insects

MDPI

Article

Reproductive apparatus, gonadic maturation, and allometry of *Cyclocephala barrerai* Burmeister (Coleoptera: Melolonthidae: Dynastinae)

Abraham Sanchez-Cruz [1] ID: 0000-0002-9035-2223, **Daniel Tapia-Maruri** [2] ID: 0000-0002-5468-5012 **and Alfredo Jiménez-Pérez** [1] ID: 0000-0002-6170-746X, *

1 Laboratorio de Ecología Química de Insectos, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, México. asanchezc1700@alumno.ipn.mx (A.S-C.), aljimenez@ipn.mx (A. J-P.).[2]
2 Laboratorio de Microscopia Avanzada, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, México. dmaruri@ipn.mx
* Correspondence: aljimenez@ipn.mx (A. Jiménez-Pérez); CEPROBI # 8, San Isidro, Yautepec, Morelos, 62731, México).

Simple Summary: *Cyclocephala barrerai* larvae feed on the roots of ornamental and commercial grasses. Most of their biology is currently unknown therefore there are no tools to manage their populations. The objectives of this work were to study the reproductive system, gonadal maturation, and allometry of C. barrerai. This is the first report that characterizes the reproductive apparatus, gonadic maturation of both sexes, allometric relationships of virgin F1 specimens and it is also the first report into the Melolonthidae family. We observed bacteria in the genital chamber of virgin females showing their relevance to the biology of this species. The presence of ultrastructure in the male genital capsule has taxonomic and evolutive value. Larvae gather reserves necessary for sexual maturation and surface to mate. Females present sclerotized accessory glands and a genital chamber with muscular fibers and bacteria. The morphology of the parameters and the antennas are under sexual selection. This is the first report of two main differe[illegible] among sexes: females are heavier while males have a longer antenna. Males and females present allometric relationships that can be used to predict the bodyweight of field-collected specimens.

Abstract: Coleoptera is a good example of morphologic specializations in the reproductive apparatus, gonadic maturation, and allometry. The female and male reproductive apparatus have been modified to ensure reproduction between individuals of the same species. The genus *Cyclocephala* has more than 500 species distributed in America and *Cyclocephala barrerai* Martínez is an economically important species in the central part of Mexico. We used optic microscopy, scanning electronic microscope and laser scanning confocal microscope to describe the reproductive apparatus and gonadic maturation of females and males. Also, the relationship between adult weight and different parts of the body was established by linear regression. Regardless the reproductive apparatus of *C. barrerai* are like those of the Melolonthidae, the genital chamber, the accessory glands type II and the ventral plaques of the female, and the ejaculator bulb and genital capsule of the males are specific for *C. barrerai*. The gonads are fully developed when 18 d old. The weight of adults of *C. barrerai* has a positive linear relationship with distinct parts of its body while the antenna of males is larger than those of the females.

Keywords: Beetles, Reproductive system, sexual dimorphism, oogenesis.

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Insects* **2022**, *13*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Gonadic maturation indicates the beginning of the insect's reproductive period [1–4]. In families such as Carabidae, Coccinellidae, Curculionidae, Dryophthoridae, Scolytidae, Silphidae, and Chrysomelidae, gonadic maturation is mainly affected by genetics, hormones (produced by the female or obtained from a male during mating), age, temperature, and food intake as larvae and adults [5–8].

Melolonthidae male's reproductive apparatus typically comprises: A) two accessory glands, B) two *vas deferens*, C) two testis with six follicles connected by the *vas efferens*, D) ejaculatory duct, E) a genital capsule and F) an aedeagus [9–11]. The genital capsule is a sclerotized structure with two plaques covering the endophallus. The most distal plaques of the genital chamber are called parameres which are used by the male to secure the female and guide the aedeagus through the female urogenital pore [12]. In Melolonthidae these parameres are ~~specie~~ specific and are of taxonomic value [9,11,13].

Female Melolonthidae reproductive apparatus typically has A) two compound ovaries with six ovarioles, B) a common oviduct, C) a *bursa copulatrix*, D) a spermatheca with a spermatic gland, E) a genital chamber F) one or two accessory glands and G) ventral plaques [9,10,14–16].

The information on female Melolonthidae gonadic maturation is scarce. Yi-Zhen et al. [17] distinguished three types of female gonadic maturation: 1) those females that emerge to mate with fully developed ovaries like *Holotrichia* sp., *Holotrichia trichopora* Fairmaire, and *Anomala exoleta* Fald, 2) those females that require some food to fully develop their gonads like *Holotrichia oblita* Faldermann and *Anomala corpulenta* Motschulsky, and 3) those females whose gonadic maturation depends on food intake as adults like *Holotrichia parallela* Motschulsky. The only information available about Melolonthidae ovary type is that for the New Zealand grass grub *Costelytra zealandica* White with a telotrophic ovary [18].

Gayon [19] defined allometry as "the changes in relative dimensions of parts of an organism that are correlated with changes in overall size". The allometry of specific morphological characters is important in Coleoptera reproduction as those characters play an important role during courtship, mate selection, mating, coercive behavior, and female manipulation during sperm transfer [20–24]. Most of the information on allometry in Melolonthidae, is that of the Dynastinae subfamily whose individuals present a strong sexual dimorphism. In males of this subfamily, there is a positive allometric relationship between size and horns, so larger males have larger horns which provide some advantage during agonistic encounters and courtship [25–27]. *Cyclocephala* presents sexual dimorphism as the ~~nail~~ of the first leg is larger in males than females [28] but its role during courtship and mating is unknown.

Regardless of the relevance of the genera *Cyclocephala* as pollinators or pests of different economically important crops [29–31], the morphology of their reproductive apparatus is not fully studied. The female reproductive apparatus has been described for *Cyclocephala jalapensis* Casey whose genital chamber and accessory glands are particular to these species, the rest of the reproductive apparatus is very similar to that of other Melolonthidae [16]. Male reproductive apparatus has been described for *C. jalapensis*, *Cyclocephala picta* Burmeister, *Cyclocephala sexpunctata* Castelnau, *Cyclocephala mafaffa* Burmeister, *Cyclocephala lunulata* Burmeister, and *Cyclocephala weidneri* Endrödi, been the parameres the main difference among these species [11].

Cyclocephala barrerai Martínez is distributed in the central part of México, and it is considered a ~~plague~~ as their larvae feed on economically important plants as grasses and maize [32–34]. At the moment there is no available management program for this pest, however, adults are attracted to volatiles produced by symbiotic bacteria from the female genital chamber [35].

The study of the morphology of the reproductive structures involved in mating will provide relevant information on the *C. barrerai* mating system, laying the foundations needed for applied research. This paper describes the gonadic maturation, the female and male genitalia, and some allometric relationships within each gender.

2. Materials and Methods

2.1. Cyclocephala barrerai insects

Cyclocephala barrerai adults were collected at "La presa" baseball park at Puebla city, México (18°58'26.0"N 98°14'46.4"W), from May to June 2020. Collected insect were reared at the Chemical Ecology laboratory facilities of the Centro de Desarrollo de Productos Bióticos of the Instituto Politécnico Nacional and were kept at 26 ± 3 °C, 70 ± 20% HR and a 12L:12D light regime.

Insects were reared according to Sanchez-Cruz et al., (in preparation). In short, a mature female and two mature males were allocated to a 50 mL cylinder plastic container filled with 25 mL of soil and covered with a cheesecloth secured by a rubber band. Eggs were collected every 24 h and each one allocated to a 50 mL plastic container (the same one mentioned above) with a nutritious substrate (25 mL) until pupation. Substrate was kept humid by spraying tap water as needed. The nutritious substrate was replaced every month.

A single pupa was allocated to a 35 mL plastic container with 29 mL of humid soil. Four of these containers were kept in a 15 cm length by 5cm high and 10 cm width squared container whose tap had four holes for ventilation. Fifty mL of water floured the container to prevent pupae dehydration. Newly emerged adults were collected 24 h after emergence and allocated to a 50 mL cylinder plastic container filled with 25 mL of soil in the above-mentioned plastic containers with the nutritious substrate.

2.2. Reproductive apparatus description

A total of 20 virgin and healthy insects (10 for each gender) of 18 d old were examined under a stereoscope. The reproductive apparatus (RA) was dissected under saline solution and stained for 5 minutes with Giemsa 1%. After this time, the RA was rinsed with tap water and temporally stored in saline solution until observation on a Zeiss Discovery-V20 (Thornwood, Nueva York, EE. UU) stereoscopic microscope. The papers by [10], [16], [12], and [11] were used to identify the reproductive structures. Drawings and all processing of images were devised with Krita 4.4.2 software (Stichting Krita Foundation, Netherlands).

The ejaculatory bulb and genital capsule from males and the genital chamber, accessory glands, and ovaries from females were dehydrated by sequential immersion in ethanol at 70% (2h), formaldehyde 40% (1 h), ethanol 90% (2 h), formaldehyde 40% (1 h), and 100% ethanol for (2 h). Then microphotographs were obtained by the methodology of [36,37]; the structures were collocated in aluminum stubs with double-sided carbon adhesive and viewed using an Environmental Electronic Scanning Microscope (ESEM) Model EVO LS10 Life Science (Carl Zeiss, Germany). The genital chamber and accessory glands were stained using 1% DAPI solution for 1 h, after that, the solution was removed with PBS. The genital chamber and accessory glands were observed in Laser Scanning Confocal Microscope (LSCM) model CLSM LSM 800 (Carl Zeiss, Germany).

2.3. Male gonadic maturation

To study the male gonadic maturation a total of 30 males were sacrificed at 0, 10 and 18 d after emergence (n=10), and their RA (accessory glands, *vas efferens* and testis) was dissected and cleaned under saline solution as mentioned before. The RA was stained with Giemsa 10% for 5 min and observed under the stereoscope previously mentioned. The sperm was obtained from the *vas efferens* and allocated to 1.5 mL Eppendorf tubes plus 200 μm of distilled water before manual stirring and observation under an ESEM.

To study the female gonadic maturation, the RA (ovaries and oocytes) of 10 females of 0, 6, 12, and 18 d old was dissected with saline solution as mentioned before, stained for 5 min with 10% Giemsa and observed under the stereoscopic microscope and was classified according to the [38] classification. The ovaries of 12 d old were dissected to

separate the ovarioles. The ovarioles were dehydrated at ethanol series (70 %, 96%, and 100%) prior observation under the above mentioned LSCM.

2.4. Allometry

All *C. barrerai* pupae were weighed using an analytical balance (Ohasus Explorer, 0.0001 gr precision, Zurich, Switzerland). All newly emerged adults were weighted on the same scale and sexed according to the nail of the first legs where males present a larger nail than females. A total of 31 males and 36 females of 18 d old were sacrificed by freezing.

To study the relationship between body parts and size, we separated the hind-tibia, middle-tibia, and the external-lamella of the antenna of both sexes and the genial chamber and *bursa copulatrix* in females and the tarsal nail (width and length) of the front legs of the male. All the structures were measured and photographed under a stereoscope Zeiss Discovery-V20 (Thornwood, Nueva York, EE. UU). The photos were analyzed with ImageJ software (Scion Corporation, USA) [39].

2.4.1. Statistical analysis

The relationship between the body parts and the size or the length of the structures was analyzed by linear regression. We compared the measurements of the different body parts between sexes with a t-test. Unless stated otherwise, all reported data is the mean ± ESM. All analyses were carried out on SigmaPlot 14.0 (Systat Software, Inc., USA).

3. Results

3.1. Male reproductive apparatus

The male RA consists of two accessory glands, two glandular duct, two testes each with six testes follicles, six *vas efferens*, two *vas deferens*, one ejaculatory duct, one ejaculatory bulb, one genital capsule, and one aedeagus (Figure 1).

3.1.2. Accessory glands

The accessory glands are long ducts with thick walls. The superior zone is small, and no fluid is observed. The subsequent zone is thick with a fluid. The accessory glands are connected to the ejaculatory duct (Figure 1).

3.1.3. Testes, vas efferens and deferens

The testis is compound of six free follicles, lobed, dorsoventrally flattened, in form of a "flower", every testis follicle is connected to a *vas efferens*. The *vas efferens* is a small and thin duct connected to the *vas deferens*. The *vas deferens* are long, with thicker walls, the diameter of its anterior part is smaller than the posterior (Figure 1).

3.1.4. Ejaculatory duct and ejaculatory bulb

One side of the ejaculatory duct is connected to the accessory glands and the *vas efferens* while the other side connects to the ejaculatory bulb (Figure 1). The ejaculatory bulb is a membranous sac with fluted like muscular tissue (Figure 1).

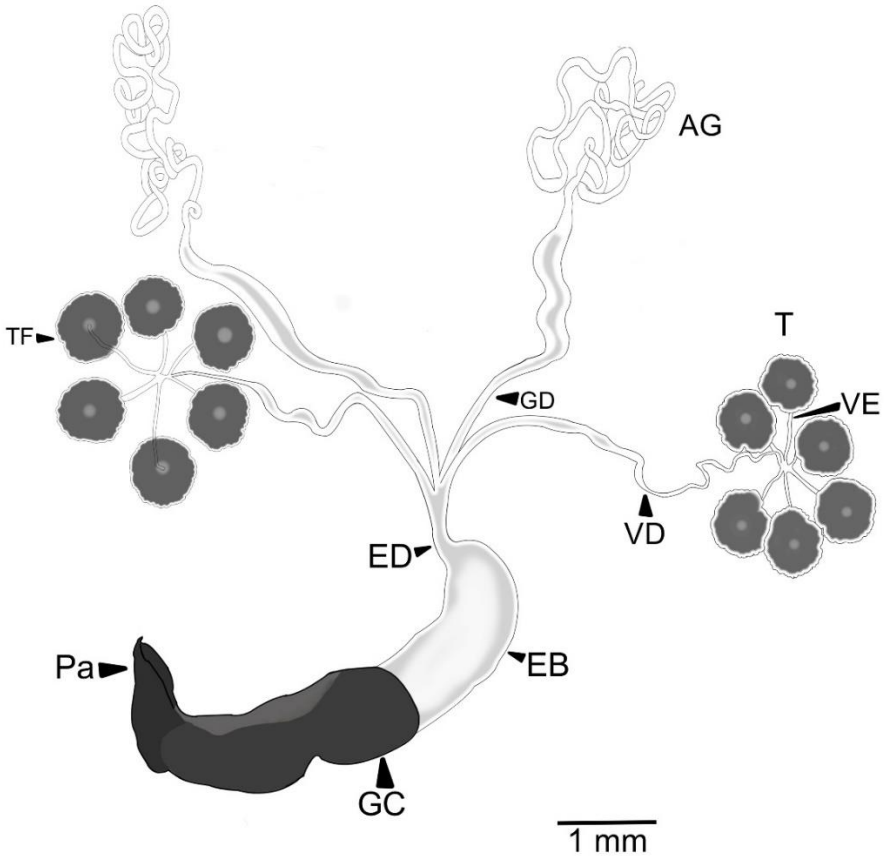

Figure 1. Reproductive apparatus of 18 d old *Cyclocephala barrerai* male. AG = accessory glands, GD = glandular duct, T = testes, TF = testis follicles, Vd = *vas deferens*, Ve = *vas efferens*, ED = ejaculatory duct, EB = ejaculatory bulb, GP = genital capsule, Pa = parameres.

3.1.5. Endophallus

The endophallus is a "Membranous sac lying within median lobe contiguous with ejaculatory duct" [12] (Figure 2 A) and it is involved by the genital capsule (Figure 2 B).

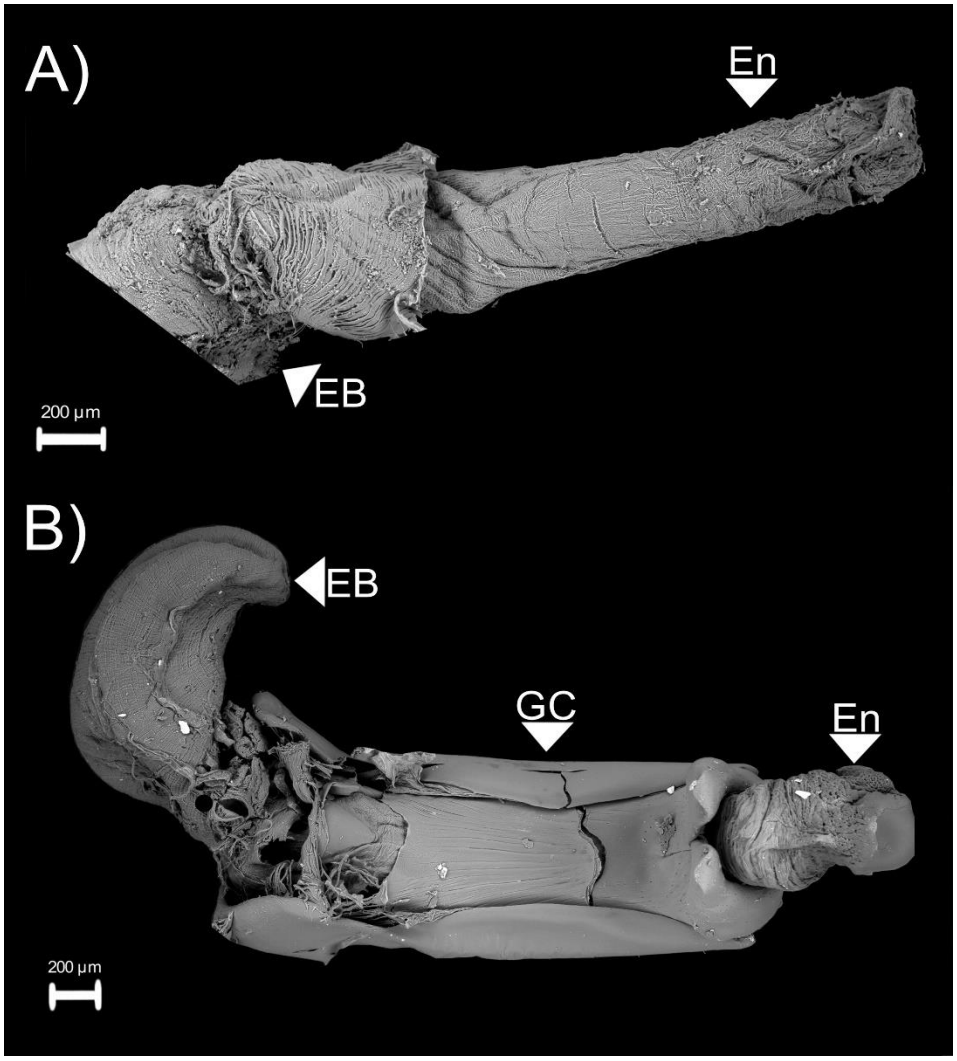

Figure 2. Endophallus of 18 d old *Cyclocephala barrerai* male. A) endophallus and ejaculatory bulb, B) genital capsule and endophallus. En = endophallus, EB = ejaculatory bulb, GC = genital capsule.

3.1.6. Genital capsule

The genital capsule "is a structure formed of the sclerites of abdominal segment" [12] (Figure 3 A) and it has two symmetrical plates:

1) Connecting membrane (Figure 3 A). It is in the ventral zone, connecting the phallobase and parameres.

2) Parameres (Figure 3 A, B). The parameres are at the end of the genital capsule and have a curvilinear triangle shape. The *C. barrerai* parameres are symmetric and they guide the aedeagus into the urogenital opening of the female during mating. The parameres are ornamented with small cones and pits (Figure 3 C). Some areas at the distal tip of the parameres have some pallid fine lines between the pits (Figure 3 D).

3) Posterior phallobase and anterior phallobase (Figure 3 E). Posterior phallobase is a sub cylindric structure that involves the aedeagus and connects to the parameres. It is made up of one piece and presents a high diversity of ultrastructure (Figure 3 F). The posterior phallobase has four types of ultrastructure: 1) large needle, 2) small needle, 3) pits (Figure 3 F), and 4) ~~small needle~~ with a circular base (Figure 3 G).

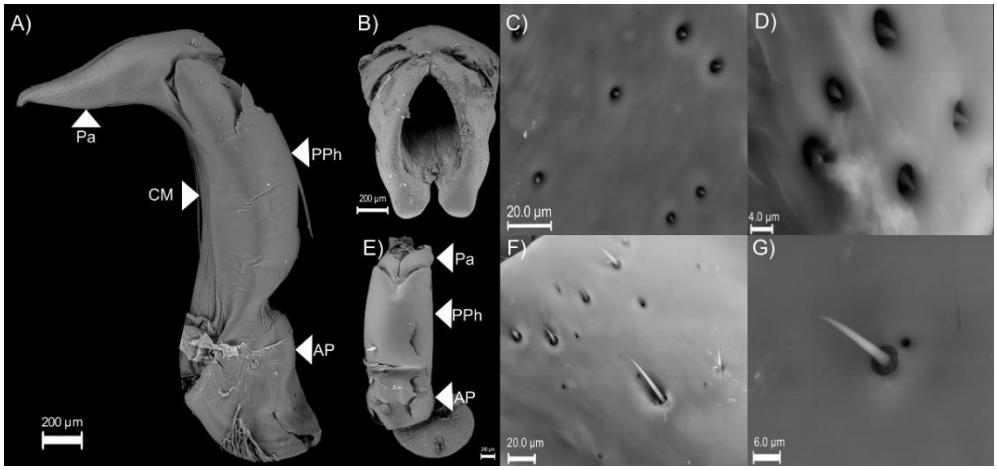

Figure 3. Genital capsule of 18 d old *Cyclocephala barrerai* male. A) genital capsule (lateral view), B) parameres (frontal view), C) ultrastructure in parameres (distal view), D) ultrastructure in parameres (proximal view), E) genital capsule (dorsal view), F) pits and ultrastructure in phallobase (proximal view), G) pits and ultrastructure in phallobase (posterior view). Pa = parameres, PPh = posterior phallobase, CM = connecting membrane, AP = anterior phallobase.

3.1.7. Gonadic maturation and sperm

Newly emerged males of *C. barrerai* present an immature reproductive apparatus (Figure 4 A). The accessory glands and *vas deferens* of newly emerged males are empty and white and clear, and their walls thickness is uniform. The accessory glands and *vas deferens* from 10 d old males are enlarged and hold a white content that does not fulfill the accessory glands and the *vas deferens*.

When males are 18 d old, the accessory glands are filled with a white content and the mature sperm is located at the *vas deferens*. Mature spermatozoids had a tubular and filamentary shape (Figure 4 B).

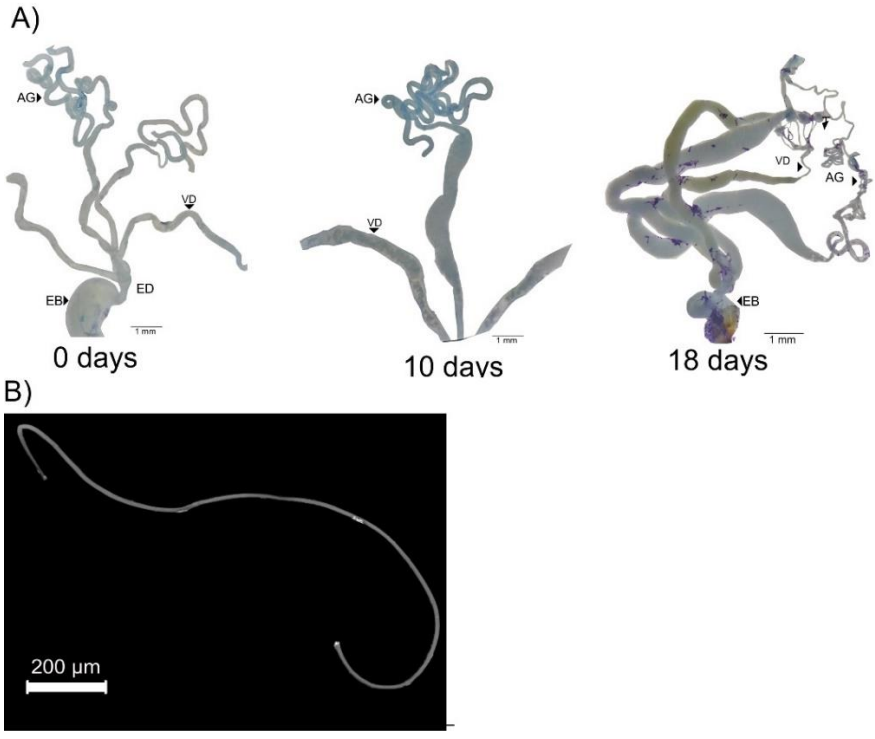

Figure 4. Development of male reproductive apparatus and sperm of *Cyclocephala barrerai*. A) development, B) sperm from an 18 d old male. AG = accessory glands, Vd = *vas efferens*, ED = ejaculatory duct, EB = ejaculatory bulb.

3.2. Female reproductive apparatus

The female RA presents a genital chamber, two pairs of genital plaques, two pairs of ventral plaques, a spermatheca with a spermathecal gland, a common oviduct, and two ovaries (Figure 5).

3.2.1. Spermatheca and spermathecal gland

The spermatheca is a tubular, sickle-shaped structure with a spermatheca muscle and spermatheca gland. The spermatheca gland is balloon-shaped and is larger than the spermatheca. Both structures join the spermatheca duct (Figure 5).

3.2.2. Bursa copulatrix

The *bursa copulatrix* is a "punch-like bimembranous structure arising anterodorsally from the genital chamber" [12]. It is bigger than the genital common oviduct (Figure 5).

3.2.3. Common oviduct

It is a "Tubular structure connecting posteriorly to the anteroventral area of the genital chamber and bifurcating anteriorly to the paired lateral oviducts that connect to the paired ovaries" [12] (Figure 5).

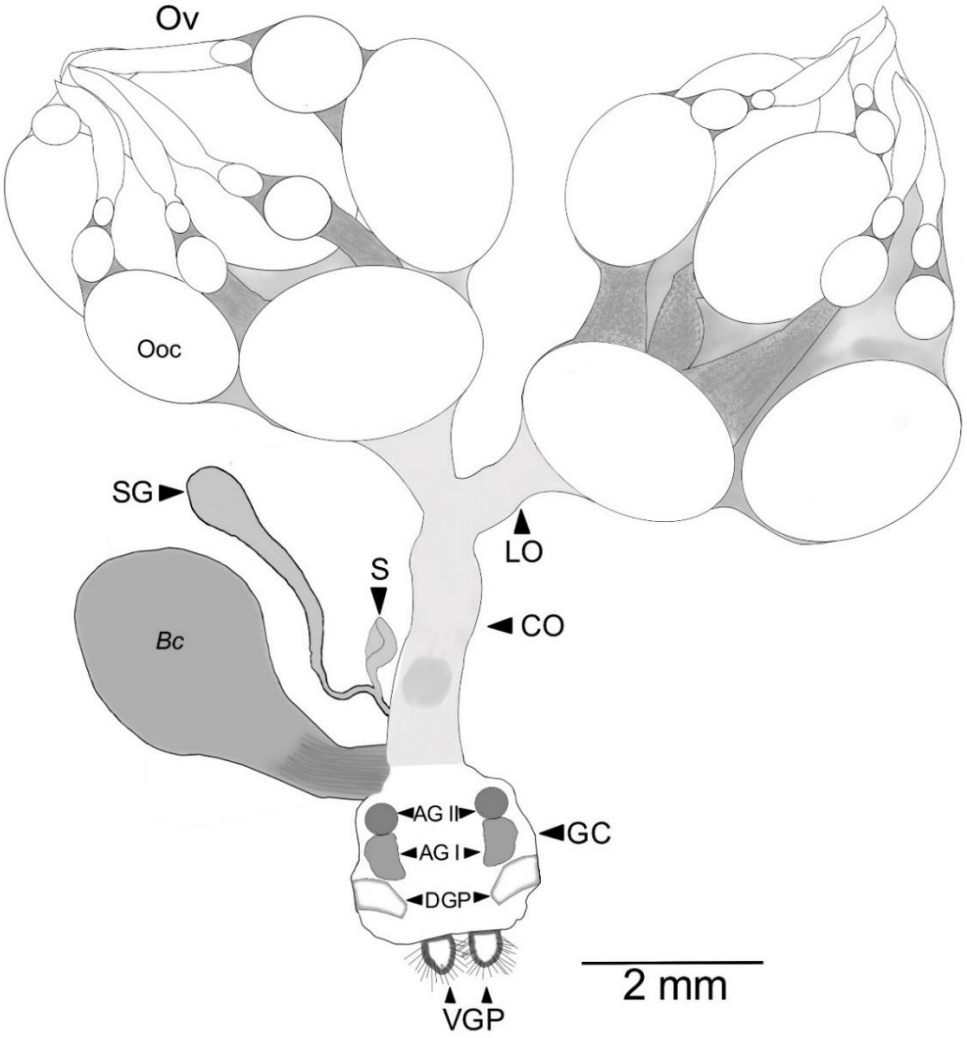

Figure 5. Reproductive apparatus of 18 d old virgin *Cyclocephala barrerai* female. Bc = *bursa copulatrix*, GC = genital chamber, S = spermatheca, SG = spermathecal gland, AG I = accessory gland type I, AG

II= accessory gland type II, Grm= gremarium, Oc = oocyte, Ov = ovary, CO = common oviduct, LO = lateral oviduct, DGP = dorsal genital plates, VGP = ventral genital plates.

3.2.4. Genital chamber

The genital chamber is a cylindric structure. The common oviduct, *bursa copulatrix*, and the spermatheca duct insert into the genital chamber (Figure 6 A, B). The latest is made of muscular fibers rolled up over each other (Figure 6 C, D). In a cross-section view, the tissue is fluted. The distal part, close to the genital plaques of the genital chamber, of 18 d old females has bacteria. These have a circular shape and are in the chamber inside the muscular tissue (Figure 6 E, F). They can be found alone, aggregated, or in couples or triples. The bacteria size varied from 3 to 14 µm.

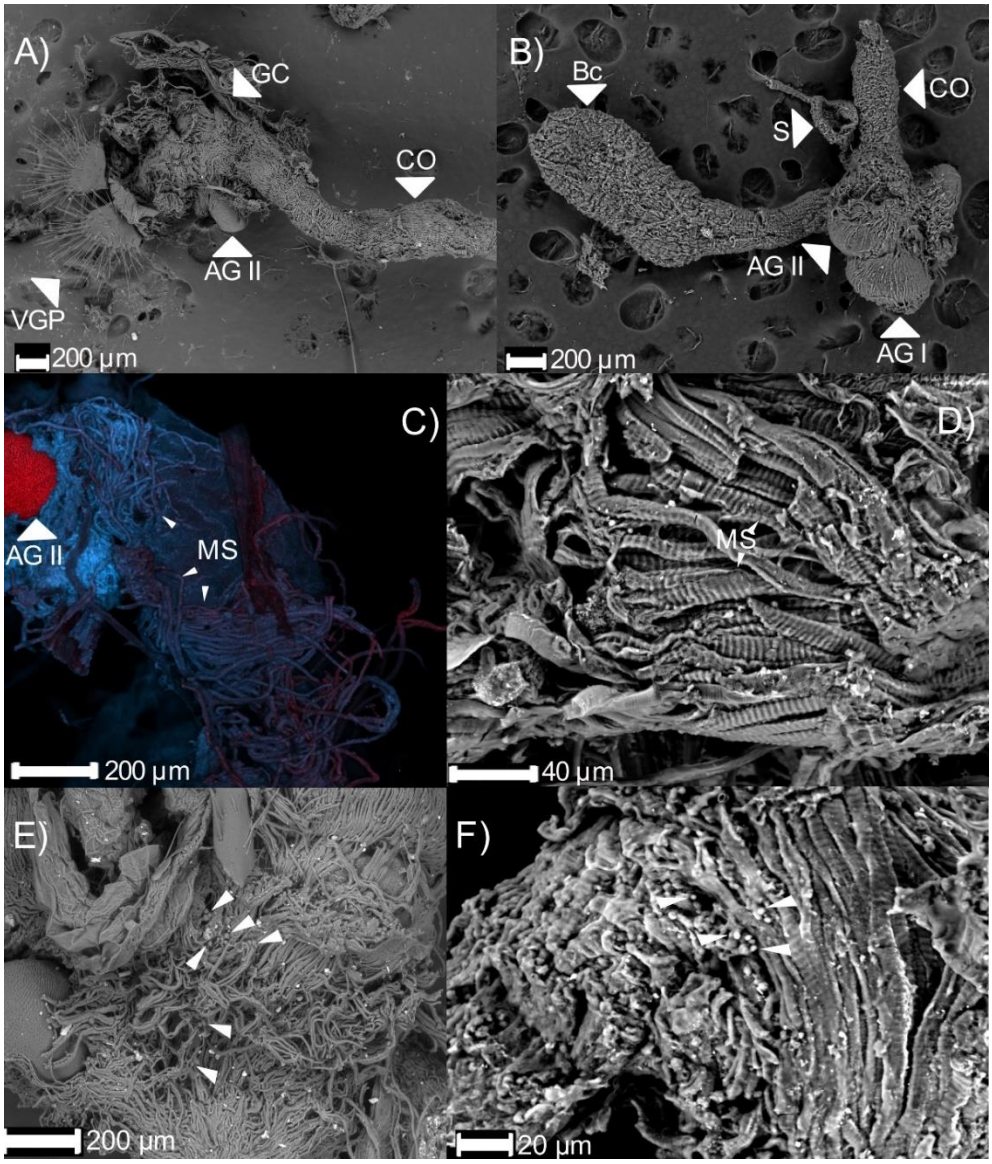

Figure 6. Genital chamber of 18 d old virgin *Cyclocephala barrerai* female. A) genital chamber (interior view), B) genital chamber (exterior view). C) muscular structure of genital chamber (confocal scanning laser microscopy view), D) cross-section, E) bacteria in the genital chamber, F) bacteria into the muscular structure of the genital chamber. GC = genital chamber, CO = common oviduct, AG = accessory glands type II, VGP = ventral genital plates, MS = muscular structures.

3.2.5. Genital plates

The genital chamber has two types of genital plates.

1) Dorsal plates. They are sclerotized rectangular shape structures. They insert near the accessory glands, around the genital chamber.

2) Ventral plates (Figure 7). They insert in the distal part of the genital chamber. The ventral plates are oval with many setae (Figure 7 A). The setae interact with the parameres of the male during mating. The ventral part has pits (Figure 7 B).

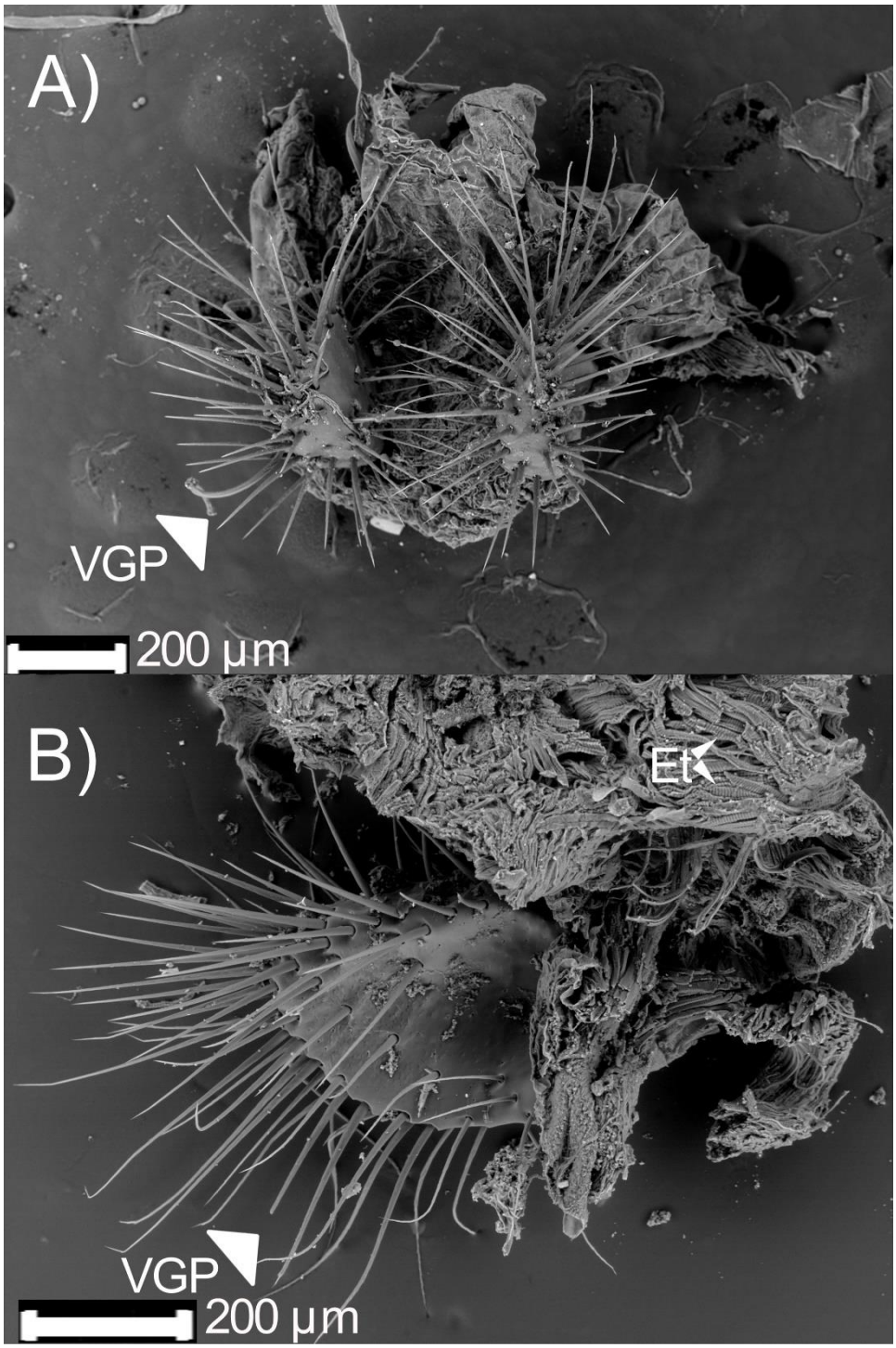

Figure 7. Ventral genital plates of 18 d old *Cyclocephala barrerai* virgin female. A) ventral genital plates (front view), B) ventral genital plates (lateral view). VGP = ventral genital plates.

3.2.6. Accessory glands

We observed two types of accessory glands: type I and type II.

1) Type I is oval shaped, and it inserts in the distal zone of the genital chamber.
2) Type II is sclerotized (Figure 8 A) and has a spherical shape. It is formed by triangular plates (Figure 8 B, C and D).

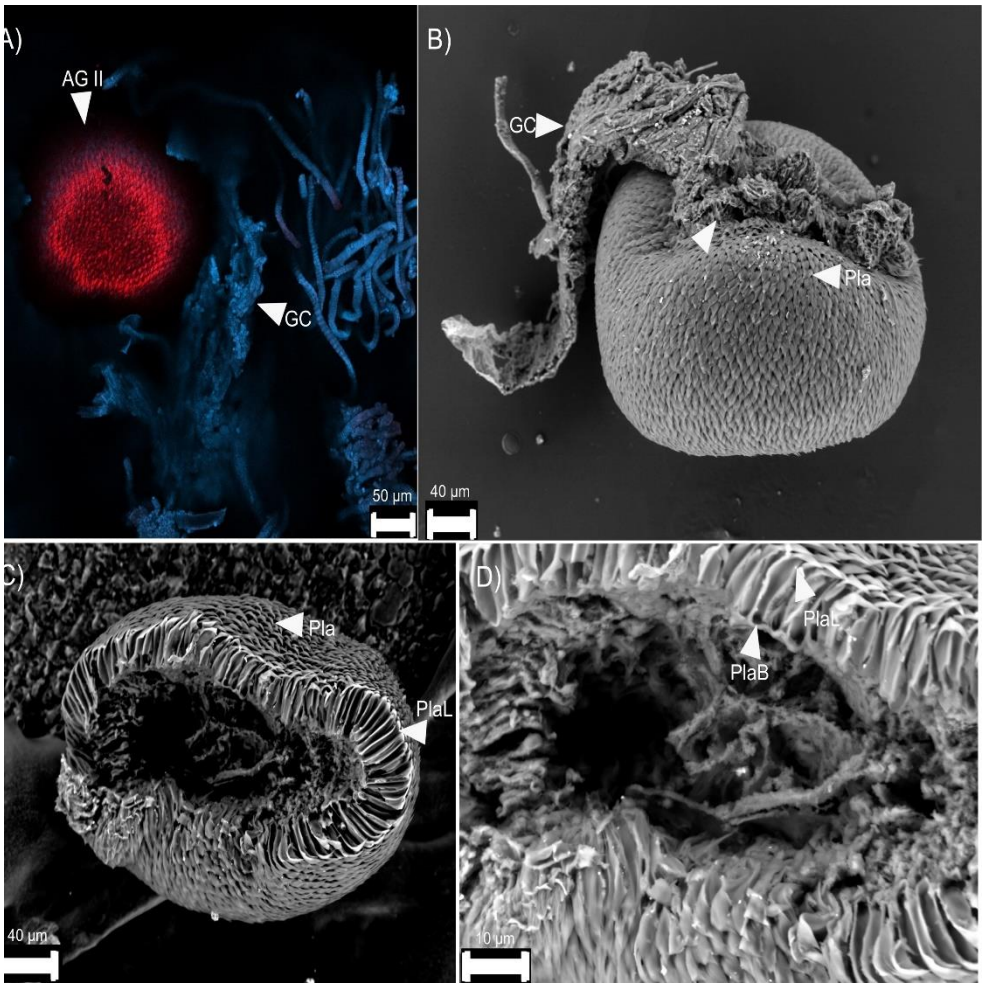

Figure 8. Accessory glands type II of *Cyclocephala barrerai female*. A) tissue in blue and keratinized structure in red (confocal scanning laser microscopy), B) accessory gland type II with tissue of the genital chamber, C) accessory gland type II (internal view), D) plates in the accessory gland type II (close-up), AG II = accessory glands type II, GC = genital chamber, Pla = plates, PlaL = plates (lateral view), PlaB = plates (ventral view).

3.2.7. Ovary type and ovarian development

The females of *C. barrerai* present a telotrophic, compound two-ovary with six ovarioles (Figure 9). The compound ovariole of the germarium, the vitellarium, and the mature oocytes are in the oviduct (Figure 9 A, B). The germarium contains the germinative cells that will produce gametes (oogonia) (Figure 9 C, D). The vitellarium contains the immature oocytes (ovarian follicles) (Figure 9 E).

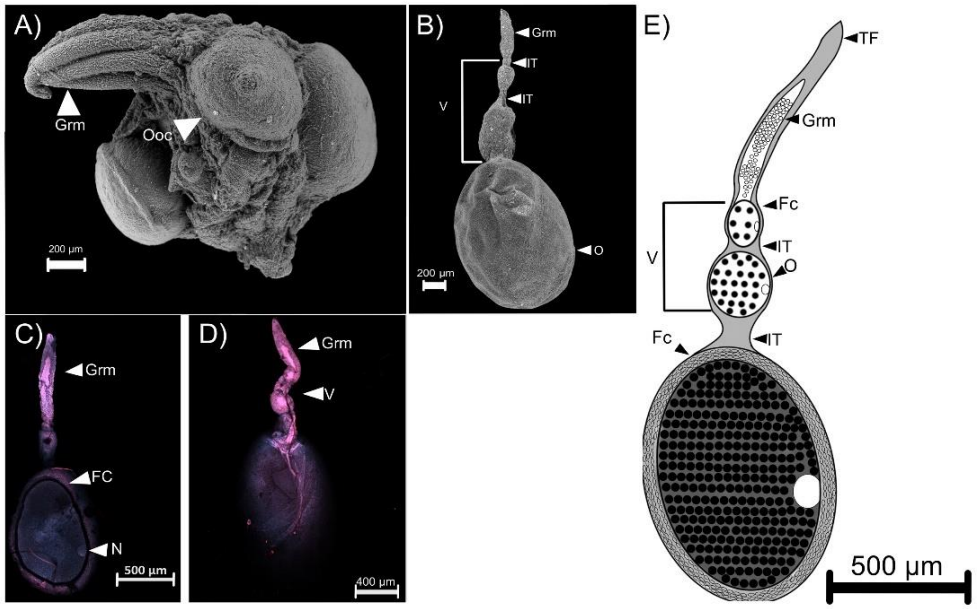

Figure 9. Telotrophic ovary of *Cyclocephala barrerai*. A) ovary of 6 d old female, B) ovariole of 12 d old female, C) and D) ovarioles (confocal scanning laser microscopy view). E) scheme of ovary. Grm = gremarium, Ooc= oocyte, Fc= Follicular cells, V = vitellarium, TF = terminal filament, N = nucleus, IT = interfollicular tissue.

The ovarian development of *C. barrerai* (Figure 10) presents three nulliparous stages and a parous (with ovulation) one (Figure 10). On day 0 (emerged from pupae) the ovary is in nullipara 1 stage; "where the germarium and vitellarium are not differentiated and are without follicles" [8]. On day six the ovary is in nullipara 2 stage: "well-defined ovarioles with multiple follicles, the proximal follicle transparent indicating that it had not completed maturation" [8]. On day 12 the ovary is in nullipara 3 stage; "proximal follicle more opaque [mature] and close to ovulation" [8]. On day 12, the ovary is in the parous stage.

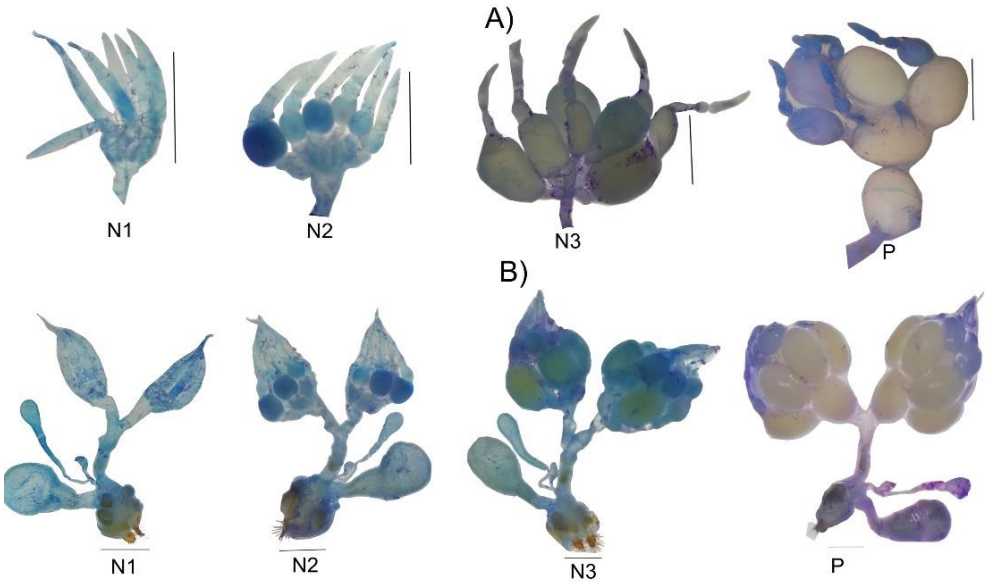

Figure 10. Ovarian development of *Cyclocephala barrerai* female. A) ovarioles individual maturation, B) ovary maturation N1 = nulliparous 1, N2 = nulliparous 2, N3 = nulliparous 3, P = parous. Bars = 2 mm.

3.3. Allometry in Cyclocephala barrerai

3.3.1. Relationships between body parts and weight

Measures of the different body parts used in this study are presented on Table 1. *Cyclocephala barrerai* lab-reared has a lineal and positive relationship among pupal weight and female ($R2 = 0.820$, $F = 254.2$, $df = 1, 57$, $p < 0.001$) and male ($R2 = 0.833$, $F = 279.531$, $df = 1, 57$, $p < 0.001$) weight. Female weight and her middle-tibial length ($R2 = 0.566$, $F = 44.28$, $df = 1, 35$, $p < 0.001$, Figure 11 A) and hind-tibial length ($R2 = 0.437$, $F = 25.584$, $df = 1, 34$, $p < 0.001$, Figure 11 B) have a linear and positive relationships. Similar relationships occur among male weight and his middle-tibial length ($R2 = 0.566$, $F = 44.28$, $df = 1, 35$, $p < 0.001$, Figure 11 C) and his hind-tibial length ($R2 = 0.437$, $F = 25.584$, $df = 1, 34$, $p < 0.001$, Figure 11 D).

Table 1. Measurement of different body parts of *Cyclocephala barrerai* adults.

Structure	**Lab-reared adults mean ± SEM (n)**	
	Female	**Male**
Pupa weight	0.308 ± 0.006 gr (58)	0.291 ± 0.006 gr (58)
Adult weight	0.188 ± 0.004 gr (58)	0.175 ± 0.004 gr (58)
Middle-tibial length	2.625 ± 0.051 mm (36)	2.770 ± 0.022 mm (33)
Hind-tibial length	3.025 ± 0.054 mm (35)	3.077 ± 0.048 mm (36)
Lamella length	1.236 ± 0.089 mm (21)	1.876 ± 0.024 mm (20)
Lamella area	0.383 ± 0.015 mm^2 (16)	0.775 ± 0.014 mm^2 (20)
Tarsal nail 1 length	-	1.199 ± 0.0218 mm (16)
Tarsal nail 1 width base	-	0.452 ± mm 0.007 (16)
Tarsal nail 2 length	-	0.839 ± 0.065 mm (15)
Tarsal nail 2 width base	-	0.0388 ± 0.008 mm (15)
Genital chamber length	1.993 ± 0.109 mm (19)	-
Genital chamber area	3.875 ± 0.291 mm^2 (16)	-
Bursa copulatrix length	4.223 ± 0.145 mm (27)	-
Bursa copulatrix area	5.089 ± 0.357 mm^2 (26)	-

Standard error of mean = SEM.

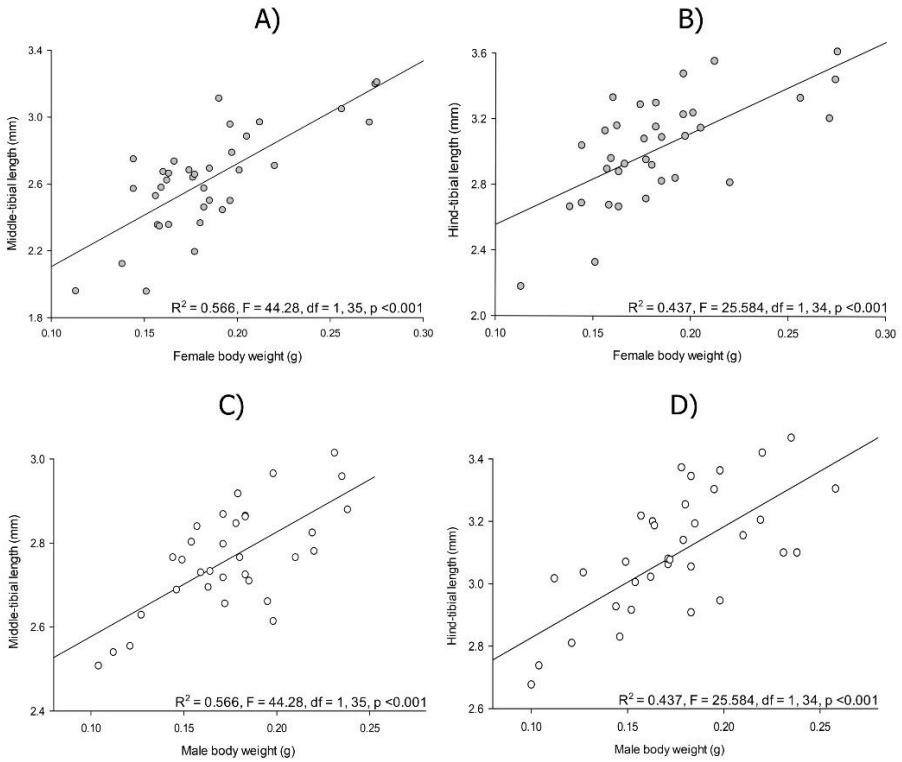

Figure 11. Relationships between body weight and tibial length on *Cyclocephala barrerai*. female body weight and A) middle-tibial length and B) hind-tibial length, male body weight and C) middle-tibial length and D) hind-tibial length. female = empty dots, male = solid dots.

The forelegs have two nails which were named as tarsal nail 1 and tarsal nail 2 (Figure 12 A). The tarsal nails of the forelegs in males are thicker than the other nails (Figure 12 B). The underside of the tarsal nail 1 has an opening with channels and there is a protuberance at the tip of the nail (Figure 12 C). Tarsal nail 2 is thinner than the first one and has no evident structures except from some light lines along the nail (Figure 12 D).

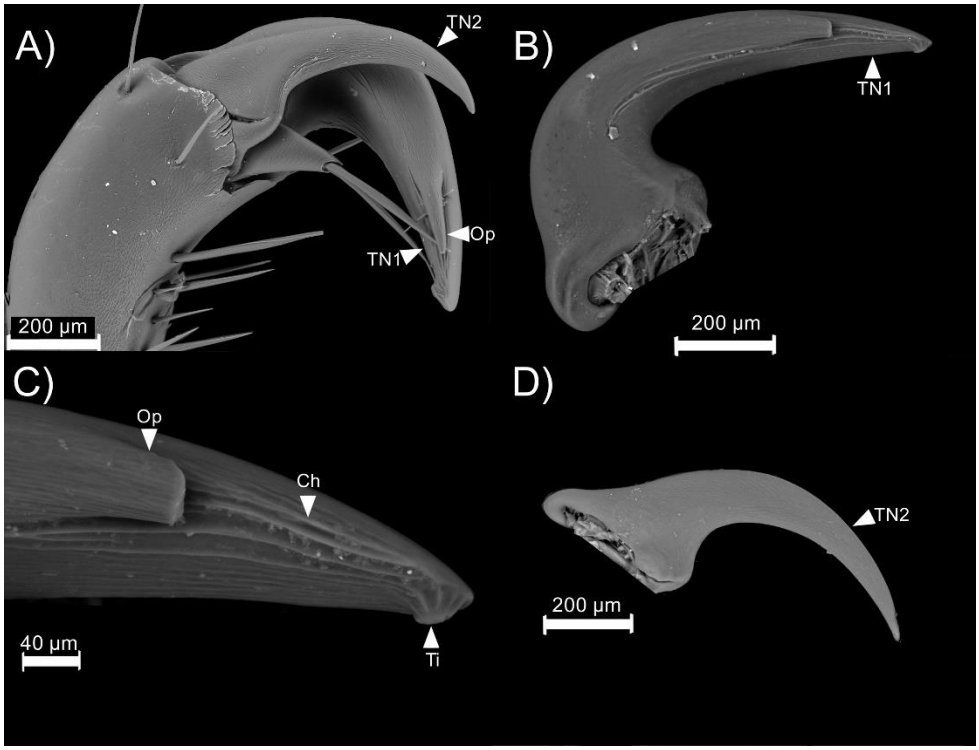

Figure 12. Tarsal nail of the first pair of legs of *Cyclocephala barrerai* males A) general view of the tarsal nail composition, B) tarsal nail 1, C) close-up of tarsal nail 1, D) tarsal nail 2.TN1 = tarsal nail 1, TN2 = tarsal nail 2, Op = opening, Ch = channel, Ti = tip.

Cyclocephala barrerai male lab-reared weight and lamella length (R2 = 0.772, F = 60.842, df = 1, 19, p <0.001, Figure 13 A), lamella area (R2 = 0.512, F = 18.867, df = 1, 19, p <0.001, Figure 13 B) have a linear and positive relationship. Similar relationships occur among male weight and the width of the base of their tarsal nail 1 (R2 = 0.605, F = 21.472, df = 1, 16, p <0.001, Figure 13 C) and tarsal 1 nail length (R2 = 0.432, F = 10.662, df = 1, 16, p <0.001, Figure 13 D).

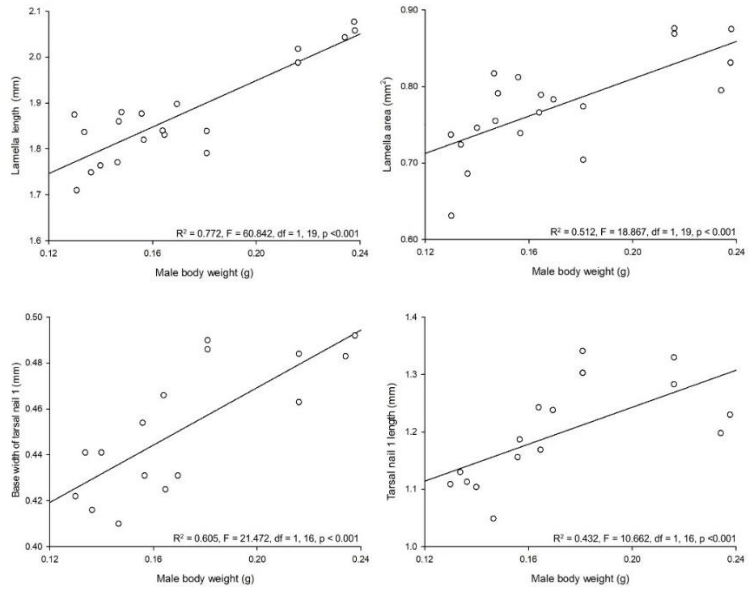

Figure 13. Relationships between body weight and different body parts on *Cyclocephala barrerai* males. Body weight and A) lamella length, B) lamella area, C) width of the base of tarsal nail 1 and, D) tarsal nail 1 length.

Cyclocephala barrerai female lab-reared body weight and genital chamber area (R2 = 0.696, F = 32.05, df = 1, 15, p <0.001, Figure 14 A), *bursa copulatrix* length (R2 = 0.185, F = 5.678, df = 1, 26, p = 0.025, Figure 14 B) lamella length (R2 = 0.615, F = 30.334, df = 1, 19, p <0.001, Figure 14 C), and lamella area (R2 = 0.474, F = 12.619, df = 1, 15, p = 0.003, Figure 14 D) have a linear and positive relationship.

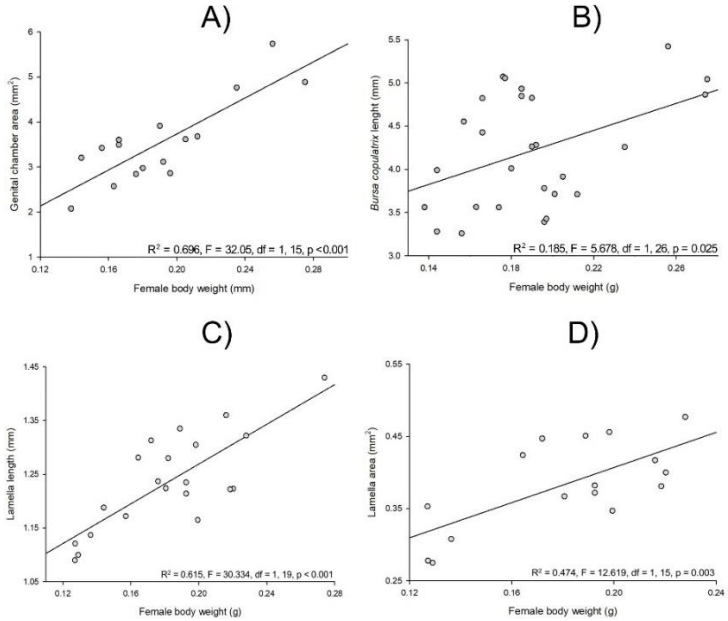

Figure 14. Relationship between body weight and body parts on *Cyclocephala barrerai* females. Body weight and A) genital chamber area, b) *bursa copulatrix* length, C) lamella length and D) lamella area.

There was no relationship between *C. barrerai* male lab-reared weight and the width of the base of tarsal nail 2 (R2 = 0.101, F = 1.456, df = 1, 15, p = 0.249) and tarsal nail 2 length (R2 = 0.167, F = 2.610, df = 1, 15, p = 0.130). No relationship was observed between lab-reared female weight and the *bursa copulatrix* area (R2 = 0.0702, F = 1.813, df = 1, 25, p = 0.191) and the genital chamber length (R2 = 0.0546, F = 0.983, df = 1, 18, p = 0.335).

3.3.2. Sexual dimorphism in lab-reared adults

The weight from pupae that produced females did not statistically differed from that that produced males (t = 1.839, df = 57, p = 0.069). However, at emergence, adult females were heavier than adult males (t = 2.112, df = 57, p = 0.037, Figure 15 A). The lamella length of adult lab-reared males, (t = 20.796, df = 39, p <0.001, Figure 15 B) and the lamella area (t = 18.632, df = 34, p <0.001, Figure 15 C) were bigger than females.

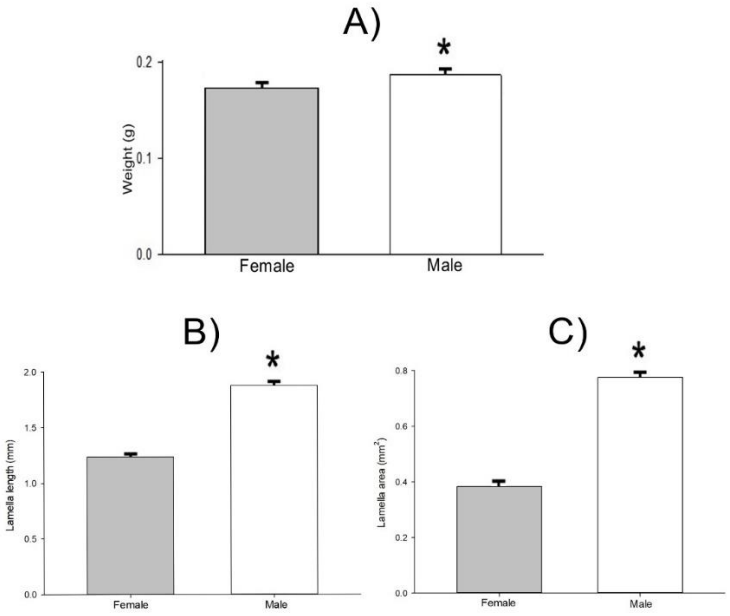

Figure 15. Mean ± ESM of weight (A), lamella length (B) and lamella area (C) of lab-reared males and females of *Cyclocephala barrerai* adults. t-test, * = significance at 0.05.

4. Discussion

The male reproductive apparatus of *C. barrerai* presents a similar arrangement and organization of the Melolonthidae: testis, accessory glands, and *vas efferens* and *vas deferens* [9,11,14]. Like the six *Cyclocephala* species described by Martínez et al. [11], the male reproductive apparatus lacks a glandular reservoir and seminal vesicles, but the ejaculator duct and ejaculatory bulb present well developed muscular fibers. The spermatophore is formed at the two lattes structures by fusing the sperm and the content of the accessory glands before it is transferred to the females [2].

The presence of a glandular reservoir and seminal vesicles is not common in Melolonthidae, but their presence has been reported for *C. zealandica* [40], *Hoplia* spp. [14], and *Macrodactylus mexicanus* Burmeister [10]. Seminal vesicles are related to the life history of the species and are usually found in long-lived adults, these seminal vesicles store mature sperm when the mating season is interrupted [41–43], for example, *M. mexicanus* adults may live up to 3 months and can mate at different times [44].

Like other beetles, *C. barrerai* male gonads mature after emergence, adults do not feed and are sexually active. This situation also has been reported for *Paraglenea fortunei* Saunders (Cerambycidae) [45], *Hylobius pales* Herbst (Curculionidae), [46] and *Oemona hirta Fabricius* (Cerambycidae) [47].

Due to its taxonomic relevance, the parameres of *Cyclocephala* have been extensively described. Breeschoten et al. [48] analyzed and compared the parameres of 48 species identifying three different types: 1) symmetric, 2) minor asymmetry, and 3) asymmetric. The same author indicates that species subject to higher interspecific selection pressure, like sympatric species of the same genera, present asymmetric parameres. Males of *C. barrerai*, have a genital capsule type 1 formed for three plates with symmetric parameres, indicating that this attribute is not under interspecific selection pressure, as this ~~specie~~ is the only one ~~populating~~ the collecting area.

The form and shape of the ultra-structures found at the phallobase are like the trichoid sensilla and the ultra-structures described at the parameres of the basiconic ones; both sensilla have been reported at the antenna of the Melolonthidae [49,50]. Ultra-structures called pores have been reported in the genital capsule of species of the Chrysomelidae but no explanation of their diversity or function has been proposed so far [51–53].

In agreement with Sanmartín & Martín-Piera [9] for species of the Pachydeminae subfamily, we hypothesized that the ultrastructure of the genital capsule of *C. barrerai* has a mechanoreception function like that performed by the ultrastructure of the genital capsule that facilitates the recognition and sexual coupling in *Drosophila melanogaster* Meigen [54]. This hypothesis also explains the function of the setae of the ventral genital plaques found on *C. barrerai* females as the genital plaques and the genital capsule have an active role during mating [13]. The parameters of the male provide support during mating and in conjunction with the female genital plaques facilitate the insertion of the penis and sperm transfer [55].

The reproductive apparatus of females of *C. barrerai* has the general arrangement of the Melolonthidae family [10,14–16]. The *bursa copulatrix* and the spermatheca connect to the genital chamber, storing and nourishing the sperm inside the female body until fecundation [12,56]. The genital chamber presents muscular fibers as mentioned for *C. lunulata* (Benítez-Herrera, unpublished data), but does not have pheromone glandular cells [10,15,49].

The female genital chamber presents muscular fibers and its static positive allometry indicates a female cryptic sexual selection process. This provides the female some control over sperm transfer to avoid mating to unfit individuals and to reduce the risk of hybridization [23,57]. The ultrastructure of parameters is the result of the coevolution of the male and female genitalia [13,23,48,58].

The presence of microorganisms in the female genital chamber agrees with that reported by [35] that identified *Klebsiella oxytoca* and *Citrobacter freundii* in *C. barrerai* wild females. The presence of microorganisms in lab-reared females indicates that these might be transferred by the mother or acquired during the larval period and not transferred by the male during mating. The composition of the genital chamber and the presence of the microorganisms suggest that they are relevant during attractants production [35,57].

Cyclocephala barrerai and *C. jalapensis*, have accessory glands type II. These are highly sclerotized superimposed plaques [16] whose function is unknown. This contrasts with those observed in *Phyllophaga* [15], *Macrodactylus* [10], and *C. zealandica* [18], where the accessory glands type II is semi-sclerotized, and the cells form conducts to transport pheromones.

Cyclocephala barrerai female and male gonadic maturation initiate once the adults emerge from the pupae and when they are sexually mature, they reach the surface and search for females as reported by Yi-Zhen et al. [17] for *Holotrichia titanis* Reitter, *Holotrichia trichopora* Fairmaire, and *Anomala exoleta* Faldermann. *C. barrerai* adults from the lab colony were not fed and their digestive system was filled with a dark liquid. Most Coleoptera adults depend (partially or completely) on food for gonadic maturation [8,59–61]. *C. barrerai* must obtain and store as larvae, all the nutrients needed for metamorphosis and gonadic maturation, explaining their voracious appetite as larvae [61].

There is a strong relationship between the hind-tibia and the body weight in *C. barrerai*, therefore the hind-tibia length can be used to assess the bodyweight [62]. *Cyclocephala barrerai* females are heavier than males, however in *C. borealis* is the other way around; males are heavier than females [28]. This could be related to the life history of each ~~specie~~. In addition, males' lamellas have a larger surface and are longer than those of females. According to the males' drawings of the lamellas for *Cyclocephala borealis* Arrow by Johnson [28] and for *Cyclocephala signaticollis* Burmeister by Carne [63] males are larger than those of females. Unfortunately, there is no quantitative information on lamella length and surface reported for any other *Cyclocephala*.

In species where mate location relays on the prompt detection of pheromones, those individuals with larger, more, or more sensitive sensilla have a clear advantage over the rest of the sexually active population. The males of *C. barrerai* present a static positive allometry due to their strong relationship between antenna length and body size [64] indicating that this relationship is under a strong sexual selection process [23,65], therefore heavier males have an adaptative advantage over lighter males during mate location and

mating [66]. Mate location on *Cyclocephala* depends on the prompt detection of receptive females in the vicinity as a contest between males is rare [67–70]. *C. barrerai* adults are short-lived and do not feed, so gathering reserves as larvae and reproduction must be paramount. These compensatory strategies are common among the Coleoptera [22,71].

Supplementary Materials: This paper doesn't have supplementary materials.

Author Contributions: Conceptualization, A.S.C. and A.J.P.; methodology, A.S.C., D.T.P. and A.J.P.; formal analysis, A.S.C. and D.T.M.; investigation, A.S.C. and D.T.M. resources, A.J.P.; data curation, A.S.C. and D.T.M; writing—original draft preparation, A.S.C.; writing—review and editing, D.T.M and A.J.P.; visualization, A.S.C., D.T.M and A.J.P.; supervision, A.J.P; project administration, A.J.P.; funding acquisition, A.J.P. All authors have read and agreed to the published version of the manuscript".

Funding: This research was supported by grant SIP 20210746 and 20220763 to AJP. Sanchez-Cruz A. acknowledges funding from Consejo Nacional de Ciencia y Tecnología (CONACyT) and Beca de Estímulo Institucional de Formación de Investigadores (BEIFI) del IPN, México.

Institutional Review Board Statement: This study did not require ethical approval.

Data Availability Statement: All the data presented in this study are in the manuscript.

Acknowledgments: Authors acknowledge Y.X. Malanco-García, P. Villa-Ayala and M. Hurtado-Salgado for help during data collection and lab maintenance.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Thornhill, R.; Alcock, J. *The Evolution of Insect Mating Systems*; Harvard University Press, 1983.
2. Chen, S. Biochemistry of insect male accessory glands. *Annu. Rev. Entomol.* **1984**, *29*, 233–255.
3. Honěk, A. Intraspecific variation in body size and fecundity in insects: A General Relationship. *Oikos* **1993**, *66*, 492, doi:10.2307/3544943.
4. Roff, D.A. *Life history evolution*; Sinauer: Sunderland, 2002; ISBN ; 0878937560. ; 9780878937561.
5. Wilson, D.S.; Knollenberg, W.G. Food Discrimination and ovarian development in burying beetles (Coleoptera: Silphidae: Nicrophorus). *Ann. Entomol. Soc. Am.* **1984**, *77*, 165–170, doi:10.1093/aesa/77.2.165.
6. Gillott, C. Male accessory gland secretions: modulators of female reproductive physiology and behavior. *Annu. Rev. Entomol.* **2003**, *48*, 163–184, doi:10.1146/annurev.ento.48.091801.112657.
7. Zhao, M.-T.; Wang, Y.; Zhou, Z.-S.; Wang, R.; Guo, J.-Y.; Wan, F.-H. Effects of periodically repeated heat events on reproduction and ovary development of *Agasicles hygrophila* (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **2016**, *109*, 1586–1594, doi:10.1093/jee/tow093.
8. Vega-Petlacalco, M.; Arzuffi, R.; Valdez, J.; Rodríguez-Monroy, M.; Jiménez-Pérez, A.; Robledo, N. Food quality influences ovarian development in *Scyphophorus acupunctatus* (Coleoptera: Dryophthoridae). *Florida Entomol.* **2018**, *101*, 447–452, doi:10.1653/024.101.0301.
9. Sanmartín, I.; Martín-Piera, F. First phylogenetic analysis of the subfamily Pachydeminae (Coleoptera, Scarabaeoidea, Melolonthidae): The Palearctic Pachydeminae. *J. Zool. Syst. Evol. Res.* **2003**, *41*, 2–46, doi:10.1046/j.1439-0469.2003.00179.x.
10. Benítez-Herrera, L.N.; Martínez-Morales, I.; Romero-López, A.A. Anatomía del aparato reproductor de *Macrodactylus mexicanus* (Coleoptera: Scarabaeoidea: Melolonthidae) y su posible participación en su comunicación química sexual. *Southwest. Entomol.* **2015**, *40*, 189–198, doi:10.3958/059.040.0117.
11. Martínez-Morales, I.; Rivera-Gasperín, S.L.; Carrillo-Ruiz, H.; Morón, M.A. Comparative aspects of the internal reproductive system of males in species of Melolonthinae, Dynastinae, and Rutelinae (Coleoptera: Scarabaeoidea) from Mexico. *Acta*

zoológica Mex. Zoológica Mex. **2021**, *37*, 1–17, doi:10.21829/azm.2021.3712419.

12. Cristóvão, J.P.; Vaz-de-Mello, F.Z. The terminalia of the superfamily Scarabaeoidea (Coleoptera): specific glossary, dissecting methodology, techniques and previously unrecorded sexual dimorphism in some difficult groups. *Zool. J. Linn. Soc.* **2021**, *191*, 1001–1043, doi:10.1093/ZOOLINNEAN/ZLAA079.
13. Richmond, M.P.; Park, J.; Henry, C.S. The function and evolution of male and female genitalia in *Phyllophaga Harris* scarab beetles (Coleoptera: Scarabaeidae). *J. Evol. Biol.* **2016**, *29*, 2276–2288, doi:10.1111/jeb.12955.
14. Carrillo-Ruiz, H.; Martínez, M.I.; Morón, M.-A. Comparative study of the reproductive system of two species of *Hoplia* (Coleoptera: Scarabaeidae: Hopliinae). *Proc. Entomol. Soc. Washingt.* **2008**, *110(3)*, 778–788, doi:https://doi.org/10.4289/07-052.1.
15. Romero-López, A.; Arzuffi, R.; Valdez, J.; Sánchez-Espíndola, E.; Morón, M.A. Tissues involved in sex pheromone production in *Phyllophaga obsoleta* (Coleoptera: Scarabaeoidea: Melolonthidae). *Ann. Entomol. Soc. Am.* **2011**, *104*, 960–965, doi:10.1603/AN10068.
16. Martínez-Morales, I.; Morón, M.A. Female reproductive systems in Melolonthinae, Rutelinae, and Dynastinae (Coleoptera: Scarabaeoidea, Melolonthidae). *Southwest. Entomol.* **2015**, *40*, 369–386.
17. Yi-Zhen, L.; Lian-Xin, L.; Yan-Jie, S. Variation in the reproduction of some phytophagous chafers and its bearing on pest control. *Acta Entomol. Sin.* **1987**, *30*, 152.
18. Stringer, I.A.N. The female reproductive system of *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae: Melolonthinae). *New Zeal. J. Zool.* **1988**, *15*, 513–533, doi:10.1080/03014223.1988.10422632.
19. Gayon, J. History of the concept of allometry. *Am. Zool.* **2000**, *40*, 748–758, doi:10.1093/icb/40.5.748.
20. Simmons, L.W.; Tomkins, J.L.; Hunt, J. Sperm competition games played by dimorphic male beetles. *Proc. R. Soc. B Biol. Sci.* **1999**, *266*, 145–150, doi:10.1098/rspb.1999.0614.
21. Okada, K.; Miyatake, T. Sexual dimorphism in mandibles and male aggressive behavior in the presence and absence of females in the beetle *Librodor japonicus* (Coleoptera: Nitidulidae). *Ann. Entomol. Soc. Am.* **2004**, *97*, 1342–1346, doi:10.1603/0013-8746(2004)097[1342:SDIMAM]2.0.CO;2.
22. Okada, K.; Miyatake, T.; Nomura, Y.; Kuroda, K. Fighting, dispersing, and sneaking: Body-size dependent mating tactics by male *Librodor japonicus* beetles. *Ecol. Entomol.* **2008**, *33*, 269–275, doi:10.1111/j.1365-2311.2007.00965.x.
23. Cayetano, L.; Maklakov, A.A.; Brooks, R.C.; Bonduriansky, R. Evolution of male and female genitalia following release from sexual selection. *Evolution (N. Y).* **2011**, *65*, 2171–2183, doi:10.1111/j.1558-5646.2011.01309.x.
24. Katsuki, M.; Yokoi, T.; Funakoshi, K.; Oota, N. Enlarged hind legs and sexual behavior with male-male interaction in sagra femorata (Coleoptera: Chrysomelidae). *Entomol. News* **2014**, *124*, 211–220, doi:10.3157/021.124.0306.
25. Grossi, P.C.; Grossi, E. A new species of *Amblyodus Westwood*, 1878 (Coleoptera, Melolonthidae, Dynastinae) from South America. *Zookeys* **2011**, *75*, 21–28, doi:10.3897/zookeys.75.884.
26. Huang, J.P. The hercules beetles (Subgenus Dynastes, genus Dynastes, Dynastidae): a revisionary study based on the integration of molecular, morphological, ecological and geographics analyses. *Misc. Publ. Museum Zool. Univ. Michigan* **2017**, *206*, 1–37.
27. Duarte, P.R.M.; Grossi, P.C. Contribution to the knowledge of Podischnus Burmeister, 1847 (Coleoptera: Melolonthidae: Dynastinae) with the description of two new species from brazilian amazon forest. *Pap. Avulsos Zool.* **2020**, *60*, doi:10.11606/1807-0205/2020.60.special-issue.22.
28. Johnson, J.P. *Cyclocephala* (ochrosidia) *borealis* in connecticut 1. *J. oj Agric. Res.* **1941**, *62*, 79–86.
29. Maia, A.C.D.; Schlindwein, *Caladium Caladium* bicolor (Araceae) and *Cyclocephala celata* (Coleoptera, Dynastinae): a well-established pollination system in the northern Atlantic rainforest of Pernambuco, Brazil. *Plant Biol.* **2006**, *8*, 529–534, doi:10.1055/s-2006-924045.
30. Duchini, P.G.; Echeverria, J.R.; Américo, L.F.; Guzatti, G.C.; Cherman, M.A.; Sbrissia, A.F. White grubs (*Cyclocephala*

flavipennis) damaging perennial winter pastures in the South Region of Brazil. *Ciência Rural* **2017**, *47*, doi:10.1590/0103-8478cr20160662.

31. Parizotto, D.R.; Grossi, P.C. Revisiting pollinating *Cyclocephala* scarab beetles (Coleoptera: Melolonthidae: Dynastinae) associated with the soursop (Annona muricata, Annonaceae). *Neotrop. Entomol.* **2019**, *48*, 415–421, doi:10.1007/s13744-018-0647-y.
32. Aragón, A.; Morón, M.A.; Tapia-Rojas, A.M.; Rojas-García, R. Fauna de Coleoptera Melolonthidae en El Rancho. *Acta zoológica Mex*. **2001**, *164*, 143–164.
33. Morón, M.A.; Lugo-García, G.A.; Aragón-García, A. Description of the third instar larvae of five species of *Cyclocephala* (Coleoptera, Melolonthidae, Dynastinae) from Mexico. *Rev. Bras. Entomol.* **2014**, *58*, 219–228, doi:10.1590/S0085-56262014000300001.
34. Moore, M.R.; Cave, R.D.; Branham, M.A. Annotated catalog and bibliography of the cyclocephaline scarab beetles (Coleoptera, Scarabaeidae, Dynastinae, Cyclocephalini). *Zookeys* **2018**, *2018*, 101–378, doi:10.3897/zookeys.745.23685.
35. Sanchez-Cruz, A.; Robledo, N.; Rosete-Enríquez, M.; Romero-López, A.A. Attraction of adults of *Cyclocephala lunulata* and *Cyclocephala barrerai* (Coleoptera: Scarabaeoidea: Melolonthidae) towards bacteria volatiles isolated from their genital chambers. *Molecules* **2020**, *25*, 1–16, doi:10.3390/molecules25194430.
36. García-Hernández, C.; Rojo-Rubio, R.; Olmedo-Juárez, A.; Zamilpa, A.; Mendoza de Gives, P.; Antonio-Romo, I.A.; Aguilar-Marcelino, L.; Arece-García, J.; Tapia-Maruri, D.; González-Cortazar, M. Galloyl derivatives from *Caesalpinia coriaria* exhibit in vitro ovicidal activity against cattle gastrointestinal parasitic nematodes. *Exp. Parasitol.* **2019**, *200*, 16–23, doi:10.1016/j.exppara.2019.03.012.
37. Delgado-Núñez, E.J.; Zamilpa, A.; González-Cortazar, M.; Olmedo-Juárez, A.; Cardoso-Taketa, A.; Sánchez-Mendoza, E.; Tapia-Maruri, D.; Salinas-Sánchez, D.O.; Mendoza-De Gives, P. Isorhamnetin: A nematocidal flavonoid from prosopis laevigata leaves against Haemonchus contortus eggs and larvae. *Biomolecules* **2020**, *10*, doi:10.3390/biom10050773.
38. Grodowitz, M.J.; Brewer, F.D. Ovarian anatomy and physiological age-grading of the female Boll Weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.* **1987**, *80*, 642–651, doi:10.1093/aesa/80.5.642.
39. Rasband, W.S. ImageJ; US National Institutes of Health: Bethesda, MD, 1997-2009, USA.
40. Stringer, I.A.N. The male reproductive system of *Costelytra zealandica* (White)(Coleoptera: Scarabaeidae: Melolonthinae). *New Zeal. J. Zool.* **1990**, *17*, 323–339, doi:10.1080/03014223.1990.10422939.
41. Brits, J.A. The anatomy, histology and physiology of the internal adult male reproductive-system of *Parastizopus-Armaticeps* Peringuey (Coleoptera, Tenebrionidae). *J. Entomol. Soc. South. Afr.* **1982**, *45*, 239–260.
42. De Los Santos, A.; Montes, C.; Ramirez Diaz, L. Life histories of some darkling beetles (Coleoptera: Tenebrionidae) in two Mediterranean ecosystems in the lower Guadalquivir (southwest Spain). *Environ. Entomol.* **1988**, *17*, 799–814, doi:10.1093/ee/17.5.799.
43. Hodek, I.; Ceryngier, P. Sexual activity in Coccinellidae (Coleoptera): A review. *Eur. J. Entomol.* **2000**, *97*, 449–456, doi:10.14411/eje.2000.069.
44. Carrillo, J.L.; William, G. Repaso de las especies mexicanas del género *Macrodactylus* (Coleoptera, Scarabaeidae), con observaciones biológicas de algunas especies. --. *Foll. técnico No. 39* **1960**, *39*, 102.
45. Wang, Q.; Zeng, W.-Y.; Li, J.-S. Reproductive Behavior of *Paraglenea fortunei* (Coleoptera: Cerambycidae). *Ann. Entomol. Soc. Am.* **1990**, *83*, 860–866, doi:10.1093/aesa/83.4.860.
46. Hoffman, G.D.; Raffa, K.F. Maturation of the Male Pales Weevil (Coleoptera: Curculionidae) reproductive system and its effect on male response to females. *Ann. Entomol. Soc. Am.* **1992**, *85*, 571–577, doi:10.1093/aesa/85.5.571.
47. Wang, Q.; Shi, G.; Davis, L.K. Reproductive potential and daily reproductive rhythms of *Oemona hirta* (Coleoptera: Cerambycidae). *J. Econ. Entomol.* **1998**, *91*, 1360–1365, doi:10.1093/jee/91.6.1360.

48. Breeschoten, T.; Clark, D.R.; Schilthuizen, M. Evolutionary patterns of asymmetric genitalia in the beetle tribe cyclocephalini (Coleoptera: Scarabaeidae: Dynastinae). *Contrib. to Zool.* **2013**, *82*, 95–106, doi:10.1163/18759866-08202003.
49. Kim, J.Y.; Leal, W.S. Ultrastructure of pheromone-detecting sensillum placodeum of the Japanese beetle, *Popillia japonica* Newmann (Coleoptera: Scarabaeidae). *Arthropod Struct. Dev.* **2000**, *29*, 121–128, doi:10.1016/S1467-8039(00)00022-0.
50. Romero-López, A.; Morón, M.; Valdez, J. Sexual dimorphism in antennal receptors of *Phyllophaga ravida* blanchard (Coleoptera: Scarabaeoidea: Melolonthidae). *Neotrop. Entomol.* **2010**, *39*, 957–966, doi:10.1590/S1519-566X2010000600018.
51. Bal, N.; Özkdikmen, H. Aedeagus structure of Cassida prasina Illiger, 1798 (Coleoptera: Chrysomelidae: Cassidinae) in Scanning Electrone Microscope (SEM) [Taramalı Elektron Mikroskobunda (SEM) Cassida prasina Illiger, 1798 (Coleoptera: Chrysomelidae: Cassidinae) 'nın Aedeagus Y. *J. Agric. Nat.* **2020**, *23 (3)*, 748–753, doi:https://doi.org/10.18016/ksutarimdoga.vi.630787.
52. Özdikmen, Ü.; Bal, N.; Amutkan Mutlu, D.; Suludere, Z. A SEM study of the aedeagus and spermatheca of *Cassida viridis* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) from Turkey. *Turkiye Entomoloji Derg.* **2020**, *44*, 355–364, doi:10.16970/ENTOTED.612447.
53. Özyurt Koçakoğlu, N.; Candan, S.; Güllü, M. Anatomy and histology of reproductive system of adult male mint leaf beetle *Chrysolina herbacea* (Duftschmid, 1825) (Coleoptera: Chrysomelidae). *Microsc. Res. Tech.* **2021**, *84*, 512–520, doi:10.1002/jemt.23607.
54. Acebes, A.; Cobb, M.; Ferveur, J.F. Species-specific effects of single sensillum ablation on mating position in *Drosophila*. *J. Exp. Biol.* **2003**, *206*, 3095–3100, doi:10.1242/jeb.00522.
55. Schubert, L.F.; Krüger, S.; Moritz, G.B.; Schubert, V. Male reproductive system and spermatogenesis of *Limodromus assimilis* (Paykull 1790). *PLoS One* **2017**, *12*, 1–17, doi:10.1371/journal.pone.0180492.
56. Pascini, T. V.; Martins, G.F. The insect spermatheca: an overview. *Zoology* **2017**, *121*, 56–71, doi:10.1016/j.zool.2016.12.001.
57. Eberhard, W.G. Rapid divergent evolution of sexual morphology: comparative tests of antagonistic coevolution and traditional female choise. *Evolution (N. Y).* **2004**, *58*, 1947–1970, doi:10.1554/04-143.
58. Shapiro, A. The Lock-And-Key Hypothesis: Evolutionary and biosystematic interpretation of insect genitalia. *Annu. Rev. Entomol.* **1989**, *34*, 231–245, doi:10.1146/annurev.ento.34.1.231.
59. Fernadez, M.; Salgado, J.; Pajares, J. The seasonal development of the gonads and fat content of *Tomicus minor* (Coleoptera Scolytidae). *Belgian J. Entomol.* **1999**, *1*, 311–324.
60. Sasakawa, K. Diet affects male gonad maturation, female fecundity, and larval development in the granivorous ground beetle *Anisodactylus punctatipennis*. *Ecol. Entomol.* **2009**, *34*, 406–411, doi:10.1111/j.1365-2311.2009.01092.x.
61. Ghoneim, K.K.; Abdel-Khaliq, A.A.; Bream, A.S.; Emam, D.M. Effects of food type on the adult performace of black blister beetle *Meloe proscaeabaeus* (Coleoptera: Meloidae). *Int. J. Biol. Biol. Sci.* **2012**, *1*, 5–17.
62. Zhou, P.; Yang, H.; Jin, D.C.; He, X.Z.; Wang, Q. Sex-Specific Allometry of morphometric and reproductive traits in oriental fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.* **2016**, *109*, 1249–1253, doi:10.1093/jee/tow022.
63. Carne, P.B. *Cyclocephala signaticollis* Burmeister, an introduced pasture scarab (Coleoptera). *Proc. Linn. Soc. New South Wales* **1957**, *81*, 217–221.
64. Stern, D.L.; Emlen, D.J. The developmental basis for allometry in insects. *Development* **1999**, *126*, 1091–1101.
65. Vera-Cano, D.A.; Álvarez, H.A.; Morón, M.A. Positive allometry of horns in the rhinoceros beetle *Golofa xiximeca* does not follow breaking-point patterns. *Southwest. Entomol.* **2017**, *42*, 933–940, doi:10.3958/059.042.0412.
66. Moczek, A.P. Pupal remodeling and the evolution and development of alternative male morphologies in horned beetles. *BMC Evol. Biol.* **2006**, *7*, 711–729, doi:10.1186/1471-2148-7-151.
67. Potter, D.A. Flight Activity and sex attraction of northern and southern masked chafers1 in Kentucky Turfgrass2. *Ann. Entomol. Soc. Am.* **1980**, *73*, 414–417, doi:10.1093/aesa/73.4.414.

68. Barbosa, C.A.F.; Rodrigues, S.R. Comportamento de cópula em *Cyclocephala verticalis* Burmeister (Coleoptera: Scarabaeidae). *EntomoBrasilis* **2016**, *9*, 158–162, doi:10.12741/ebrasilis.v9i3.581.
69. Rodrigues, S.R.; Barbosa, C.A.F.; Fuhrmann, J.; Amaro, R.A. Mating behavior and description of immature stages of *Cyclocephala melanocephala* (Fabricius, 1775) (Coleoptera: Scarabaeidae: Dynastinae), identification key and remarks on known immatures of Cyclocephalini species. *Rev. Bras. Entomol.* **2018**, *62*, 205–219, doi:10.1016/j.rbe.2018.07.001.
70. Saldanha, F.G.; Rodrigues, S.R.; Amaro, R.A.; Fuhrmann, J. Description of mating behavior, life cycle, and antennal sensilla of *Cyclocephala putrida* burmeister, 1847 (Coleoptera, Scarabaeidae, Dynastinae). *Biota Neotrop.* **2020**, *20*, 1–8, doi:10.1590/1676-0611-BN-2020-0973.
71. Knell, R.J.; Pomfret, J.C.; Tomkins, J.L. The limits of elaboration: Curved allometries reveal the constraints on mandible size in stag beetles. *Proc. R. Soc. B Biol. Sci.* **2004**, *271*, 523–528, doi:10.1098/rspb.2003.2641.