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Summary of knowledge on *Bradyporus dasypus* (Illiger, 1800) (Orthoptera: Tettigoniidae)

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Abstract

Bradyporus dasypus, only taxon of its genus, is one of the largest European apterous bush-cricket. It is coloured in black with metallic sheen bronze or copper. Its distribution is over the Balkan Peninsula. Its geographic range and altitude matches more or less with that of the Hungarian Oak (*Quercus frainetto*). *B*. *dasypus* prefers mainly semi-open biotopes, most often along forest wood (mostly oak and beech).

On the chromosomal analysis performed, we noted the presence of B chromosomes. We suggest that the total number of chromosomes varies in the *B*. *dasypus* populations.

With our rearing performed, we noted a two year long embryonic diapause on clutches, with hatchings in earliest spring. *B. dasypus* is omnivorous and information concerning its diet mainly comes from rearing experiments. We noted that a diet including animal protein was required, during the growth of the insects.

Zusammenfassung

Bradyporus dasypus, einziges Taxon in dieser Gattung, ist eine der größten flügellosen europäischen Heuschrecken. Sie ist schwarz, metallisch schimmernd, bronze oder kupferfarben. Ihre Vorkommen erstrecken sich über Teile der Balkanhalbinsel. Ihre geographische Verbreitung und die Grenzen der Höhenverbreitung sind mehr oder weniger deckungsgleich mit der ungarischen Eiche (*Quercus frainetto*). *B. dasypus* kommt vor allem auf halboffenen Biotopen sowie am Rande von Wäldern (Eichen- und Buchenwälder) vor.

Im Rahmen von Chromosomen-Analysen stellten wir die Anwesenheit von B-Chromosomen fest. Wir postulieren, dass die Gesamtzahl der Chromosomen in *B. dasypus* variabel ist.

Unsere Zucht zeigt eine zweijährige embryonale Diapause mit Schlupf schon bei den ersten Frühlingszeichen. *B. dasypus* ist omnivor und die meisten Informationen über seine Lebensweise stammen aus Zuchtversuchen. Wir haben festgestellt, dass Tierprotein-Ernährung während der Larvenzeit nötig war.

Introduction

The aim of this work is to establish an overview of knowledge on *Bradyporus dasypus* (Illiger, 1800), based on data from both literature and unpublished re-

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search done by the G.E.E.M. (Groupement d'Études Entomologiques Méditerranée) and colleagues. This species exhibits a number of quite unusual characters for the entomofauna of the Balkans. As such, we believe that a good knowledge of its ecology and its biology are useful for better understanding the operation of entomocenoses in this region of Eastern Europe.

Materials and methods

Cytogenetic analysis

The biological material

A sub-adult male collected in late May 2008 in the Greek region of Western Macedonia, in Klisoura area (021°28'05''E/40°32'19''N), at 1240 m of altitude.

Protocol

After anaesthesia with ethyl acetate, the insect was dissected to cut off the testes which were immediately dipped in tube containing a KCl solution (0.88 g in 100 ml distilled H_2O) where they were dilacerated using a pellet piston. The cell suspension was maintained in this solution for various times, but only the eight minutes treatment gave good results. Then, the KCL solution was replaced by Carnoy fixative (ethanol: 3 vol.; acetic acid: 1 vol.) by centrifugation. After one hour, the fixative was replaced by a fresh one and the spreading on wet slides was performed according to our usual techniques (DUTRILLAUX et al. 2006). The slides were stained by Giemsa. This part of the technique was performed in Larissa University, thanks to Pr. Z. Mamuris. Slide observation, and further treatments for C-banding were performed in the MNHN laboratory, as described (DUTRILLAUX et al. 2006).

Data from breeding

Biological material

Two strains from the western region of Macedonia in Greece near Klidi (021°38'04''E/40°45'31''N) and Klisoura (021°28'05''E/40°32'19''N), respectively at 1240 m and 793 m of altitude. A breeding sample collected in the Republic of Macedonia (FYROM), north of Kočani in the region of Osogovo at 894 m altitude.

Protocol

Males and females were placed by small groups of 4-5 individuals in wooden cages of various sizes, topped by a roof and covered on all four sides of canvas type net. They were installed outdoors to benefit from both shadows and sun. The bottom was covered with a thick layer of leaf litter and bark where insects could hide. A small autonomous weather station placed close to cages performed recordings regularly, to be correlated with certain behaviour and biology of the insects.

Field data

Observations were collected between 2003 and 2008, in Greek Macedonia in the prefectures of Florina and Halkidiki, and in the north-east of Republic of Macedonia (FYROM), in the area of Kočani. This work has been supplemented by data from literature or personal communications kindly provided by some colleagues.

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Characteristics of the species

Morphology

The typical morphology of Bradyporinae cannot be confused with another group (Fig. 1). In the Balkans, this sub-family is represented by the genus *Callimenus* which includes several species from Greece to Romania, and *Bradyporus dasypus* only taxon of its genus. This is one of the largest European Ensifera (body ♂ : 47-57 mm, ♀ : 46-60 mm). As opposed to *Callimenus*, the ovipositor of the female is not serrated at its tip and it is longer (Fig. 2). Furthermore, the shape of the male cerci differs by the location of the tooth internal located near the middle and not close to the apex. With its short and stocky shape, its way of moving, and its black colour with metallic sheen bronze or copper, this big apterous bush-cricket looks like big cricket. Some parts of the legs and sides of pronotum are often light-colored and Karaman (1961) indicated that this is even more obvious that the locations are hot.

Fig. 1: Male adult

Fig. 2: Ovipositor of the female

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Chromosomal analysis

The karyotype of the spermatogonia is composed of 35 chromosomes. Autosomal pairs N°1 and 2 and the X are metacentric, and all the other autosomes are acrocentric. C-banding is limited to the centromeric regions. Its intensity is very strong on pair N°1, high on pairs N 8, 9 and 12, medium on pairs 2, 4, 6, 13 and 14, low on pairs 2, 3, 5 and X, and very low on others (Fig. 3). The relative length of the eight largest chromosomes, established by measurement of five karyotyped cells, is given in table 1. The relative size of the remaining chromosomes gradually decreases from 0.03 to 0.02. This karyotype differs from that of *B. dasypus* described by WARCHALOWSKA-SLIWA (1998), with 27 chromosomes, including five metacentrics (Pairs N°1 and 2, and X) and 22 acrocentrics. It has an excess of small acrocentrics.

At diakinesis/metaphase I, the unique X chromosome differs from autosome bivalents by its compaction and staining, as in other Orthoptera species. Large autosomal bivalents exhibit a high interstitial chiasma frequency (Fig. 4). Their counting in bivalents N°1-6 from 20 cells gives an average of 18.18, which is higher or equal to the total number of chiasmata (terminal and interstitial) reported in other species such as *Locusta migratoria* (Linné 1758) (14.4 chiasmata, TEASE & JONES 1978), *Chorthippus parallelus* (Zetterstedt 1821) (14 to 17 chiasmata, JOHN & HEWITT 1966) or *Dociostaurus genei* (Ocskay 1833) (14.56 to 19.38 chiasmata, RODRIGUEZ-INIGO et al. 1998). Interstitial chiasmata are not rare in smaller bivalents, and on the whole, there is an average of 32 chiasmata or terminal associations per spermatocyte, which is about twice the number reported in other Orthoptera species. Thus, the meiotic recombination rate is quite high in *B. dasypus.* Interestingly, there is a variable number of small monovalents, hence, a variable total number of elements per diakinesis/metaphase I (table 2), with a modal number of 21 and a maximum number of 22. With a 35,X karyotype, we would expect 17 bivalents plus the X. This means that up to eight small chromosomes remain as monovalents.

At metaphase II, the X chromosome, which remains differently stained and compacted from autosomes, as described in other Orthoptera species (HEWITT 1979), can be easily identified. Chromosome segregation appears to be strongly irregular, with a total number of chromosome per spermatocyte II ranging from 16 to 20 (table 2). In the example given in figure 5, there are 18 autosomes for 17 expected in case of a correct segregation. There is an excess of small acrocentrics with a strong C-banding (N°8, 9 and 12), which indicates they are Bs.

In conclusion, the presence of a variable number of monovalents at metaphase I, and of chromosomes at metaphase II indicates the presence of B chromosomes. This suggests that the total number of chromosomes varies in the *B. dasypus* populations. These B chromosomes are small, and at least some of them carry a C-band positive heterochromatin. Here, it seems that the karyotype contains at least 8 such B chromosomes. This can explain the numerical difference between this karyotype and that published by WARCHALOWSKA-SLIWA et al. (1998).

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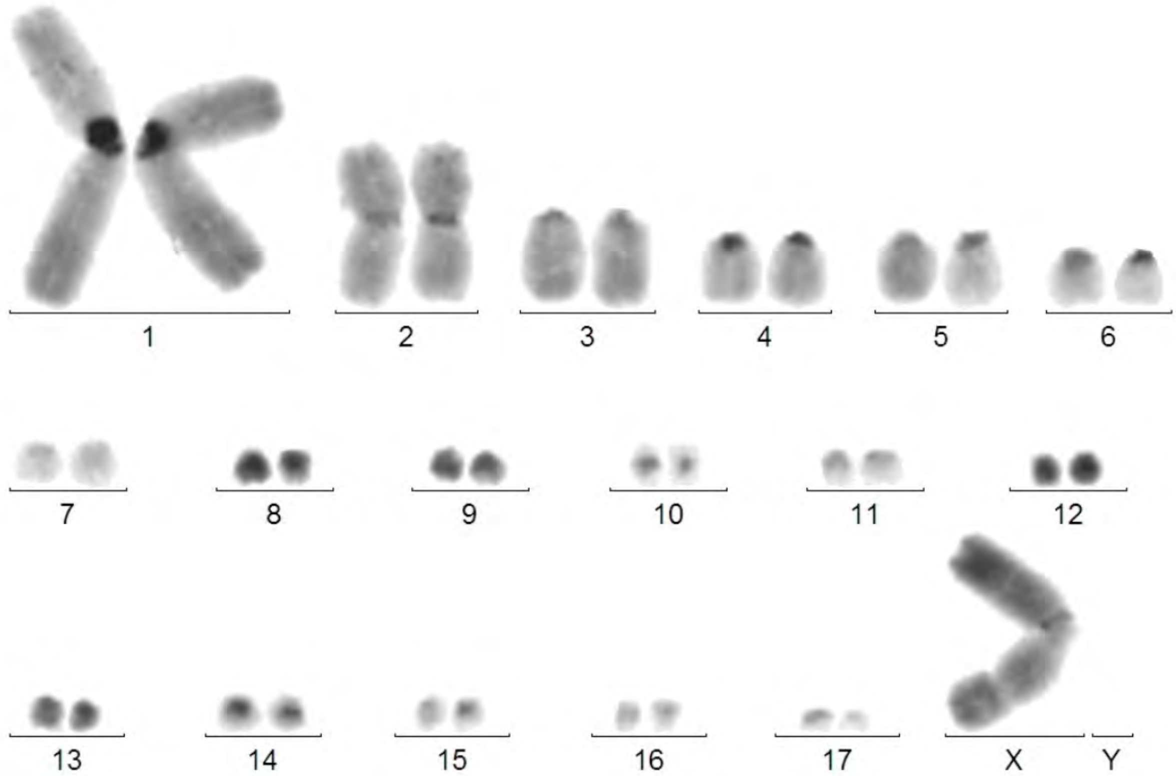

Fig. 3: C-banded mitotic karyotype of *Brachyporus dasypus*: 35,X. Some of the small acrocentrics are B chromosomes, but can hardly be identified.

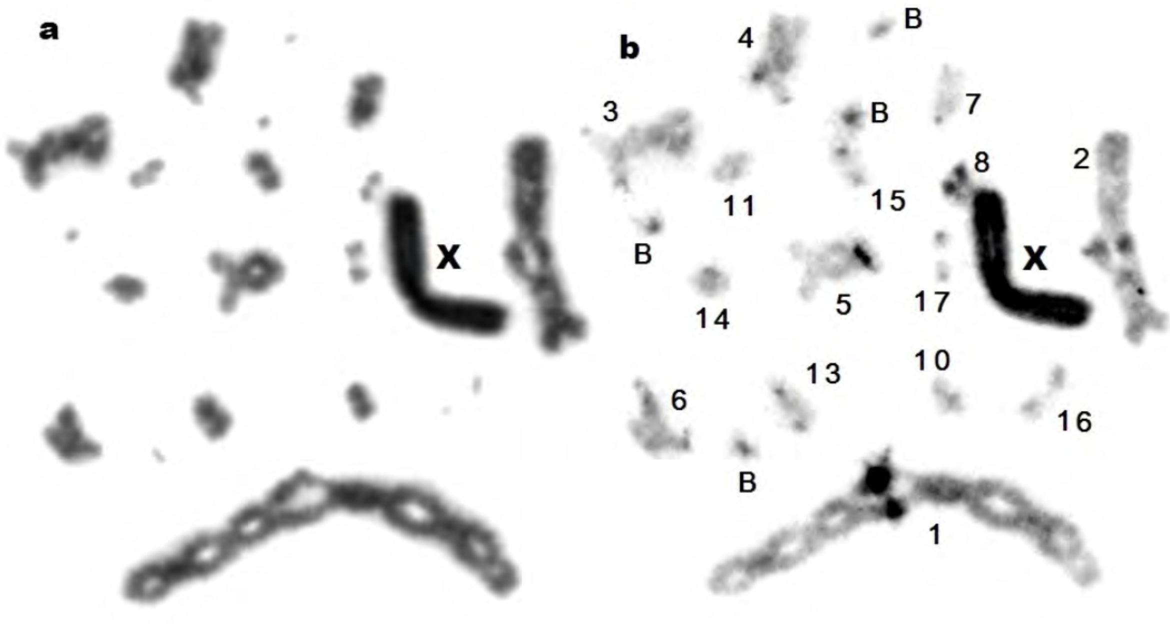

Fig. 4: Metaphase I after Giemsa staining (a) and C-banding (b). The X is easily identified by its staining. Beside 14 autosomal bivalents (tentatively identified), there are four monovalents (B). Notice the high interstitial chiasma frequency on bivalent N°1.

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Chromosome	Relative length	Standard deviation
1	29.4	1.4
2	14.9	0.9
3	8.2	0.4
4	6.3	0.4
5	5.8	0.3
6	4.6	0.3
7	3.6	0.3
X	23.14	1.4

Table 1:
Relative length, expressed in percentage of the total haploid chromosome length.

Table 2: Numbers of bivalents and monovalents observed in spermatocytes at metaphase I and chromosomes at metaphase II.

Chromosome or bivalent/monovalent numbers	Metaphases I	Metaphases II with X chromosome	Metaphases II without X chromosome
16	0	2	2
17	1	3	4
18	8	4	1
19	4	3	0
20	13	1	0
21	18	0	0
22	6	0	0
Total	50	13	7

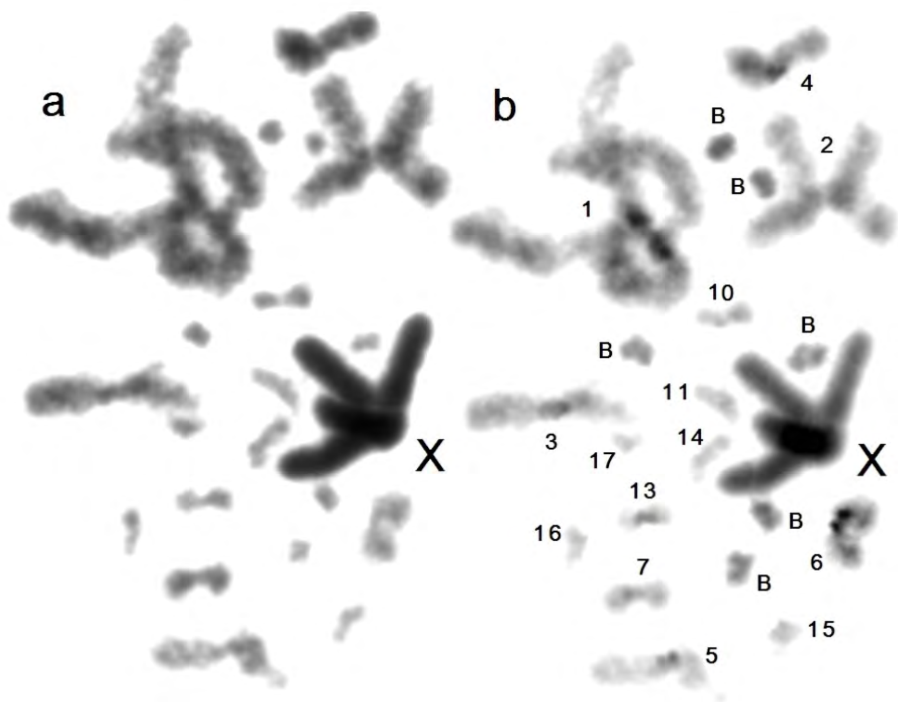

Fig. 5: Spermatocyte II metaphase, after Giemsa staining (a) and C-banding (b).It is composed of 20 chromosomes (20,X) instead of the 18 expected. There are too many strongly C-banded small acrocentrics, assumed to be B chromosomes (B). Chromosomes have been tentatively identified.

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Distribution

B. dasypus has a pontic distribution, mainly focused on the Balkan Peninsula: from northern Greece, up to Serbia and Romania, through Macedonia, Bulgaria and European Turkey. The species was reported around Budapest in Hungary (Buda Hills) at the end of the nineteenth century, but seems to have disappeared today (KOLICS et al. 2008).

The following mapping is categorized by squares of 20 minutes lat/long. The size of points represented depends on the number of stations located inside each square. The data are coming from the literature (BUNESCU 1959; BURR et al. 1923; BURESCH, & PESCHEV 1958; Collaborative workshop: Budapest Zoo 2002; Faculty of Natural and Agricultural Sciences 2004; KALTENBACH 1967; KARABAG et al. 1971; KARAMAN 1961; KIS 1962; KOLICS et al. 2008; KOVACHEV 1905; MÜLLER 1933, NASKRECKI & OTTE 1999; NEDELKOV 1907, 1909; PESCHEV 1962, 1964, 1974, 1975; PESHEV & ANDREEVA 1986; PESHEV & DJINGOVA 1974; PESHEV & MARAN 1963; POPOV & CHOBANOV 2004; SARADNIK & MATVEJEV 1967; VASILIU 1961; WEIDNER 1950; WERNER 1937b; WILHELM et al. 1959; WILLEMSE 1984), from observations of colleagues and field data from G.E.E.M.

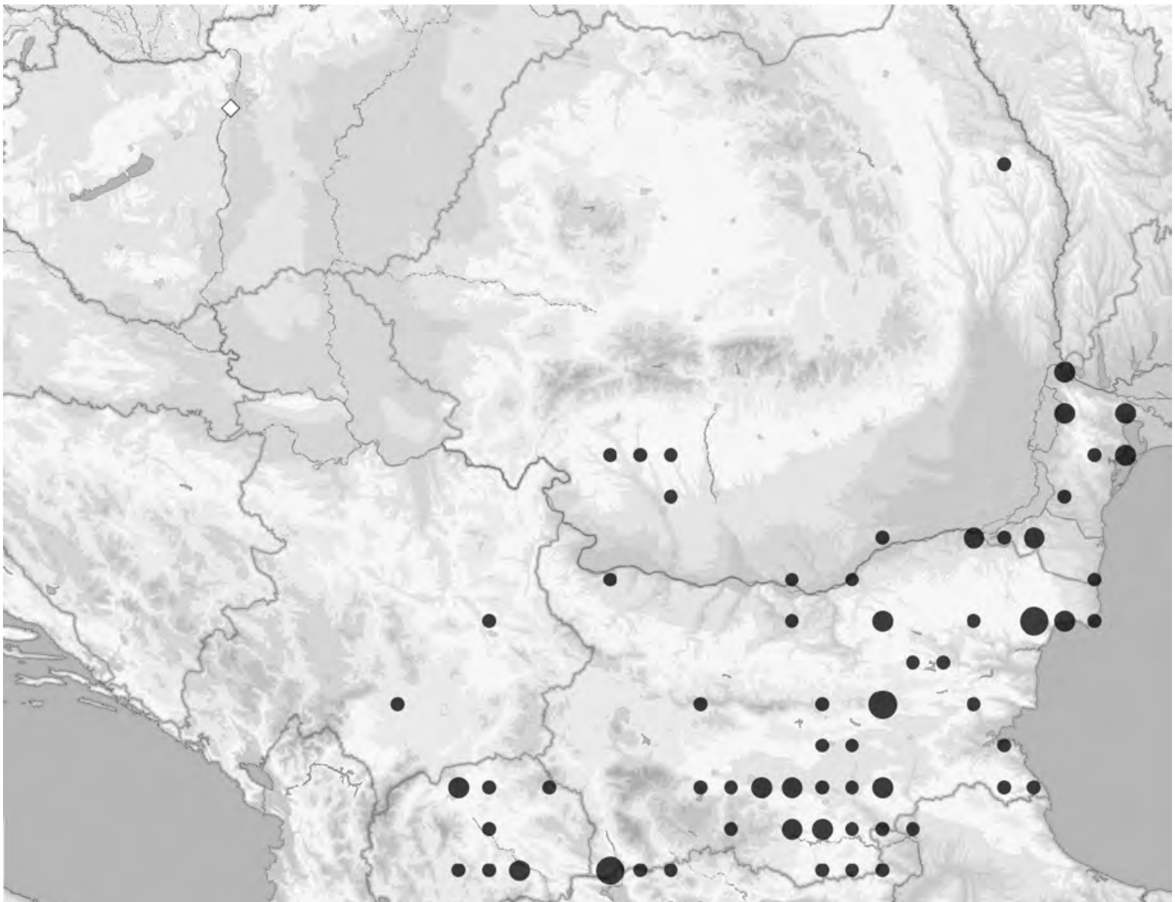

Fig. 6: Distribution map (performed with Flauna © E. Polidori, 2002-08).

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Ecology

At the biogeographic point of view (OZENDA 1994), the distribution of this Bradyporinae is mainly included in the thermo-nemorale area (Pannonian depression, Balkan oak). However, for the majority of Greek stations, it is also in the supra-Mediterranean area and oro-Mediterranean area. We also note a few forays up to the alpine level of mountain range of Rhodope and Balkan.

Bradyporus dasypus occurs from the plain up to the mountain, its upper altitude limit being likely around 1300 m, in Bulgaria and Greece. Its geographic range and altitude matches more or less with that of the Hungarian Oak (*Quercus frainetto*) (HORVAT et al. 1974). We note that its upper altitude can be locally higher in area under Mediterranean climate as observed by Klaus Reinhold (pers. comm.) at about 1650 m in the Ossa mountains, in Greece (Thessalia).

This Ensifera prefers mainly semi-open biotopes, moorlands, high grasslands, overgrown wastelands, most often along forest wood (mostly oak and beech). In these stations, the presence of a herbaceous layer or dense litter could be a constant characteristic. This would ensure the insect an effective concealment during most of the day. In plain areas, the edges of extensive cereal crops are also occupied. Usually located near hardwood, they provide a greatly appreciated secondary habitat.

Biology and Behavior

We observed a two year long embryonic diapause on clutches from individuals collected in northern Greece, at altitude of 793 m (Klidi, prefecture of Florina). This phenomenon, not unusual among Tettigoniidae (Ephippigerinae, Saginae, *Eupholidoptera* genus), has already been acknowledged for this taxon (HELLER 2006). However, we neither know the maximum duration of the diapause nor its possible variations.

Hatchings begin by the end of February, in the most xero-thermophilic plains, particularly in Bulgaria (Chobanov, pers. comm.) and in our rearing at Callian. It occurs until May in mountainous areas and the extreme north of its distribution range. The first adults appear in early May, after 6 moults and can live up to September, especially in the mountains. Our latest observations are the last week of September, at altitudes between 1100 and 1300 m.

The "swarming", which occasionally occurs in south-eastern of France (Massif des Maures) for another Bradyporidae, *Ephippiger provincialis* or the Phaneropteridae, *Barbitistes fischeri*, does not seem common among *B. dasypus*. The only report in the literature (BURR, CAMPBELL & UVAROV, in GWYNNE 2001), traces back to early 20th century.

B. dasypus is omnivorous and information concerning its diet mainly stems from rearing experiments: Willemse (pers. comm.) notes the insect attraction for watermelon. HARZ (1987) relates about leaves of *Cirsium arvense, Stellaria media, Taraxacum officinale, Lactua sativa, Sonchus arvensis, Raphanus sativus, Aegopodium podagraria* indicating that the insect also accepts animal food based on

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live caterpillars or flour of fish and crustaceans. In the field, it has even been observed on the corpse of a shrew in decomposition (Botella, pers. comm.).

In captivity, our experiences have shown that a diet including animal protein was required for good success, at least during the growth of the insect. When juveniles are fed exclusively with vegetable, even enriched with vitamins, we note a higher mortality before adulthood (40% to 60%), often caused by large rifts of chitin during moulting. In addition, cannibalism behaviour increases as already indicated HARZ (1987). An animal protein deficient food does not limit the activity of the adults and seems to have no influence over their lifetime, provided that their food was not deficient during their growth.

Acoustics and role of the song

Like the other Bradyporidae, females of *B. dasypus* can stridulate - song transmitting wings are configured reversed compared to male - but their repertoire is mainly limited to short stridulations. According to our observations when breeding them, they most often occur in response to physical stimuli such as handling or during crush among congeners. HARTLEY et al. (1974) also reported a sound exchange between males and females Bradyporinae prior to the formation of couples.

The stridulations of male *B. dasypus* occur mainly in the morning and late afternoon. Willemse (pers. comm.) observed that, in northern Greece, they regularly start singing around 5 pm. During the day, the insects remain hidden in the vegetation, usually in the herbaceous layer, but can perch on top of the ears of cereals, to sunbathe early in the day.

The duration of one sequence of the song of the male is usually between 30 seconds and one minute. The stridulating emission is composed of a set of pulses at a rhythm of 40 Hz on average: 35 Hz to 58 Hz, depending on the external temperature (HELLER 1988), each pulse has a duration of about 20 milliseconds followed by a resting phase of 5 milliseconds (figure 7). The rhythm is roughly constant throughout the sequence (figure 8) but we have always noticed a slight regular slackening. Each pulse has a relatively wide spectrum with a maximum of energy around 9 to 12 KHz (figure 9).

Reflexes and defence

Faced with a threat, the attitude most commonly adopted by *B. dasypus* is the immobility in soil, vegetation or litter. The insect seems relatively powerless against predation, because of its low cryptic coloration, its relatively heavy moving and its winglessness. Moreover, questions remain about the trigger mechanisms and the role of the auto-haemorrhage, or reflex bleeding, used in response to different stimuli, by all Bradyporinae. Even if the haemolymph of some Orthoptera can irritate the skin and mucous membranes (HOLLANDE 1912), the deterrent effect against predators is not clearly established. In both breeding and field conditions, we found that this reaction does not systematically occur in case of threat.

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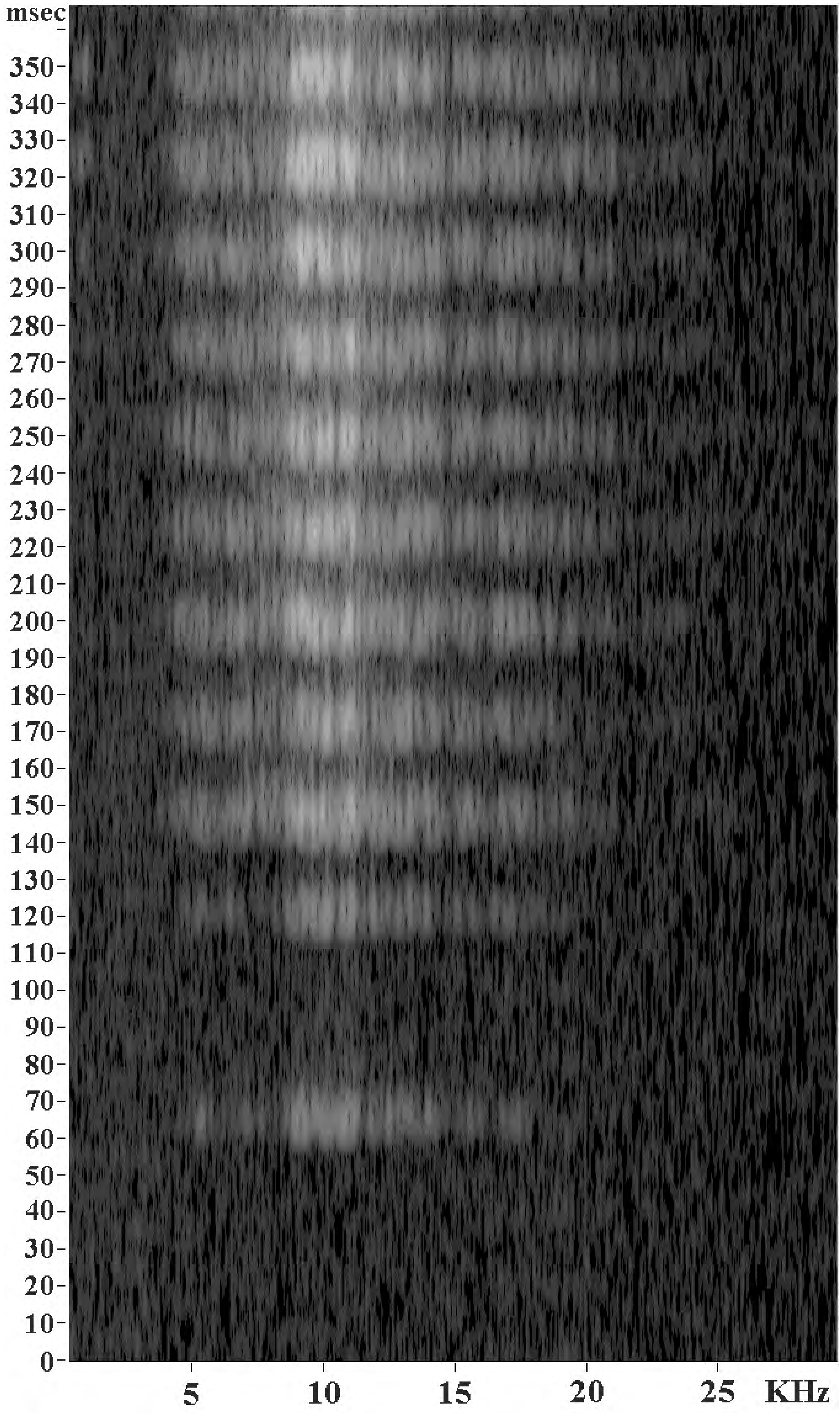

Fig. 7: Sonogram of a song sequence.

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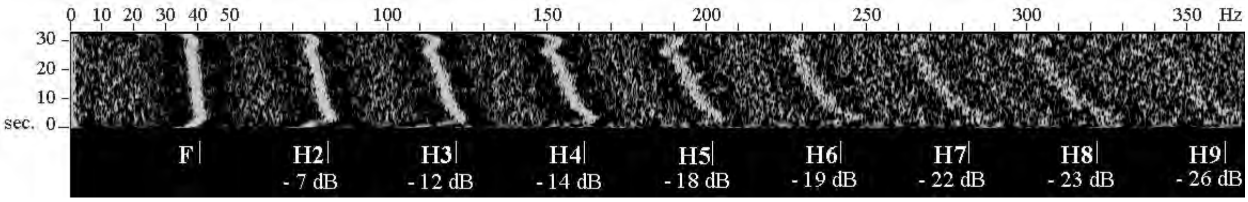

Fig. 8: Sonogram of the modulation of a song sequence.

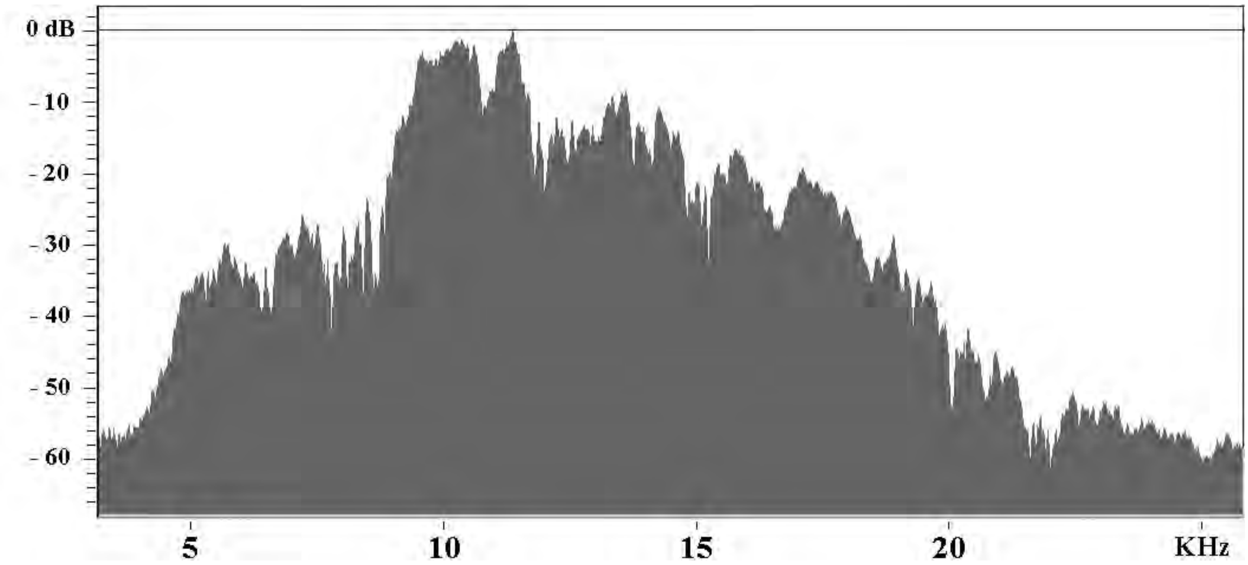

Fig. 9: Spectral distribution of the song.

Discussion

This overview shows that some aspects of biology and behaviour of *B. dasypus* are still unclear, as its annual cycle, incubation duration and hatching, post-embryonic development etc. Moreover, some cases of syntopie with *Callimenus macrogaster longicollis* Fieber 1853 are reported in Romania (KIS 1962) and in Greece with *Callimenus omniscus* Burmeister 1838 (Willemse, pers. comm.), without knowing whether the habitats are really shared and if they cross-mate. Some progresses were achieved on the genetics of these insects (WARCHALOWSKA-SLIWA 1998; TURKOGLU et al. 2002) and on the mechanism of embryonic diapause (KOLICS et al. 2008). In this study, it appears that the karyotype contains a large number of small B chromosomes, not detected before. The meaning of the presence of such chromosomes remains unclear, as that of additional heterochromatin in general. One additional B chromosome was already observed in other three Tettigoniidae species (JONES & REES 1982). Their effect on phenotype is hardly detectable, but it is admitted that when present in high frequency, Bs are generally deleterious, by slowing the embryo and larva development (HARVEY & HEWITT 1979, JONES & REES 1982). It was also noticed that their effect, principally on meiotic segregations, depends on their presence in odd or even numbers (DARLINGTON & UPCOTT 1941; CAMACHO et al. 2004). Here, they are present in even but high number and seem to be mitotically stable but meiotically very unstable. This may be a factor reducing their reproduction fitness.

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It is difficult to have a clear idea of the distribution of *B. dasypus* and assess its abundance, due to the oldness of existing data, especially in the northern part of its area (Serbia, Kosovo, Romania). This is especially true for data from large agricultural areas that have given up their traditional farming practices in favour of an intensive mode to meet growing economic constraints. Some populations have certainly not escaped from the homogenisation of environment and the increased use of pesticides (HELLER 2006). In northern Greece, in the plain of Florina, where this species was still widespread a few years ago, we noticed a sharp decline which seems to be linked to a change in cultivation practices. We cannot yet determine whether these two phenomena are actually related, it has to be analysed further in the coming years, and assessed with the benefit of hindsight.

B. dasypus has certainly taken advantage of environment changes over the centuries to colonize the traditional farming areas close to hardwood (oak, beech). Hence, it may be considered as an excellent indicator of this type of culture, such as the cropland plants. Today it is potentially threatened by changes in farming practices and it would be wise to conduct a comprehensive investigation on variation in its populations by setting up an observatory to its distribution and evolution, correlated with the environment current status.

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