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On the taxonomic status of *Cicada orni* Linnaeus (Hemiptera, Cicadidae) from Lesbos island in Greece

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Abstract

A male of *Cicada orni* Linnaeus, 1758 from the island of Lesbos (Greece) was found in a recent study to be different from the typical species on the basis of longer echemes and a higher peak frequency. As such it was described as the new subspecies *C. orni lesbosiensis* Boulard, 2000. The present study is a more thorough analysis of the calling song of further material of *C. orni* collected in the island of Lesbos as well as in the surrounding area, i.e., other Aegean islands and the Greek and Turkish mainlands. This acoustic signal was recorded, comparatively analysed in time and frequency domains and no significant differences were found between this Lesbos sample with nearby populations. Therefore, the present results do not support the designation of the Lesbos material as an independent subspecies.

Key words: Hemiptera, *Cicada orni*, taxonomic status, acoustic signals, bioacoustics, Lesbos, Greece

Introduction

As emphasized by Paterson (e.g., 1985), sexually reproducing species can be defined as a set of organisms with a common specific mate-recognition system (SMRS). Therefore, the defining properties of most species are their unique SMRSs. In many insects such as cicadas, the SMRSs are mostly acoustic, and the structure of the calling song is an important component. Thus, the evolution of such unique mate-recognition systems is the crucial event for the divergence of populations and the origin of new species (Paterson, *op. cit.*).

Several cicada studies have shown that species are usually isolated by ethological mechanisms, and good examples may be found in the genus *Cicada* L. For instance, the species *C. barbara* Stål and *C. orni* Linnaeus (e.g., Quartau & Rebelo 1994) are sympatric

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in some localities, but are ethologically isolated by the production of quite different calling songs. On the other hand, *C. cretensis* Quartau & Simões, *C. mordoganensis* Boulard and *C. orni* have both different acoustic signals, and are geographically isolated (e.g., Simões *et al*. 2000; Quartau & Simões, 2005).

Cicada orni is one of the most abundant and common circum Mediterranean cicadas with a broad distribution from the Iberian peninsula in the west to Greece and Turkey (Quartau *et al*. 1999; Pinto-Juma *et al*., 2005) and some countries in the near East (Nast 1972), and also around the Black Sea (Popov 1975). Males produce striking calling songs during summer time and can sing continuously from a single site for hours, sometimes chorusing with other males (e.g., Boulard, 1995; Pinto-Juma *et al*., 2005).

Boulard (2000a) studied a single male of *C. orni* from the island of Lesbos and found that it was distinct from the typical species on the basis of longer echemes and a higher peak frequency. Therefore, he erected the new subspecies *C. orni lesbosiensis* Boulard, 2000.

Meanwhile, the first and the third authors had the opportunity to make further field work in the same island as well as in the surrounding area, and collected a larger sample of *C. orni* .

The present paper is a detailed analysis of the acoustic signals of *C. orni* in the Aegean area, including samples from the nearby Greek and Turkish mainlands, in order to get a better understanding of the taxonomic status of the Lesbos populations.

Material and methods

Fieldwork was carried out in the Mediterranean geographical area of the Aegean Sea during the last summers and specimens of *C. orni* were collected in several localities in Greek islands, including Lesbos, and the Greek and Turkish mainlands (Fig. 1). Material examined belonging to different populations and samples are described in Table 1.

The acoustic recordings were made during the warmest hours of the day, usually at temperatures of 30–38º C and in the frequency range of 20–22 000 Hz with a TCD-D10 ProII digital Sony DAT recorder connected to a compatible dynamic microphone (frequency range 50–18 000 Hz). Specimens were later collected by hand or through a sweeping net. As in previous studies, song terminology follows that of Boulard (e.g., 1995, 2000b). Acoustic recordings and specimens are kept in the Department of Animal Biology with one of the authors (J.A.Q.)

Signals were digitized from the analog output of the DAT recorder at a sampling rate of 32 kHz and then were analysed in time and frequency domains through the software Avisoft-SASLab Pro (Specht 2002). For each specimen, whenever possible, a one-minute recording was used to produce oscillograms, spectrograms, and mean amplitude spectra computed with a Fast Fourier Transform (FFT) using a resolution of 512 points of a Hamming Window size with 50% overlap.

With a view to discriminate acoustic differences, the following 13 acoustic variables were measured (Table 2): number of echemes/s, echeme duration, inter-echeme interval, echeme period, ratio echeme/inter-echeme interval, peak frequency, bandwidth (at -20dB), quartile 25 %, quartile 50 %, quartile 75 %, quartile 75 % – quartile 25 %, minimum frequency, and maximum frequency.

Statistical tests were made using STATISTICA 6.0 software (StatSoft 2001). Reduction of dimensionality of the data matrix was made through R-type principal component analysis based on standardized data in order to test for intraspecific acoustic differentiation between specimens.

TABLE 1. Samples of *C. orni* L. with number of males recorded for sound analyses (N) and dates of recording.

Populations	Region	N	Dates of recording
Andros (Greece)	Island (Lat. 37º 55 N, Long. 24º 48 E)	3	3–4. vii .1999
Athens (Greece)	Mainland (Lat. 37º 58 N, Long. 23º 43 E)	19	9–10.vii.1997; 15.vii.1998; 13.vii.1999
Evia (Greece)	Mainland (Lat. 38º 27 N, Long. 24º 4 E)	12	29. vi .2002
Itea (Athika, Greece)	Mainland (Lat. 38º 26 N, Long. 22º 25 E)	24	26 and 29. vi .2002
Kosmas (Greece)	Mainland (Lat. 37º 6 N, Long. 22º 44 E)	2	24. vi .2002
Lesbos (Greece)	Island (Lat. 39º 11 N, Long. 26º 2 E)	3	12–13. vii .1999
Naxus (Greece)	Island (Lat. 37º 6 N, Long. 25º 28 E)	4	6. vii .1999
Neapolis (Greece)	Mainland (Lat. 35º 31 N, Long. 24º 1 E)	7	25. vi .2002
Paralio (Greece)	Mainland (Lat. 37º 15 N, Long. 22º 52 E)	7	24. vi .2002
Skala (Athika, Greece)	Mainland (Lat. 38º 40 N, Long. 23º 5 E)	4	29.vi . 2002
Skyros (Greece)	Island (Lat. 38º 54 N, Long. 24º 32 E)	17	28. vi .2002
Assos (Turkey)	Mainland (Lat. 39º 36 N, Long. 26º 27 E)	1	27. vi .2003
Total		103	

Results

All together, the calling songs of 103 males of *C. orni* were recorded in the field. The calling song can be described as being made up of a regular repetition of echemes, which in turn are composed of a variable number of groups of pulses (Fig. 2).

Table 3 gives a summary of the time and spectral characteristics of the male calling songs that were measured for the Lesbos population and the remaining 11 other populations from Aegean islands and the Greek and Turkish mainlands. Considering average values, this signal can be described in the time domain as having a regular repetition of echemes with 0.07 ± 0.02 (average ± standard deviation) seconds of duration separated by intervals of 0.19 ± 0.07 seconds (Table 4). The spectral characteristics of the signal showed a peak frequency of 5015 ± 507 Hz and a bandwidth (at -20dB) of 7565 ± 1400 Hz.

FIGURE 1. Samples of *C. orni* investigated (see Table 1 for details).

Therefore, mean acoustic values of specimens from Lesbos fall into the intraspecific variation for most variables, namely the echeme duration, peak frequency and bandwidth (Tables 3 and 4).

Moreover, in order to test for intraspecific acoustic differentiation a principal component analysis was performed and four components were extracted accounting for 92.2% of the total variation (C1= 42.3%; C2= 22.6% C3= 15.3%; and C4= 12%). More than half (64.9%) of the variation in the study was explained by the first two components. No evident pattern of variation in *C. orni* was found, specimens tending to form a homogeneous group (Fig. 3). There is just a slight tendency in some populations for individuals to form clusters but these do not appear to be isolated, overlapping instead. With respect to the specimens from Lesbos, despite tending to appear close to each other in the upper right of the diagram, they overlap with different populations such as those from Athens and Kosmas (Fig. 3).

Discussion

Our acoustic results in time and frequency domains reveal some variation in the calling song among the populations of *C. orni* investigated in the Aegean area (Fig.1), but they are similar and within the range of variation for the species as observed by previous authors for other areas (e.g., Popov 1975; Joermann & Schneider 1987; Fonseca 1991; Boulard 1995; Pinto-Juma *et al*. 2005). The new specimens from Lesbos appear to be within the limits of variation found in those of the other populations investigated, i.e., other Aegean islands and the Greek and Turkish mainlands, and are not in concordance with the previous results by Boulard (2000a) based on a single male. Therefore, the present results do not support the designation of the Lesbos material as an independent subspecies.

TABLE 2. Description of the acoustic variables analysed in *C. orni* L.

Variables	Description
No. echemes/s	Number of elements per second
Echeme duration	The duration of each element from start to end
Inter-echeme interval	The duration between the end of one element and the start of the following one
Echeme period	The duration between the start of one element and the start of the following one
Ratio echeme/inter-ech. interval	Ratio between the echeme duration and the inter-echeme interval
Peak frequency	Frequency of maximum amplitude on the spectrum
Minimum frequency	The lowest frequency having an amplitude exceeding the threshold (-20dB)
Maximum frequency	The highest frequency having an amplitude exceeding threshold (-20dB)
Bandwidth	Difference between maximum frequency and minimum frequency (at -20dB)
Quartile 25%	Frequency below which there is 25% of the total energy of the spectrum
Quartile 50%	Frequency below which there is 50% of the total energy of the spectrum (mean frequency of the spectrum)
Quartile 75%	Frequency below which there is 75% of the total energy of the spectrum
Quartile75%–quartile25%	Difference between upper (quartile 75%) and lower (quartile 25%) quartiles of the spectrum (measure of the pureness of the sound)

TABLE 3. Average values of the 13 acoustic variables in the samples of *C. orni* L. studied. The time and frequency characteristics are in seconds and in Hz, respectively.

	Andros	Athens	Evia	Itea	Kosmas	Lesbos	Naxus	Neapolis	Paralio	Skala	Skyros	Assos
No. echemes/s	5.25	3.10	4.34	4.08	4.28	5.60	4.86	3.72	5.23	5.54	4.08	4.30
Echeme duration	0.06	0.08	0.06	0.08	0.15	0.09	0.06	0.08	0.05	0.06	0.07	0.05
Inter-echeme interval	0.14	0.24	0.18	0.18	0.09	0.09	0.16	0.2	0.14	0.12	0.19	0.18
Echeme period	0.20	0.33	0.24	0.26	0.24	0.18	0.21	0.28	0.19	0.18	0.26	0.23
Ratio echeme/inter-ech. interval	0.50	0.45	0.39	0.53	1.93	0.98	0.50	0.65	0.45	0.57	0.42	0.31
Peak frequency	4589	4866	4781	5344	4650	4479	5147	4840	5057	4438	5300	4269
Bandwidth (-20dB)	6846	6300	7095	8613	6233	4932	6234	8051	8199	8913	8015	7626
Quartile 25 %	4527	4603	4567	5115	4492	4492	5034	4827	4935	4556	4949	4357
Quartile 50 %	5171	5356	5466	6067	5019	5033	5672	5746	5837	5772	5894	5152
Quartile 75 %	6713	6408	6831	7846	6368	6303	7755	7476	7426	7962	7395	6078
Quartile 75 %-quartile 25 %	2186	1805	2263	2731	1876	1811	2722	2649	2492	3406	2446	1721
Minimum frequency	2156	2049	1980	2298	2234	2528	2505	2246	2206	2007	2187	1897
Maximum frequency	9005	8445	9080	10923	8473	7464	8743	10272	10409	10925	10208	9527

TABLE 4. Descriptive statistics of the 13 acoustic variables investigated in *C. orni* L. The time and frequency characteristics are in seconds and in Hz, respectively.

	N	Mean ± SD	Range
No. echemes/s	103	4.15 ± 1.13	2.23–7.08
Echeme duration	103	0.07 ± 0.02	0.04–0.17
Inter-echeme interval	103	0.19 ± 0.07	0.06 -0.35
Echeme period	103	0.26 ± 0.07	0.14–0.45
Ratio echeme/inter-ech. interval	103	0.52 ± 0.37	0.21–2.48
Peak frequency	103	5015.36 ± 507.28	4061.71–6584.29
Bandwidth (-20dB)	103	7565.95 ± 1400.63	4391.51–11280.30
Quartile 25 %	103	4817.92 ± 359.92	4047.52–5784.14
Quartile 50 %	103	5687.44 ± 442.46	4812.96–6640.51
Quartile 75 %	103	7211.54 ± 849.99	5597.78–9948.06
Quartile 75 %-quartile 25 %	103	2393.62 ± 669.89	1257.78–5205.60
Minimum frequency	103	2179.28 ± 289.94	1244.27–3235.48
Maximum frequency	103	9751.75 ± 1432.80	6966.84–13347.91

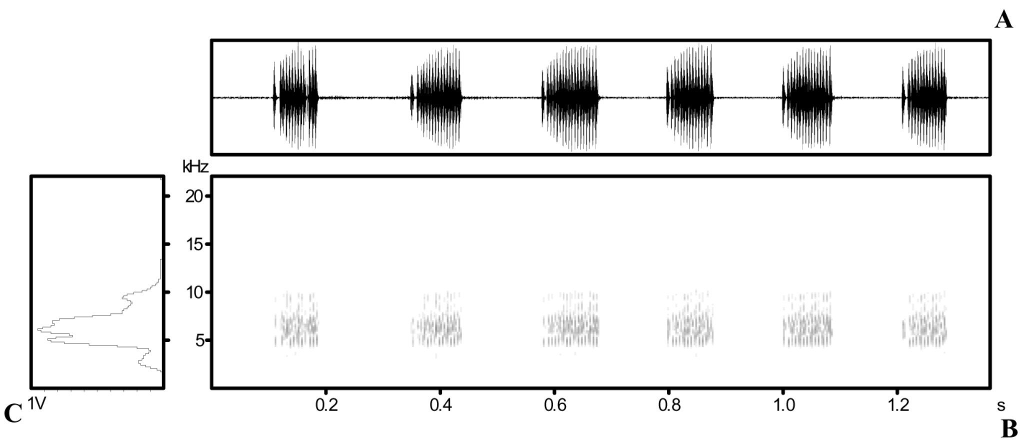

FIGURE 2. *C. orni* L. calling song profile (specimen from Athens). A - Oscillogram (amplitude *vs*. time), B - spectrogram (frequency *vs*. time), and C - mean amplitude spectrum (frequency *vs*. amplitude).

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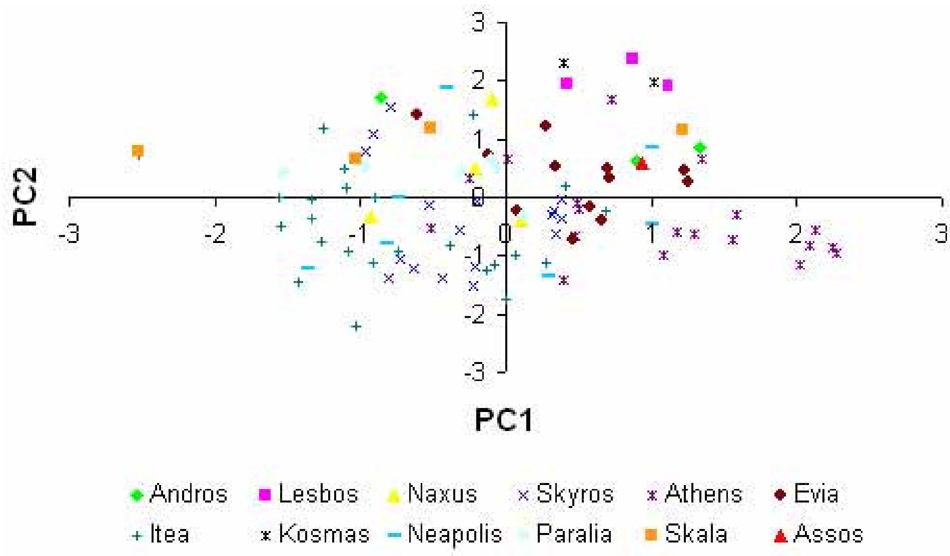

FIGURE 3. Bidimensional diagrams of relationships between specimens of *C. orni* L. (103 OTUs) of a principal component analysis based on a correlation matrix between 13 acoustic variables (standardized data). The first and second axes explain 42.3% and 22.6% of the total variation, respectively.

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