars stridens* characteristics and the calling song as diagnostic traits is essential in the *Gryllus* taxonomy.

KEY WORDS: Insecta, bioacoustic, *pars stridens*

The Grylloidea superfamily includes a fairly diversified group of insects, commonly called crickets and widely distributed throughout the world. They are usually nocturnal and occupy the most diverse habitats.

The *Gryllus* genus (Gryllidae family) is outstanding because it presents many species (about 80) distributed throughout the world. It is one of the most complex in the Orthoptera systematic field, because it has a set of cosmopolite and cryptic species that have been classified and identified by different researchers according to different taxonomic standards. Another element that contributes to their taxonomic problems has been the presence of intra-specific variation in the population of which polymorphism for wing length is the most characteristic; both micropterous and macropterous individuals are observed in some populations (Harrison 1979, Roff 1986, Walker 1987, Roff & Simons 1997).

The *Gryllus* fauna of South America have been little explored by entomologists. The catalogues and synopses proposed are old and some are based on museum materials elaborated according to the external morphological and phallic complex characteristics that do not vary greatly in most species of the group.

Crickets present the most complex acoustic system among the invertebrates and some repertoires include four to six functional types of distinct signals and thus form a good element for analysis of evolutionary mechanisms (Alexander 1962) mainly when associated to observation of the species behavior during acoustic communication and the *pars stridens* morphology.

Thus this study characterized the *Gryllus* species that occur on the UNESP campus in Rio Claro (SP) using bioacoustics and *pars stridens* analysis in their identification.

Material and Methods

The *Gryllus* examples analyzed in this study were captured monthly from March 2000 to July 2001 in nocturnal collections or by traps buried in the soil on the UNESP campus in Rio Claro (SP). The crickets were kept in terrariums for about 10 days until they began to emit singing or specific sounds.

The songs were recorded on magnetic tape rolls on a NAGRA E recorder with MS88 microphone and analyzed by the Avisoft program.

The right tegmen of the individuals was extracted to count the teeth of the stridulator apparatus and for morphometric analysis. The morphology of the teeth that form the *pars stridens* was analyzed under a scanning electronic microscope. Descriptive statistical methods were used, including the mean of the total number of *pars stridens* teeth (obtained with the help of a Axiohome microscope) and number of teeth per millimeter. These data were submitted to the Kruskal-Wallis test and multiple comparison involving all the pairs of treatments (Campos 1983).

Results

No striking external differences were observed among the individuals in the sample and they were all very similar for the aspects normally used for cricket species description, such as the structures of the phallic complex. After the different groups had been distinguished, variations were detected in the size and coloring of the specimens within each group.

The calling song or specific sound analysis showed that eight specimens (G4, G6, G7, G8, G17, G18, G20 and G26 - one of them in Fig. 1A) emitted the calling song characterized by the presence of eight to nine notes, with frequency between 3,5 kHZ and 4 kHz (Fig. 2A). Three different rhythms were observed, that is, the individuals presented different quantities of sets of note per second.

Another 14 species (G1, G2 G3 G5, G10, G19, G21, G23, G24, G25, G27, G28, G31 – one in Fig. 1B) emitted calling song in groups of three notes, with frequency between 5 kHz and 5.5 kHz (Fig. 2B). Three different rhythms were also observed.

A

B

C

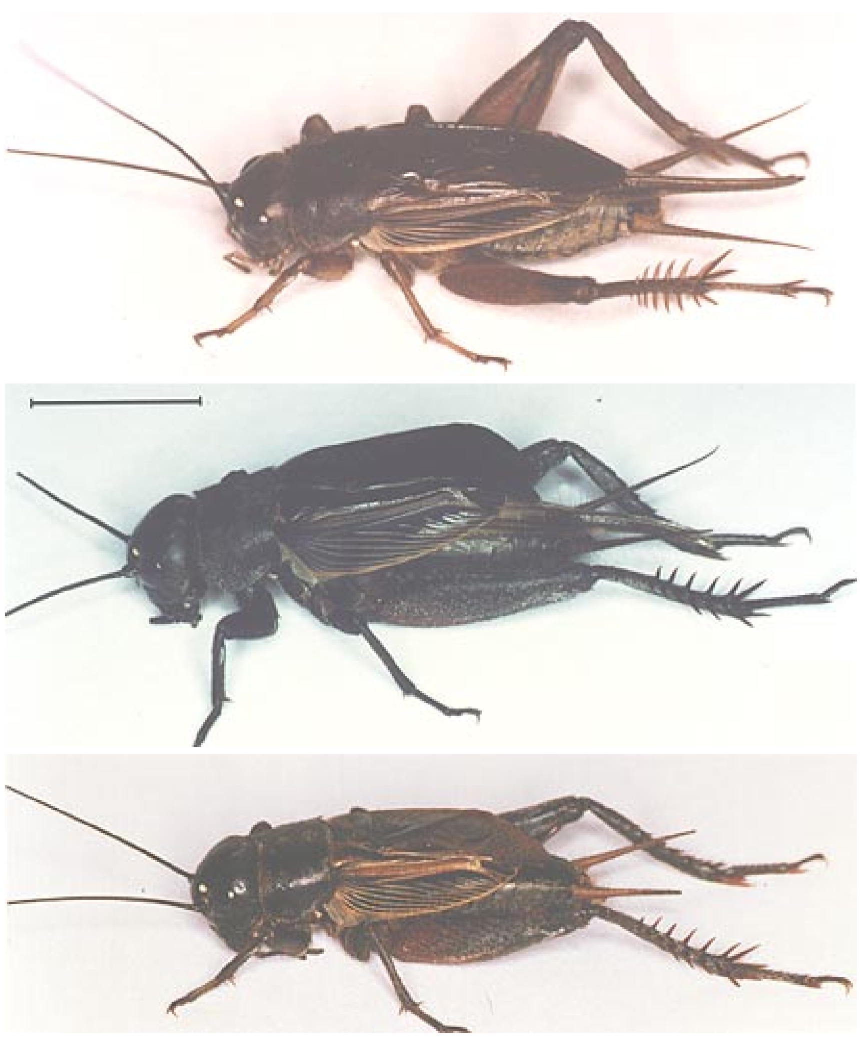

Figure 1. *Gryllus* spp. specimens. A. Species A; B. Species B; C. Species C. Scale = 1 cm

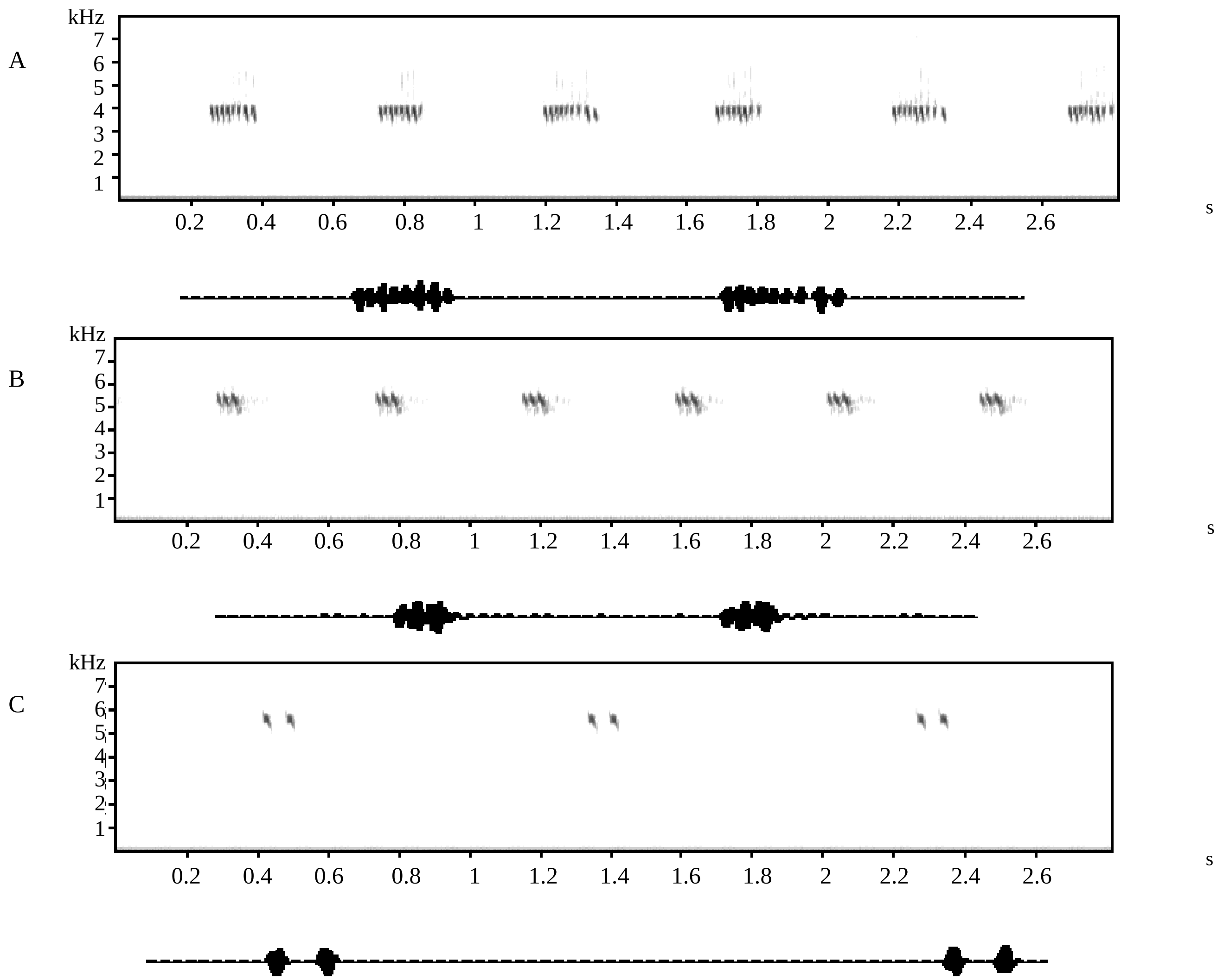

Figure 2. *Gryllus* spp. sonograms. A. Species A; B. Species B; C. Species C.

Two of the specimens (G14 and G30 – Fig 1C) emitted the calling song in groups of two notes, with frequency between 5.2 kHz and 6 kHz (Fig. 2C).

The difference in the number of notes that composed the calling song distributed the examples in three species: species A (G4, G6, G7, G8, G17, G18, G20 and G26), species B (G1, G2, G3, G5, G9, G10, G19, G21, G23, G24, G25, G27, G28 and G31) and species C (G14 and G30); these three groups were also well characterized and distinguished by the *pars stridens* morphological and morphometric analysis.

The *pars stridens* consists of small teeth placed in the ventral region of the right tegmen, which are scraped by a 'scraper' present in the left tegmen edge, working as a washboard. In *Gryllus* the *pars stridens* teeth generally present triangular, uniform and inclined morphology and gradually decrease in size at both ends.

The comparative analysis of the teeth that form the *pars stridens* shows small differences among the specimens studied. Species B specimens (Fig. 3B) and species C (Fig. 3C) present teeth with a wider base than those observed in species A (Fig. 3A). The ultrastructure of the *pars stridens* of the individuals in species C showed some differences when compared with that of the specimens from species B; in species C the teeth are more arched and wider sideways and more flattened dorsoventrally.

A mean of 117 teeth was counted in species A distributed at 34.2 per mm and a mean *pars stridens* length of 3.4mm was observed. In species B, there were 140.3 teeth on average, distributed at 32.7 per mm, in a total mean *pars stridens* length of 4.2 mm. The specimens from species C presented 199.5 teeth, distributed in a 4.5 mm long *pars stridens* with a mean of 44.3 teeth per mm (Table 1).

The data obtained in the *pars stridens* morphometric analysis were submitted to the Kruskal-Wallis test (Campos 1983) to ascertain the difference among the species; difference was observed among at least two species for the number of teeth and *pars stridens* length (Fig. 4A, B); however, there was no significant difference among them regarding the number of teeth per millimeter (Fig. 4C).

A multiple comparison involving all the pairs of species

A

B

C

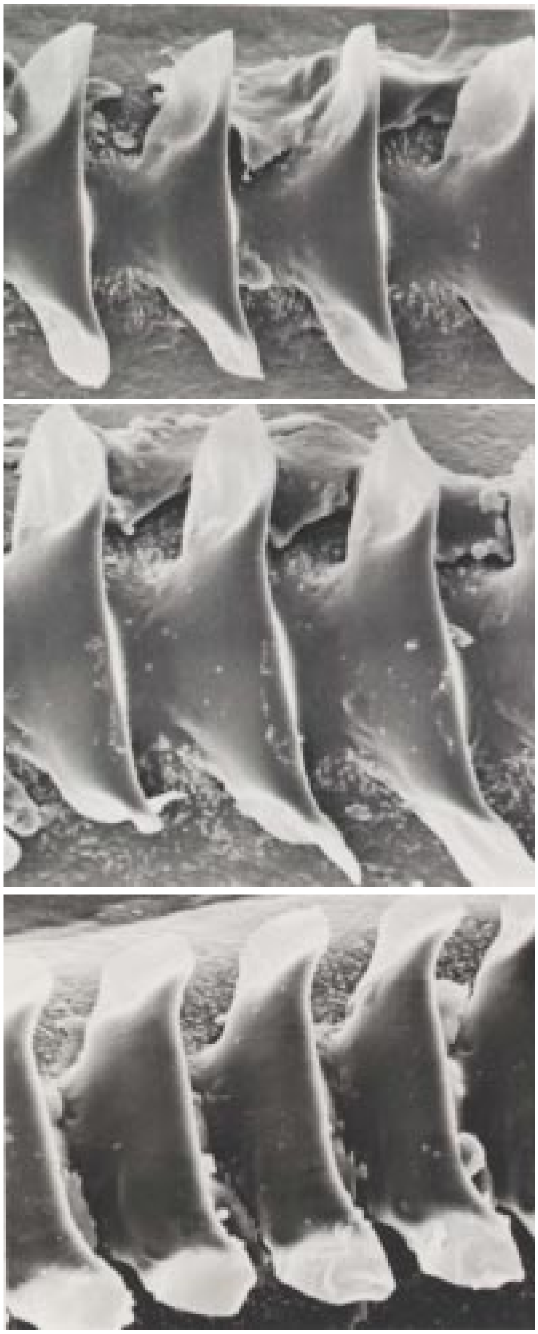

Figure 3. Mid portion of the *Gryllus* spp. *pars stridens*. A. Species A; B. Species B; C. Species C. Increases: A, B,C = 500X

was used to identify the differences among the pairs of samples (Campos 1983). This comparison showed significant difference among all the pairs of species for the number of teeth (Fig. 4A) while for the *pars stridens* length, significant difference was only shown between species A and B.

Discussion

Fulton (1932) was the first person to use sound to differentiate crickets. While studying *Gryllus assimilis* Fabricius in North Carolina, USA, he was able to distinguish four species that had different song emissions, but all thought to be G. *assimilis*.

The *Gryllus integer* Scudder species was first identified in California, but several authors found what they thought to be the same species in other locations in Texas. After studying the song emissions from samples collected in California and Texas it could be concluded that the two regions have different species that did not even generate hybrids in laboratory. Cade and Otte (2000) published a study to validate the name *Gryllus texensis* Cade & Otte, for the species that occurs in the Texas region and describe the difference in the calling song of the two species.

Gray & Cade (2000) studyied *G. texensis* and *Gryllus rubens* Scudder and submitted female *G. texensis* to the calling song their own species and to that of *G. rubens*; they observed a clear preference of the females for the song of their own species that prevented mating between individuals of different species. It is known, however, that there can be intercrossing among the species in the laboratory with the formation of viable hybrids, with intermediary calling song, showing a deficiency in the mechanism of post-mating reproductive isolation mechanism.

Doherty (1985) reported that in *Gryllus bimaculatus* De Geer the temperature variation has a great effect on the series of note repetitions, but their duration, number or fundamental frequency are not altered. This would be a factor that could explain the different rhythms found in the examples analyzed here, therefore forming intra-specific variations.

The life cycles also have been used as important factors in *Gryllus* species distinction. In 1967, Alexander and Meral analyzed song emission taking into consideration the seasonal and daily cycles of *Gryllus veletis* Alexander and *Gryllus pennsylvanicus* Burmeister. *G. veletis* stridulate from mid May to the beginning of August and *G. pennsylvanicus* stridulate from the beginning of August to mid November.

During the collection period of this study, three types of calling song were heard at the same time. This suggests

Table 1. Analysis of the mean and the standard deviation of *pars stridens* of the individuals of the three *Gryllus* species. x = mean; DP = standard deviation; r = variation amplitude

Species	N	Number of teeth			*Pars stridens* length			Teeth per mm		
		x	DP	r	x	DP	r	x	DP	r
A	8	117.0	± 6.02	(106-122)	3.4	± 0.13	(3.2-3.6)	34.2	± 1.55	(31.2-36.5)
B	14	140.3	± 6.93	(124-147)	4.2	± 0.22	(3.7-4.6)	32.7	± 2.34	(28.3-39.1)
C	2	199.5	± 2.12	(198-201)	4.5	± 0.40	(4.2-4.8)	44.3	± 3.39	(41.3-46.1)

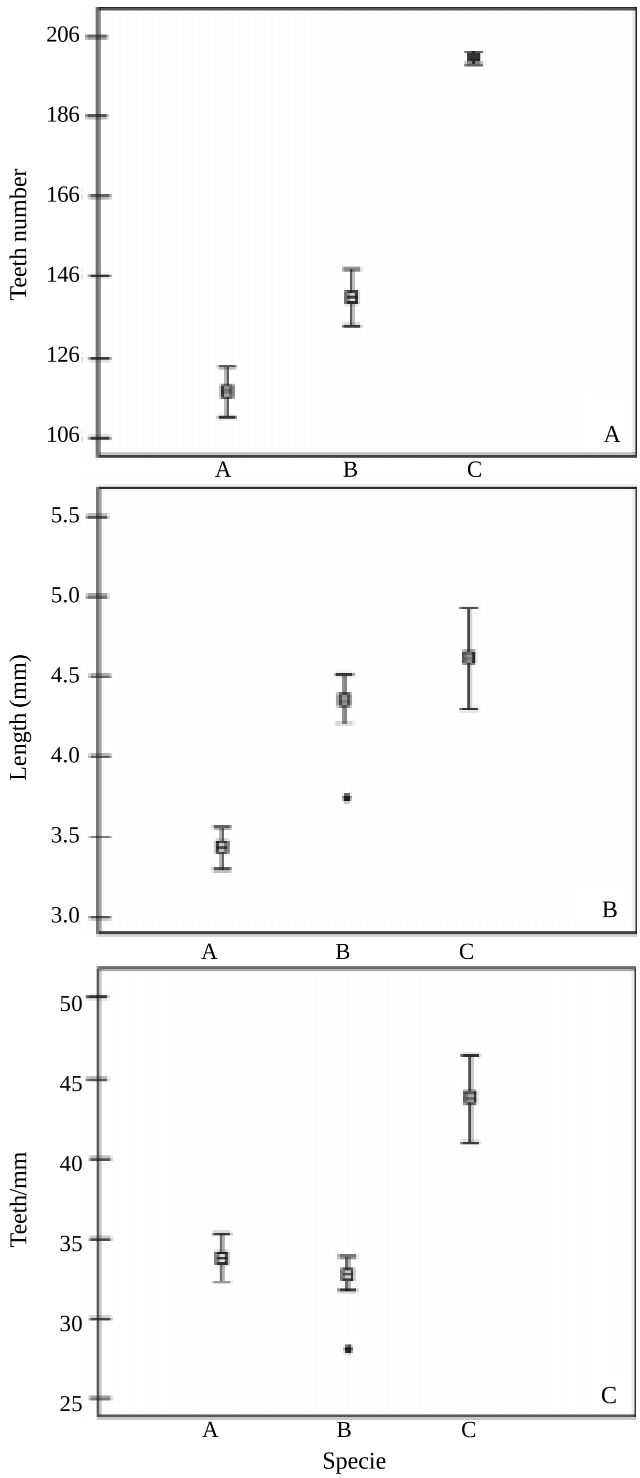

Figure 4. Graphs obtained from the *pars stridens* morphometric analyis. ⌶ = standard deviation, ■ = mean, ● = extreme values (outliers).

that the groups formed different species because the role of acoustic behavior is always clear and defined in sexually active species at the same time and place (Alexander 1962). We could suggest the action of an ethological reproductive isolation process of these species that would include basically differences in the calling song emission.

According to Walker (1964), after recognizing cryptic species by song, it is easier to locate morphological differences among these species; in the case of *Gryllus* differences can be observed in the *pars stridens*.

Pars stridens morphometry has been used as a differentiating factor in crickets because each species has a specific number of teeth, *pars stridens* length, and number of teeth per millimeter (Weissman *et al.* 1981, Otte *et al.* 1988, Alexander 1991, Mafla & Cevallos 1991). In this sense, Desutter (1990) studied South American *Gryllus* and observed that the animals in her sample could be divided into two groups according to the number of teeth on the *pars stridens,* those with many teeth (180 to 220) and those with few teeth (110-160). The analysis of significance of the results obtained here reinforces this idea, because it demonstrated that the number of teeth is the only trait that was highly significant in separating the species. However, the results observed in the three species for *pars stridens* morphology and morphometry are good examples of how a population that apparently consists of a single species, has characteristics that permit its individualization after separating the individuals in groups with similar song characteristics.

When our data are compared with those in the literature, we observe that species A fits the description of *G. assimilis.* On the other hand, species B and C do not fit the description of other species and probably represent new species. Since differences were not observed among the morphological traits of the specimens analyzed, we could quote them as typical examples of the concept proposed by Walker (1964) for cryptic species.

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