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Morphology and structure of adult male genitalia of *Brachytrupes megacephalus* Lefebvre, 1827 (Orthoptera, Gryllidae) in the southeast of Algeria

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Abstract
Depending to the few and old data which have been conducted on the body morphology of *Brachytrupes megacephalus* Lefebvre, 1827 (Orthoptera, Gryllidae), we have investigated the structure of male genitalia in Algerian Sahara species to establish a basis for morphological study. The study was conducted in the laboratory of entomology in the experimental station of Sidi Mehdi.
After isolation of the male genital apparatus, we find that its structure is consisted by the testis, accessory gland, ejaculatory duct and the phallic complex. The last organ is composed of epiphalus, guiding rod, ectoparamere, endoparamere, dorsal and median pouch. The spermatophore produced by *Brachytrupes megacephalus* was investigated in detail. Besides the study of the spermatophore size (8 mm), morphology, and formation, spermatophore is composed of the ampulla (3.9 mm), attachment plate (2.8 mm) and the tube (1.5 mm). The present paper aims to improve this information, on the species of *B. megacephalus*.

Keywords: Genitalia; *Brachytrupes megacephalus;* phallic complex; Spermatophore; Algerian Sahara.

Introduction
The final molt in insect development results in the adult instar responsible for the reproduction. While there are some groups that are capable of copulating immediately after emergence [1], many insects have a period of sexual maturation with the adult instar.
After the track made within four years of study (from 2011 to 2014), we noticed that *B. megacephalus* has an annual life cycle with five larval stages with an imago stage; it goes through an imaginal-post hibernation. In fact, all the females captured and dissected in this period have ovarioles full with ovocytes. During this period, most collected individuals are mature males, against the period from August until September; all captured adults (male / female) are not sexually mature and they go through a hibernation stage to get out in the mid-March as a mature males [2-5]. From mid-March to mid-April, the males began stridor (continuous stridor without interruption); this behavior shows that imagoes are sexually mature. It is the breeding season [6, 3] with a post-emergence maturation after six months of hibernation which is very long compared to that of other insects like *Schistocerca* Stal. (Orthoptera, Acrididae) with about 30 days [7].
Historically, the gonads have been considered the only developing structures responsible for delayed sexual maturation [8]. All samples captured in spring period have gonads. In many insects whose external morphology is similar, differences in male genitalia are often the only reliable species- diagnostic characters. Naturally, male genitalia occupy a special place in insect systematics, and their taxonomic value has shown to be enormous in major lineages of insects [9, 10].
The present study is based on the conventional as well as genitalic characters, for a better understanding of the significance of morphological structures.

Material and methods
Site presentation
This study was conducted in the region of Sidi Mehdi, Which is an area located in the

Southeastern part of Algeria (Fig. 1). It is a saharian region with one dry period throughout the year (Fig. 2). This very low region is located at an altitude of 69 m at 06°4' E and 33°7'N. This area is approximately 07 km of Touggourt on the road leading to the airport.

Fig 1: Study area

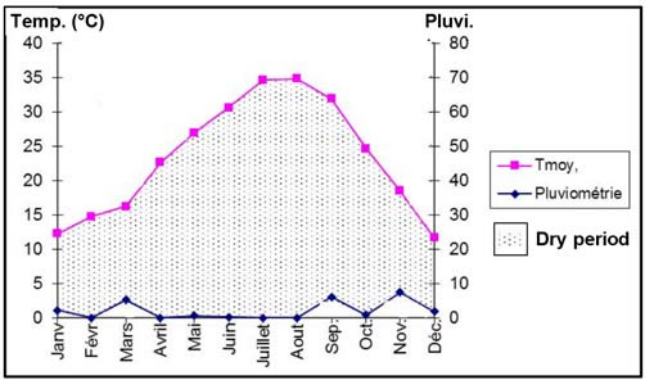

Fig 2: Ombrothermic diagram of Gaussen in the region of Touggourt region in 2014

Biological materials

Crickets of *B. megacephalus* (Fig. 3) were reared in a climate chamber at the laboratory of entomology in the institute of INRAA, station of Touggourt. The adults were separated individually into glass bottles to avoid a cannibalism phenomenon [2].

Fig 3: Adult of *B. megacephalus*

Method of light trap

According to the farmers of Touggourt region, this cricket is attracted to light houses (Fig. 4). That is why the lamps were placed in the experimental site. The individuals of *B. megacephalus* regrouped under light traps. This method has been used particularly in the spring.

Fig 4: Method of light trapping of *B. megacephalus*

Individual breeding in glass bottle

This method was very practical because we had the ability to control individuals (Fig. 5). The samples were placed inside the bottles for 4 months. In addition, the maintenance of the bottles was very easy and we have not seen any kind of disease.

Fig 5: Breeding of *B. megacephalus* adult in glass bottles

Dissection method of adult male (genital apparatus)

The dissection was made in tub of water (Fig. 6), we set the male and fixed it with insect pins onto a cork plate in a dorsal-up position after the abdomen had been opened. The cerci, epiproct and testis were removed, and the abdominal cavity was filled with insect saline. The genital apparatus is located in the abdominal area, so, we need to remove the digestive apparatus to observe the genital apparatus in its Global form (Fig. 7).

Fig 6: Dissection of *B.* megacephalus

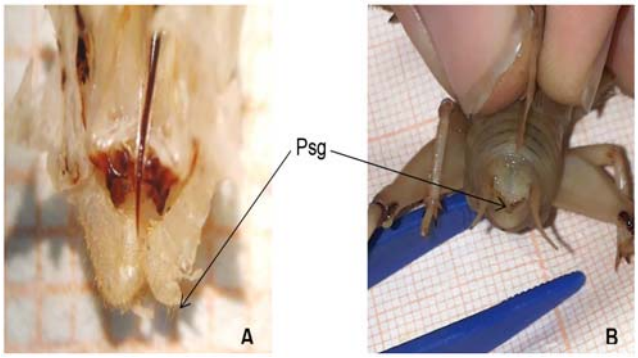

Fig 7: Psg: Sub genital plate (A: internal; B: external)

In order to study the male genitalia, a total of 30 male specimens were dissected. Insects were killed each day by freezing at –20°C for 30 minutes to collect developmental data. From a whole specimen, phallic complex was dissected by cutting through the membrane between seventh and eighth abdominal segments. Each dissected specimen was placed in a weak KOH solution for about 4 h to dissolve muscle tissues, rinsed in ethanol and preserved in glycerol in glass flasks. Because a phallic complex is a three-dimensional structure which is difficult to position, extra care was taken to position specimens in the same manner. Digital photographs of the reproductive system phallic complex and spermatophore were taken with each structure in the plane of the image. Digital photographs of the correctly positioned genital specimens were taken using a SONY Lens 20.1 mounted on OPTIKA binocular.

Results

For a better understanding of the significance of genitalic structures, we have studied the genitalia and spermatophore forms of *B. megacephalus* males.

The genitalic structure

The male genital organ complex comprises two important parts. One part includes the testis, vas deferens, accessory gland and ejaculatory duct for the production and transport of spermatophore materials (Fig. 8).

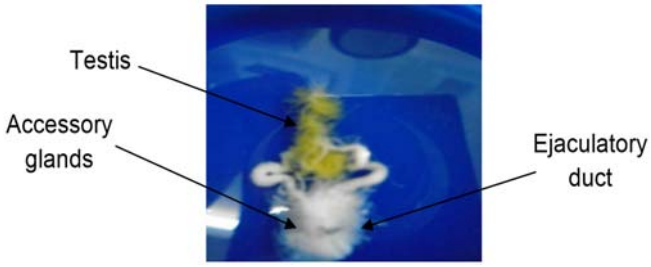

Fig 8: Genitalic apparatus of *B. megacephalus*

- **Testis**

Form an irregular mass with a yellow color shaped hundreds of follicles (Fig. 9).

Fig 9: Testis (G: X 10)

- ***Accessories glands***

They are evaginations with a tube form (with a white color) (Fig. 10) ejaculatory duct. They emit secretions that mix the spermatozoa and play an important role in the manufacture of spermatophores capsules containing sperm.

- ***Ejaculatory duct***

It has a white color with a length of 5 mm; it receives spermatophore material from the accessory glands (Fig. 10).

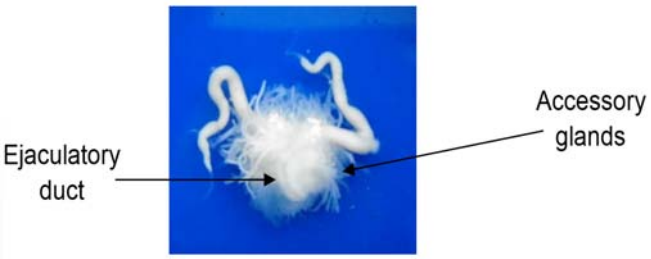

Fig 10: Accessories glands and ejaculatory duct

The other part, here termed the phallic complex, includes the dorsal pouch, guiding rod, epiphallus, ventral lobes and median pouch for the formation, extrusion and transfer of the spermatophore.

Skeleton of the phallic complex

The skeleton of the phallic complex is composed of two kinds of cuticle. The epiphallus, ectroparameres, latch-like sclerite, lateral arms, W-shaped sclerite and guiding rod are made of hard cuticle, while the median grooved fold (MGF) in the

dorsal pouch and the connection between the lateral arm and the anterior edge of the proximal region of the epiphallus are made of soft cuticle (Fig. 11). This phallic complex contains the following organs.

- ***Dorsal pouch***

The dorsal pouch consists of muscles, membranes, the MGF and some hard sclerites. Transparent membranes cover both sides of the MGF and the space between the MGF and the epiphallus (Fig. 11).

- ***Guiding rod***

The sclerotized guiding rod is a narrow grooved structure in the midline on the wall of the MGF with a brown color and 8 mm of length, and its posterior tip (Figs. 11, 12), protrudes through the neck region of the genital cavity.

- ***Epiphallus***

It's the most evident portion of the genital complex which is a sclerite developed on the dorsal and ventral surfaces of the dorsal phallic lobe with a caramel brown color, the wall of the genital cavity have bristle hairs of different lengths (Figs. 11, 12).

- ***Ventral lobes***

The ventral lobes are flexible structures resembling a pair of gloves fused laterally. They are composed of membranes, striated muscle fibers and soft cuticle. The proximal regions of the ventral lobes are continuous with the lateral arms, and the peripheral regions form a pair of flaps joined.

- ***Median pouch***

An inversible lobe lying on the floor of the genital chamber between the lateral phallic lobes and bearing on its dorsal surface a molding sclerite (Fig. 11).

- ***Ectoparamere***

A scleritized lobe of the ventral surface of the dorsal phallic lobe (Fig. 11); visible in the ventral aspect of the genital complex.

- ***Endoparamere***

A sclerite developed on the dorsal surface of the spermatophore sac and later evaginated into the body cavity (Fig. 11).

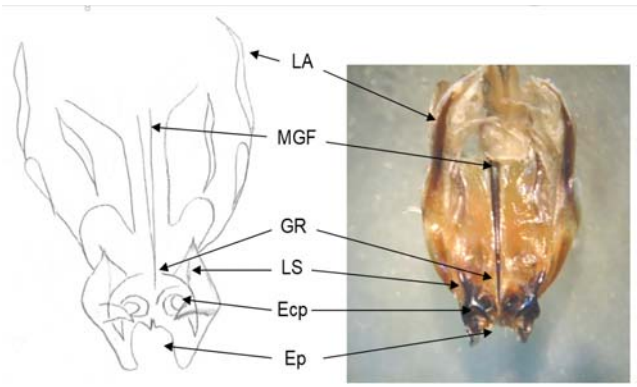

Fig 11: Quiescent pre-copulatory state of the phallic complex. (Ecp: ectoparamere; Ep: epiphallus; GR: guiding rod; LA: lateral arm; LS: latch-like sclerite; MGF: median grooved fold)

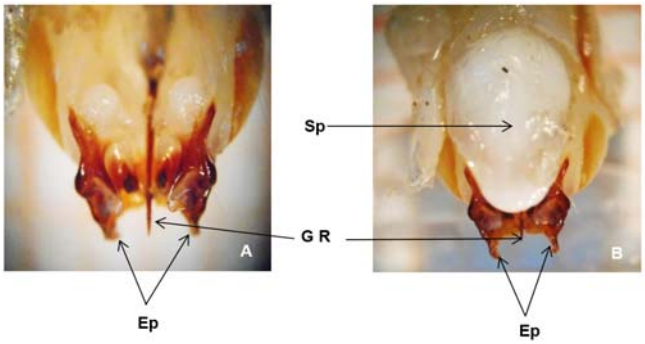

Fig 12: Quiescent pre-copulatory state of the phallic complex (A: dorsal view; B: ventral view (Sp: Spermatophore; GR: Guiding Rod; Ep: Epiphallus)

The spermatophore structure

In order to understand the spermatophore morphology, the pressure bodies are responsible for emptying the spermatophore after its transfer to the female; that's why we suggested a morphological study on it which was necessary to remove fully developed spermatophore from sexually mature male of *B.* megacephalus.

Morphology and size of the spermatophore

The spermatophore produced by male *B. megacephalus* is represented schematically in Fig. 14. In general, the studied spermatophore consists of three components: The ampulla, the tube and the attachment plate (Figs. 13, 14), acting as a device for fixing the spermatophore in the genital tract of the female.

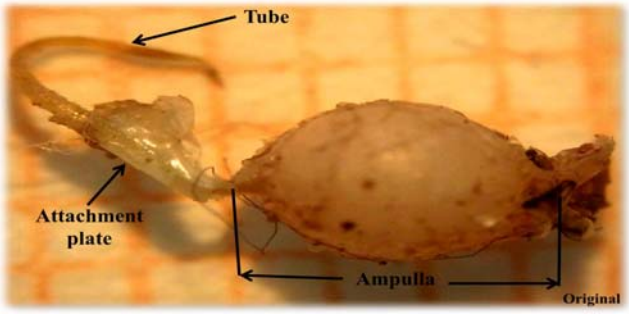

Fig 13: Spermatophore of mature male of *Brachytrupes megacephalus*

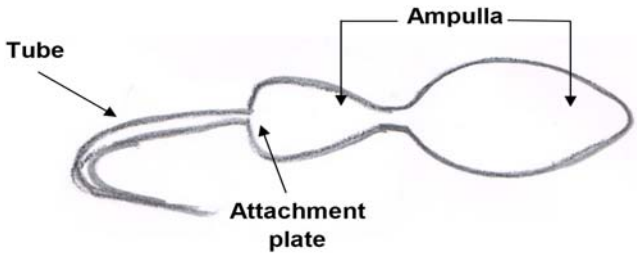

Fig 14: Scheme of the spermatophore of *B. megacephalus*

In the study presented here, 10 individuals were dissected. The table presented below shows the size of the parts contain in the spermatophore produced by male of *B. megacephalus*. Its size was about 8 mm. The first part of the spermatophore which is the ampulla, measure 3.9 mm in length and 2.8 mm in width. Secondly, the attachment plate is 1.5 mm in length and 1 mm in width. Finally, the tube has a length of 2.8 mm and a width of 0.3 mm (Tab. 1).

Table 1: Morphometric results for the spermatophore of *B. megacephalus*

N = 10	Average (mm)	St. dev. (mm)
Total length	8	0.4
Ampulla		
Length	3.9	0.17
Width	2.6	0.13
Tube		
Length	2.8	0.23
Width	0.3	0.07
Attachment plate		
Length	1.5	0.39
Width	1	0.12

St. Dev: Standard deviation

Discussion

According to [11], the description of *Brachytrupes membranaceus* includes morphological study of many specimens (male) who says that the male genitalia is similar to *B. megacephalus*. According to the work done by Kumashiro & Sakai [12] on *Gryllus bimaculatus* we noted that this species has the same description of the phallic of *B. megacephalus*.

In many insects, particularly orthopterans, the male transfers a spermatophore often consisting of a sperm-filled ampulla and a proteinaceous spermatophylax, which is attached to the female's genital opening [13].

The present study could clearly demonstrate that the spermatophore produced by male *B. megacephalus* is very similar to respective devices of other cricket species. The investigated spermatophore has three structural main parts (ampulla, attachment plate, and tube) and therefore unequivocally follows the scheme that was previously described for other gryllidae [14].

According to [15], the spermatophore of the black field cricket *Teleogryllus commodus* (Orthoptera, Gryllidae) has a length of 3.85 mm and contains the same three parts of our cricket which are: the ampulla (1.56 mm), the attachment plate (0.76 mm) and the tube (2.29 mm).

Conclusion

The result of our dissection of male *Brachytrupes megacephalus* shows that the genitalic apparatus contains in one part: testis, vas deferens, accessory gland and ejaculatory duct for the production and transport of spermatophore materials, the other part, here termed the phallic complex, includes the dorsal pouch, guiding rod, epiphallus, ventral lobes and median pouch for the formation, extrusion and transfer of the spermatophore. The morphological description of the spermatophore has a length of 8 mm and devised to three parts which are: the ampulla (3.9 mm), the attachment plate (1.5 mm) and the tube (2.8 mm).

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