# DESCRIPTION OF THE FEMALE GENITAL TRACT OF *Macroperipatus* aff. *geayi* (ONYCHOPHORA, PERIPATIDAE).

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## RESUMEN

**TITULO:** DESCRIPCIÓN DEL APARATO REPRODUCTOR FEMENINO DE *Macroperipatus* aff. *GEAYI* (ONYCHOPHORA, PERIPATIDAE)*

**AUTOR:** OSCAR YESID HERNÁNDEZ LAGOS**

**PALABRAS CLAVES:** ONYCHOPHORA, PERIPATIDAE, VIVIPARIEDAD, SUPERFETACIÓN.

**DESCRIPCIÓN:**

Los onicóforos o gusanos aterciopelados son macroinvertebrados, los cuales habitan bosques húmedos templados o tropicales. Actualmente son reconocidas 205 especies agrupadas en dos familias, Peripatidae y Peripatopsidae. La familia Peripatidae se distribuye principalmente en el Neotrópico, donde las especies son estrictamente vivíparas y las hembras presentan diferentes generaciones o cohortes en el interior de su útero a modo de 'collar de cuentas'. A pesar de que estas características particulares asociadas con la viviparidad no son comunes entre macroinvertebrados, solo unas pocas especies de esta familia han sido estudiadas. Se analizó el aparato reproductor femenino en distintos estadios de actividad reproductiva de 32 especímenes, adicionalmente se midieron 83 embriones para evaluar el incremento en longitud y área en los diferentes estadios de desarrollo. El tracto genial de *Macroperipatus* aff. g*eayi* está conformado por un ovario y órganos pareados como embudos ováricos, receptáculos seminíferos y úteros; este último se fusiona en la parte más distal del cuerpo para formar la vagina que conecta con el exterior a través del poro genital. Esta organización en el sistema genital femenino sigue el patrón descrito para la familia Peripatidae. Hembras juveniles presentan el útero completamente vacío, que ocupa $^{1}/_{5}$ de la cavidad corporal; el estadio de madura joven presenta un útero que ocupa ½ de la cavidad corporal, el útero ascendente puede tener hasta 5 cámaras embrionarias y su útero descendente se encuentra vacío. Finalmente, hembras maduras viejas exhiben un útero que ocupa $^{2}/_{3}$ de la cavidad corporal, ocupado por cámaras embrionarias en la primera parte y fetos en diferentes estadio de pigmentación en la segunda. Encontramos que el índice de superfetación para hembras maduras viejas de *M.* aff. g*eayi* es de 12-16 cohortes; adicionalmente, los embriones exhiben un incremento de 100 veces en tamaño desde el estadio de blástula hasta feto pigmentado.


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* Bachelor Thesis

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## ABSTRACT

**TITLE:** DESCRIPTION OF THE FEMALE GENITAL TRACT OF *Macroperipatus* aff. *geayi* (ONYCHOPHORA, PERIPATIDAE)*

**AUTHOR:** OSCAR YESID HERNÁNDEZ LAGOS**

**KEYWORDS**: genital tract, superfetation, superfecundity, embryo development, Peripatidae, maternal investment.

**DESCRIPTION:**

The onychophorans display remarkable reproductive strategies among their two major subgroups, Peripatidae and Peripatopsidae. Neotropical species of Peripatidae shows a viviparous mode of reproduction and also a particular arrangement of a number of generations developing simultaneously along the uterus. There is extensive data on a limited number of species about the reproductive organs along postnatal reproductive stages. In this study the female genital tract of *Macroperipatus* aff. *geayi* is described in a series of reproductive stages with an examination of the embryonic development within the reproductive tract; also, viviparity, superfecundity, and superfetation are discussed in the context of maternal care. The genital tract of *M.* aff. *geayi* consists of an ovary and paired organs like: ovarian funnels, seminal receptacles and the uterus; the latter organ fuses in the posterior region of the body to form the vagina, which opens to the exterior by the genital opening. The female genital system of *M*. aff. *geayi* shows characteristics similar to those described from other species of Peripatidae. The uterus in juvenile females is short and empty; later, embryonic chambers appear in the ascendant uterus of young and old mature females, while the latter additionally have developing fetuses in the descendant uterus. The embryonic development has two noticeable events, the first is characterized by segment formation, these embryos remain attached via a dorsal stalk to the chamber until the segment formation has ended, then the embryos are freely located in the chamber; the second event is characterize by pigmentation stages in the fetuses. In general, we found a superfetation index of 12-16 broods in large old mature females of *M*. aff. *geayi,* and a 100-fold increment in offspring area and length from blastula or early gastrula to the most developed fetus; these features suggest an important maternal investment in reproduction by this species.


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## INTRODUCTION

Onychophorans are soft-bodied, terrestrial invertebrates closely related to Arthropods (Ghiselin, 1984; Leishman & Eldridge, 1990; Wright, 2012). They comprise 205 species grouped into two families: Peripatopsidae, with 121 austral species, and Peripatidae with 84 tropical species (Oliveira et al., 2012). In spite of the vast diversity of taxa, there are few works regarding important aspects of the onychophoran biology like reproduction and morphology as well as the phylogenetic relationships inside each family.

Both onychophoran families exhibit similar external morphology; nonetheless, they might differ in some aspects of their reproductive biology, such as the reproduction mode, the organization of the genital system, position of the genital opening and, the ovarian type. The Neotropical species of Peripatidae present a viviparous mode of reproduction and the genital pore is positioned in the penultimate pair of legs. Within the ovary, the developing oocytes show an endogenous arrangement, i.e., they are found below the surface of the ovary and show little yolk formation (Bouvier, 1905; Clark, 1915; Anderson, 1966, 1973; Ruhberg, 1985; Storch & Ruhberg, 1993; Reid, 1996; Mayer, 2007; Mayer & Tait, 2009).

The anatomy of the female genital system of Peripatidae has been described in few species *Macroperipatus torquatus, Epiperipatus edwardsii, E. biolleyi*, *Plicatoperipatus jamaicensis* and *E. acacioi*. In general, the reproductive tract consists of a pair of ovaries, oviducts, ovarian funnels, seminal receptacles, and uterus (Von Kennel, 1883, 1885; Huebner & Lococo, 1994; Campiglia, 1995; Walker, 1998; Brockmann, 1999). The latter organ is of particular interest in viviparous species of onychophorans because it is closely associated with the growing embryos, which appear in different stages of development according to their position along the uterus. The portion of the uterus nearest to the ovary holds early embryos in cellular division stages like blastula or gastrula, followed by

embryos undergoing segment formation. Finally, the distal region of the uterus contains fetuses in pigmentation stages which lie freely. Several stages of development have been described, which are based on the external characteristics of the embryos: blastula, gastrula, stalked vesicle, coiled segmented, semi-coiled, flexed embryo, straight unpigmented and pigmented fetus. Embryos are contained within embryonic chambers while fetuses lie freely in the lumen of the uterus (Anderson, 1972; Anderson & Manton, 1973; Walker & Campiglia, 1988, 1990; Campiglia & Walker, 1995).

This study provides an account of the general morphology of the female genital tract in different reproductive stages in the Neotropical viviparous species *Macroperipatus* aff. *geayi* and compares it with other oviparous and viviparous species from both onychophoran families described in the literature. Also, we calculated an index for the number of embryos (i.e., broods) that a mature female can carry inside its uterus simultaneously, and their increase in area and length among the development stages. This information can shed light about the sequence of changes in the organs that comprise the female genital system, the development of the embryos inside the uterus and the implications of carrying multiple broods for maternal investment.

## 1. MATERIAL AND METHODS

We will refer to the onychophoran population from the study site as *Macroperipatus* aff. *geayi* given the uncertainty over its taxonomic determination. In Colombia this taxon was first reported from Sierra Nevada de Santa Marta, Magdalena Department (Clark, 1946; Peck, 1975) and then reported from Los Santos municipality, Santander Department (Jerez-Jaimes & Bernal-Pérez, 2009). Since a holotype has not been designated in the original species description and since the putative sintype deposited in the Muséum National d'Histoire Naturelle de Paris, France lacks precise collecting data, this species cannot be formally revised and has been regarded as a *nomen dubium* (Oliveira et al., 2012).

For this study we used 32 females and 2 male specimens of *Macroperipatus* aff. *geayi*. Females were grouped into the following reproductive activity stages: 5 virgin juveniles, 9 young-mature and 18 old-mature females. All the specimens were collected in 2013 from La Hacienda el Roble, an organic coffee shade plantation in the municipality of Los Santos, on the western slopes of the Cordillera Oriental of the Colombian Andes (Department of Santander, Colombia, 06°54' N, 73°03' W; 1640 m.a.s.l.; Fig. 1a). The specimens were found under dead plantain trees, rotten logs, and leaf litter. The animals were transported alive to Universidad Industrial de Santander and maintained in a terrarium inside a wine cooler at 16°C until required. We measured the females with a digital caliper (±0.02mm), from the head to the anus, and took the weight with a Precisa ® digital scale (±0.001 g).

The specimens were observed and photographed under a stereomicroscope to check for characters of sexual dimorphism. Then, the individuals were euthanized by immersion in a lethal dose of 2% liquid Lidocaine. The females were dissected ventrally from the mouth to the genital pore using microsurgical scissors and then observed under a Nikon stereomicroscope (Nikon® SMZ-1000) to visualize the internal organization and features of the reproductive organs in the different

reproductive activity stages. Afterwards, the genital system was removed from the body cavity of the dissected specimens. The descriptions of the genital tract of *M.* aff. *geayi* were compared with previous descriptions for the members of the family Peripatidae; the anatomical description of the genital tract followed the nomenclature of Campiglia & Walker (1995). Photographs were taken using a Nikon stereomicroscope equipped with a Canon EOS Rebel XS® digital camera. Some photographs were used to draw diagrams of the female genital tract. After the dissection, the material was fixed in 10% buffered formalin or Bouin's fixative and then preserved in 70% ethanol. Individuals were finally placed in the Museo de Historia Natural, Universidad Industrial de Santander.

We established and described three reproductive activity stages according to the characteristics of each female uterus and body size. Juvenile virgin females are similar in size to the late unborn pigmented fetuses but without embryonic chambers in their uteri (Fig. 2a and 3a). Young adult females have up to 8 uterine chambers in their ascendant uteri and the descendant uterus is empty (Fig. 2a and 3b). Finally, old adult females have up to 8 chambers in the ascendant uteri and at least 4 fetuses in the descendant uteri (Fig. 3c and 4).

The embryos were gently removed from their embryonic chambers, and then put into a concentrated solution of Lugol staining to facilitate the observation under the stereomicroscope. The development stages were established following Anderson & Manton (1973) and Walker & Campiglia (1988, 1995). We calculated the area and length of the growing embryos and fetuses inside each uterus, obtained from a total of 14 females, using the software ImageJ. The resulting data was used to describe the increment in size of the embryos, from one stage to the following.

## 2. RESULTS

Females of *Macroperipatus* aff. *geayi* are easily distinguished from males because the latter show particular accessory organs such as crural glands (cr), and anal glands openings (ag) on the posterior region of the body. The crural tubercles are present in the genital leg pairs, whereas the paired anal glands openings are located nearing the anterior border of the anus and show a reddish coloration in freshly prepared material (Fig 1 c, d).

The female genital tract in *Macroperipatus* aff. *geayi* consists of a single tubular ovary connected to paired successive organs: oviduct, ovarian funnel, seminal receptacle, and uterus coupled to a single vagina (Fig. 2). The ovary lies dorsal to the gut in the haemocoel and is loosely attached to the dorsal portion of the body by a long, white, and smooth ligament, which is bifurcated at the distal portion of the ovary. The fresh ovary is white-translucent but turns white-yellowish in formalin-fixed specimens. The ovarian funnel is wide at the base and narrow at its distal region, and the seminal receptacle has a spherical form.

The white-translucent oviduct is connected anteriorly to the seminal receptacle and posteriorly to the uterus; the latter lies freely in the body cavity and can be situated either ventrolaterally or ventrally in the body. The first region of the uterus (ascendant uterus) runs anteriorly and bears embryonic chambers with early developing embryos, while distally, towards the mid-body region, the uterus bends posteriorly to join the vagina; this latter portion of the uterus (descendant uterus) contains fetuses in pigmentation stages. The vagina is found ventral to the digestive system and connects the reproductive tract with the outside through the genital pore, which is found at the level of the penultimate pair of legs, anterior to the anus (Fig. 3c,d).

## 2.1 CHANGES IN THE GENITAL SYSTEM WITHIN REPRODUCTIVE STAGES.

**2.1.1 Juvenile females** At this stage, females of *Macroperipatus* aff. *geayi* range from 20-22 mm (SD=0,454) long from the head to the anus; this length is similar to late fetuses found nearest to the vagina in mature females. The surface of the ovary exhibits a coloration pattern consisting of several yellowish dots over a whitish background as seen in formalin-fixed specimens. The uterus is about 7 mm and reaches only a fifth part of the body length; at this stage the uterus looks straight, it is not yet divided into an ascendant and descendant uterus; however, the uterus is not uniform, there is a clear difference between the anterior portion and the wider posterior region. The uterus does not contain embryonic chambers at this stage (Fig. 4a).

**2.1.2 Young adult females** These females are approximately 40-50 mm (SD= 7,17) long from the head to the anus. In this stage specimens are characterized by the presence of 3-4 embryonic chambers in each ascendant uterus. This portion of the uterus reaches half the body length and bends backwards to join the vagina. There is an equal number and position of embryonic chambers in each uterus (Fig. 3b).

**2.1.3 Older adult females** These adult females ranged from 50-85 mm (SD=5,49) long from the head to the anus. Each uterus reaches 2/3 of the body length; it shows approximately 8-9 embryonic chambers in each ascendant uterus as well as one unpigmented and two pigmented fetuses in each descendant uterus. The chambers are translucent and the embryo can be observed inside. Fetuses are not contained within embryonic chambers. Instead, they are found freely within the lumen of the uterus; the descendant uterus shows irregular thickness rings in the head region of the fetuses. The fetuses contact each other with the upper or lower fetuses showing that they are freely positioned in the uterine lumen (Fig. 3c,d).

## 2.2 STAGES OF EMBRYO DEVELOPMENT

The uterus of old adult females of *Macroperipatus* aff. *geayi* can bear up to 10 embryonic chambers and three generations of fetuses in each uterus. The embryos look light-red when fresh and purple when fixed in formalin. Each chamber contained an embryo in a different developmental stage.

The first two embryonic chambers show an external pink coloration bearing constriction rings in the middle region of the chamber. These chambers seemed empty under the stereo microscope, probably because embryos were still very small, or in early cellular development (Fig. 4a,b). The next chamber appears pale-pink and contains a visible vesicle, attached to the inner wall of the uterus by a stalk (Fig. 4d, 5c); also, the constriction rings are no longer in the middle region of the chamber, instead they are found at both ends.

The adjacent chamber shows an embryo starting segment formation with a prominent cephalic region corresponding to a pair of preoral segment and around 4-5 segment buds. The first and second segment buds correspond, respectively, to the forming jaws and oral papillae (slime glands), while the last three segments correspond to the developing anterior limbs. In the rest of the body segmentation folds are noticeable. These embryos appear in a coiled position and remain attached to the uterus by a short stalk, located in the upper back of the embryo (Fig. 4e, 5e).

The following chamber contains an embryo exhibiting 7-9 paddle shaped limb buds, which are more developed anteriorly than posteriorly. At this stage the stalk is only visible as a small remnant found dorsally behind the preoral segments, whereas they are no longer visible in the embryonic chamber or in the back of the embryo.

In the next chamber the embryo shows twenty-five pairs of legs and, at the distal region next to the anus, two pairs of paddle-shaped limbs that are still developing. The formation of limbs exhibit different shapes under their development, ranging from fold lines segments, limb buds, paddle-shaped limbs to the adult conical limbs (Supporting Information). Dorsally, in the cephalic region the antennae begin to differentiate behind the preoral segments and appear in an ellipsoid form. Also, the oral papillae, the antennae, and the first 3 pairs of limbs, nearest to the cephalic region, exhibit a pattern of annuli ornaments on their surface.

The following embryo exhibits a longer body and all the legs are formed. These embryos adopt a flexed posture; the only region that remains coiled is the posterior end. The oral papillae show an opening to the outside. The embryos remain attached to the chamber, until the segment formation has ended; then, the embryo breaks its embryonic sac up, and it is freely located in the chamber.

The first fetus can remain slightly flexed or in a straight posture, depending on whether the fetus is located in the bent portion of the ascendant uterus or if it has already passed it. This fetus is translucent, allowing observing the gonads through the skin; therefore it is possible to determine the gender of fetuses at this stage of development. Some of these fetuses show their heads pointing towards the ovary or the vagina of the mother; externally, the fetus has completed its development and all the legs bear a pair of claws (Fig. 5i). The second fetus exhibits the adult pigmentation except in the posterior region, which shows lighter pigmentation. The last fetus, which is nearest to the vagina, shows the complete adult pigmentation (Fig. 5j).

### 2.3 SUPERFETATION INDEX AND EMBRYO GROWTH

Superfetation is a condition in which females of some viviparous species carry a variable number of embryos in different development stages, growing

simultaneously inside the uterus (Zuñiga-Vega et al., 2007; Pollux et al., 2009). We found that this condition is exhibited by females of *Macroperipatus* aff. *geayi* because of the large number of offspring that can be found inside the uteri of a single female, each representing a different development stage and thus a distinct brood, ranging from early cellular division stages and segment-forming embryos, to early and late-developing fetuses. We calculated the superfetation index as the mean number of broods for a total of 18 old-mature females of *M.* aff. *geayi*. Our results showed that these females can hold an average of 14 cohorts at the same time (min. 12 and max. 18, see table 1).

We measured the area and length of 83 embryos of *Macroperipatus* aff. *geayi* in five distinct development stages: stalked vesicle, coiled and flexed embryos, unpigmented and pigmented fetuses. The average length and area for each stage was calculated and registered in table 2. Overall, there was a noticeable increment in these variables from one stage to the next. The values of area and length ranged, respectively, from 0.30 to 30.198 $mm^2$, and 0.19 to 20.198 mm, from the stalked vesicle to the pigmented fetus. In total, the embryos of *M*. aff. *geayi* experimented a 100-fold increment in size and length along their development. This increment was particularly notable in the transition from stalked vesicle to the coiled stage in length, and from coiled to flexed stage in area.

## 3. DISCUSSION

Our results show that females of *Macroperipatus* aff. *geayi* present the same organ arrangement previously described for other Peripatidae species like *Macroperipatus torquatus, Epiperipatus edwardsii, E. biolleyi*, *Plicatoperipatus jamaicensis* and *E. acacioi*; this includes the presence of paired reproductive organs (i.e., endogenous ovary, oviduct, ovarian funnel, seminal receptacle and uterus) and the formation of a single vagina by fusion of the posterior ends of both uteri. This condition differs in some respects from Peripatopsidae species, which can show either an exogenous or pseudoendogenous ovary as well as accessory pouches (Huebner et al., 1994; Campiglia, 1995; Walker & Campiglia, 1998; Brockmann et al., 2001; Brockmann et al., 1999; Walker et al., 2006; Mayer, 2007; Mayer & Tait, 2009).

The uterus of *Macroperipatus* aff. *geayi* varies in size along the different reproductive activity stages, starting with a short and smooth uterus in the juvenile stage; in the following young adult stage, this organ is approximately half the length of the body. Finally, old mature females present a disproportionately large uterus when compared to the other reproductive organs and the body length; this is a consequence of the large size and number of embryos and fetuses present inside the uterus. Both uteri are bent 180° to be able to fit within the body cavity. Our results indicate that *M.* aff. *geayi* contains approximately 4 or 5 embryonic chambers along the ascendant uterus in young and old mature females; however, the latter additionally displays both unpigmented and pigmented fetuses in the descendant uterus. This arrangement of the developing offspring and their position along the uterus has been well-described in other viviparous species of Peripatidae (Anderson & Manton, 1972; Campiglia & Walker, 1988, 1990, 1995, Brockmann, 1999).

Viviparous species of Peripatidae are known to exhibit a close relationship between their uterus and the developing offspring. In this sense, the uterus can be regarded as a complex structure that shows several morphological features associated with the developmental stages of the embryos present throughout its length. These characteristics comprise changes in coloration patterns, variation in the epithelium structure and composition, the presence of big vacuolated cells throughout the entire length of the uterus and the formation of placentation zones during the first cellular and segment-forming stages (Anderson & Manton, 1972; Campiglia & Walker, 1995; Brockmann et al., 1999). These characteristics can be used to identify the different stages of the developing offspring. In *M*. aff. *geayi* the developmental stages are divided in three categories; the first comprise early cellular division stages, which ultimately form a vesicle that is attached to the inner uterine wall by means of a hollow stalk of embryonic origin (Campiglia & Walker, 1995); this region of the uterus is characterized by having a red pigmentation and embryonic chambers in the form of swellings (Fig. 4c,d and 5d).

Further development is characterized by embryos starting segment formation; segments are formed following an anterior-posterior pattern. In this region, the uterus appears horizontally and vertically distended showing a light-pink coloration on its surface (Fig. 4e,f and 5f, h). The last category includes unpigmented and pigmented fetuses that have completed segment formation and resemble the adult form; this part of the uterus is fully distended, and exhibits a very thin and striated wall; also, the uterus exhibit some rings that are consider as a vestiges of previous placentation zones (Anderson & Manton, 1972) (Fig. 4g,h and 5i,j).

Females of Peripatidae species are known to store sperm for long periods of time within their seminal receptacles, as evidenced in females of *Epiperipatus acacioi* (Campiglia & Walker, 1995; Walker & Campiglia, 1998). Furthermore, given the mechanism of sperm delivery in Peripatidae, which implies spermatozoa passing through the entire uterine lumen, it is impossible for a gravid female to copulate

with multiple partners (Havel et al., 1989; Campiglia & Walker, 1995). For this reason, it has been proposed that the mating event occurs only once during the juvenile stage (Havel et al., 1989). In this sense, superfetation in *M*. aff. *geayi* might be a consequence of sperm storage, because females are able to release fertilized eggs to the uterus in different periods of time and therefore, it is possible to find embryos in distinct development stages growing simultaneously within the uteri of a single female.

The concept of superfetation has been used principally for some species of fish and mammals (Yamaguchi et al., 2006; Zuñiga-Vega et al., 2007; Pollux et al., 2009); however, this phenomenon is poorly studied among invertebrate taxa. Superfetation is widespread among Neotropical species of onychophorans; in spite of this, only a few authors have discussed about this topic and its implications for maternal effort (Havel et al., 1989). Our calculated superfetation index for *M*. aff. *geayi* was of 14 broods (SD = 1,893), this finding is similar to that reported for other members of Peripatidae, which exhibit a superfetation index that ranges from 8-26 broods per female, in contrast with some oviparous species of Peripatopsidae that are known to retain up to 100 eggs inside the uterus until their development is complete (see table 1). Additionally, the rates of birth in Peripatidae range from 8-20 juveniles per year, this vary according to the species, but there is always one newborn per parturition event (Havel et al., 1989; Campiglia & Walker, 1995). Some Peripatopsidae species deposit batches of 15-45 eggs that hatch a few days later, but because most of these eggs are found in early development stages, they are usually aborted (Brosius-Roggenbuck & Ruhberg, 2000).

Viviparity as a reproductive strategy is costly and involves significant demands of energy and space to maintain the growing embryos (Trexler et al., 2003; Timothy et al., 2008; Bleu et al., 2013). In the case of viviparous onychophorans, the resources needed to nourish the embryos during gestation come directly from the mother, and the costs of reproduction are high as a consequence of superfetation

and females giving birth throughout the year (Walker & Campiglia, 1988; Havel et al., 1989 Campiglia & Walker, 1995). In addition, embryos in different development stages may have specific energy requirements, resulting in resources being allocated proportionally to the stage of development; therefore, old mature females of *Macroperipatus* aff. *geayi* carrying up to six fetuses in their uteri may exhibit higher energetic costs compared to young mature females, which only hold early stage embryos. As a means to reduce such energy requirements, viviparous onychophorans give birth to only one juvenile at a time (Havel et al., 1989; Campiglia & Walker, 1995); we think that this process should be regulated probably in response to physiological triggers rather than environmental stimuli; in this way, the presence of a fetus in an advanced development may induce the remaining embryos to enter in a stage of diapause or arrested development, allowing fetuses to complete their development before successive embryos can attain this stage. This phenomenon of physiological dormancy has been previously described for oviparous onychophorans and is relatively common among invertebrate and vertebrate species (Monge-Nájera, 1994; Gilbert, 2010).

The reproductive strategies displayed by *Macroperipatus* aff. *geayi* and other Neotropical Peripatidae include: viviparity, long-term sperm storage, superfetation, matrotrophy, and postpartum maternal care (Morera et al., 1988; Havel et al., 1989; Campiglia & Walker, 1995; pers. obs.). Additionally, some authors consider Peripatidae species to have a limited fecundity because of the long gestation periods and the low annual birth rates (Havel et al., 1989); nevertheless, the fact that females can fertilize its own eggs throughout many years from a unique mating event can be considered as superfecundity (Havel et al., 1989). All the factors above mentioned can be interpreted in terms of maternal investment as each of them have positive implications for maximizing offspring survival.

## 4. CONCLUSIONS

The genital tract of *Macroperipatus* aff. *geayi* follows the same arrangement of the organs in the genital system that was previously described for Neotropical species of the family Peripatidae. Additionally the genital tract exhibits changes among the different reproductive activity stages; principally in the uterus, which presents a disproportional change in the size from young females to old mature females. This organ presents is arrange into specialized regions for embryo and fetuses development.

The reproductive strategies used in *M.* aff. *geayi* includes: superfetation, matrotrophy and take care of newborns; all this strategies are related to parental investment, which has a positive effect in the offspring survivorship.

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## TABLES

**Table 1**. Superfetation index, n = females reviewed. * Unknown species of Peripatidae captured near to the collecting site.

| Species | Superfetation index (n) | Reference |
|---|---|---|
| **Peripatidae** | | |
| *Macroperipatus* aff. *geayi*. | 14 (18) | Present study |
| *Peripatidae sp1 | 26 (1) | Personal observation |
| *Peripatus* sp | 8-10 (3) | Sclater, 1988 |
| *Epiperipatus acacioi* | 16 (40) | Campiglia & Walker, 1988, 1990, 1995 |
| *Epiperipatus trinidadensis* | 10 (4) | Anderson & Manton, 1972 |
| *Macroperipatus torquatus* | 16 (4) | Anderson & Manton, 1972 |
| *Plicatoperipatus jamaicensis* | 16 (-) | Huebner & Lococo, 1994 |
| **Peripatopsidae** | | Brosius-Roggenbuck & Ruhberg, 2000 |
| *Austroperipatus eridelo* | 100 | |
| *Peripatoides novaezealandiae* | 10 | Brosius-Roggenbuck & Ruhberg, 2000 |

**Table 2**. Average length and area along of the embryos along the different developmental stages. n= number of embryos reviewed.

| Developmental stage (n) | length (mm) | area ($mm^2$) |
|---|---|---|
| stalked vesicle (8) | 0,1938 | 0,3162 |
| coiled (19) | 2,971 | 0,648 |
| flexed (10) | 5,679 | 2,073 |
| unpigmented fetus (18) | 10,528 | 12,085 |
| pigmented fetus (28) | 20,898 | 30,125 |

**FIGURES**

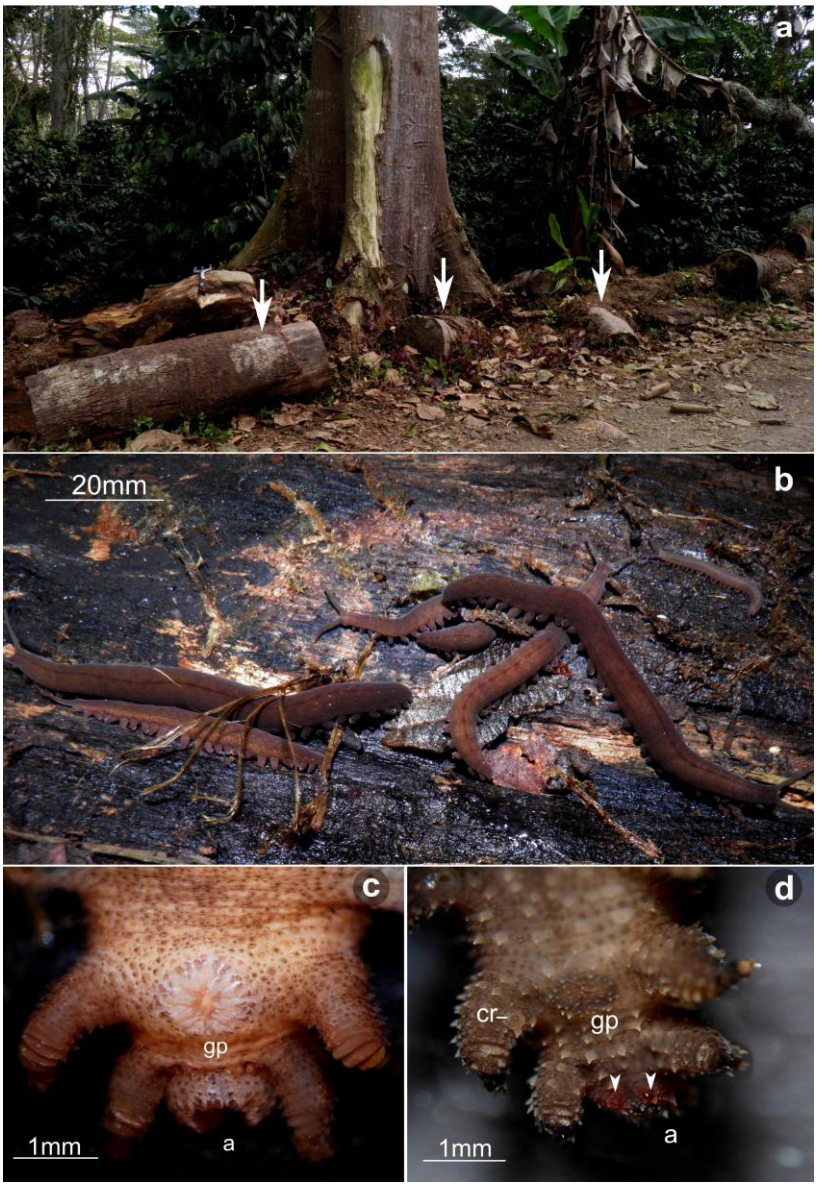
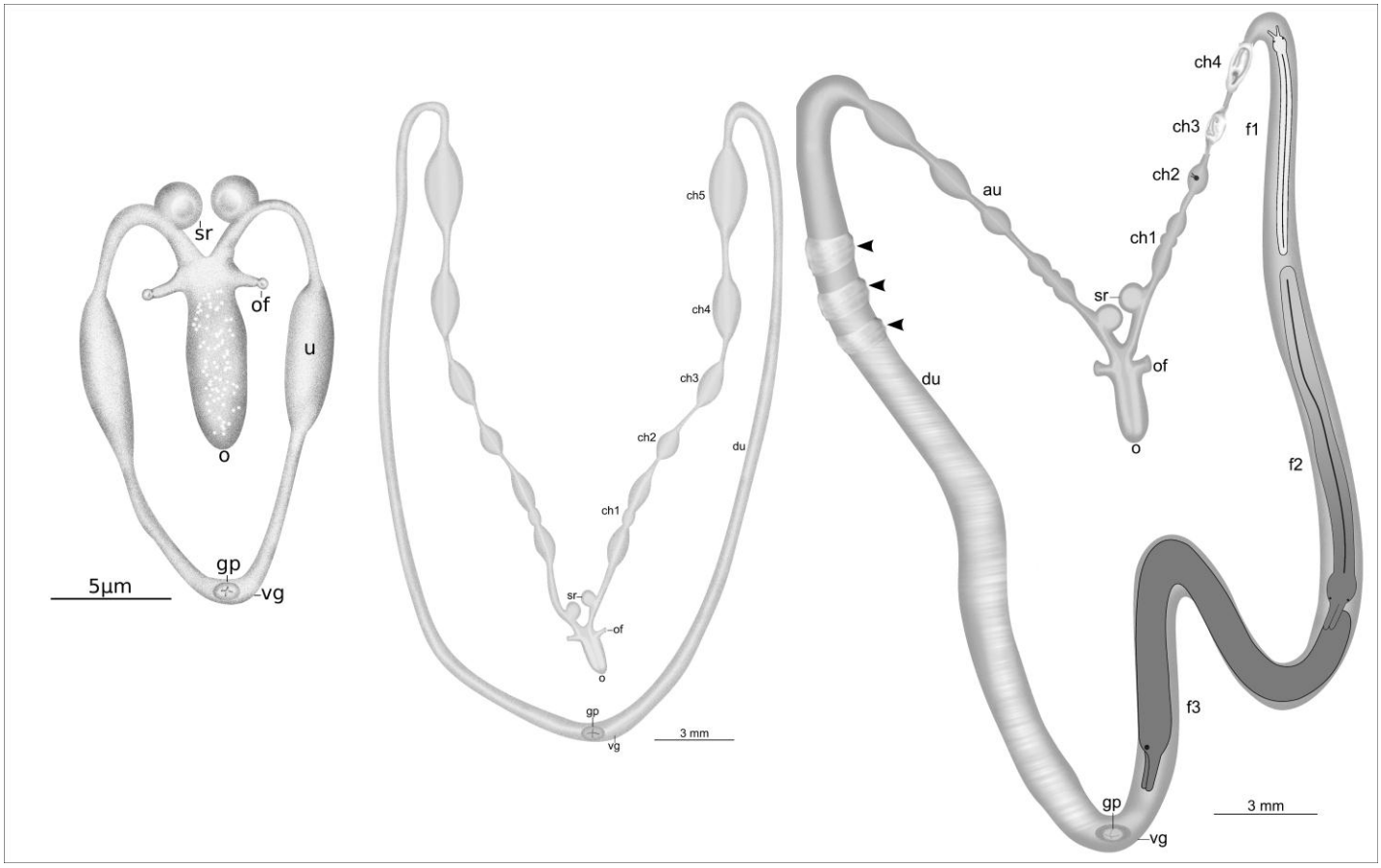



***Figure 1.*** *Microhabitat and life coloration of* Macroperipatus *aff.* geayi.

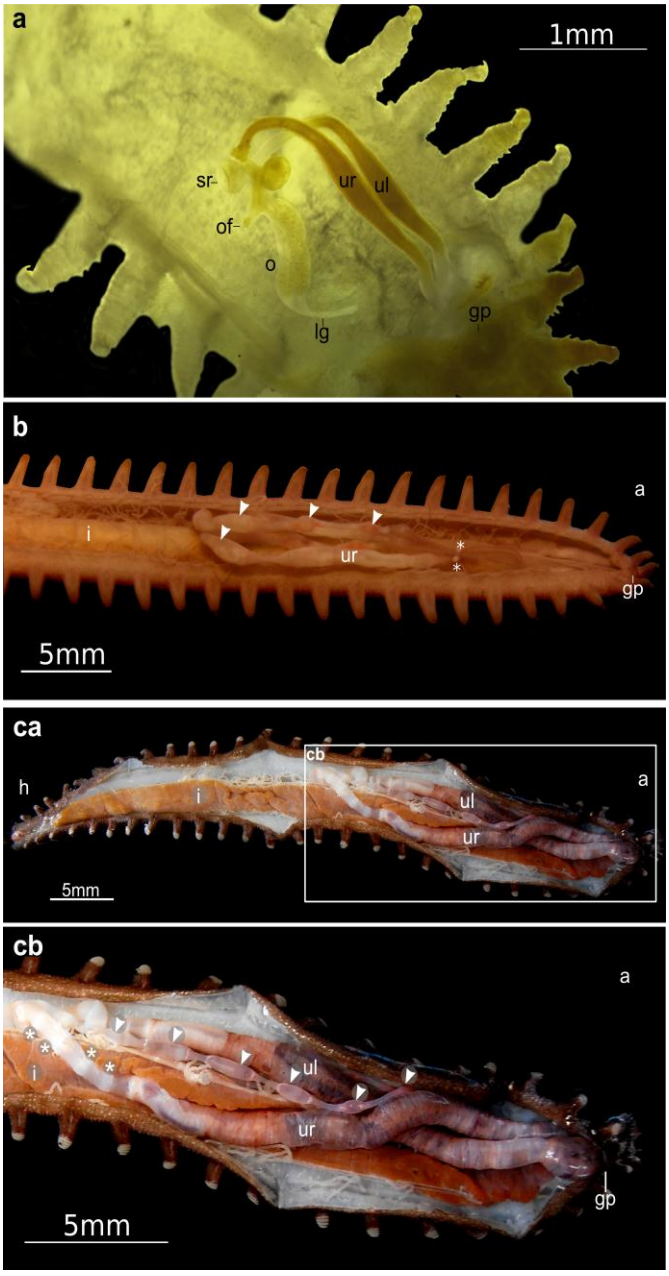
a) Typical habitat, a shadow coffee plantation in Santander department, Colombia at El Roble farm. Arrows indicate decaying logs where specimens of Macroperipatus aff. geayi are usually found. b) Life specimens of Macroperipatus aff. geayi, mature females and one juvenile. c) Photograph of the posterior region of the female body, notice the genital pore between the penultimate pair of legs typical feature of the family Peripatidae. d) Photograph of the posterior region of the male body, arrows point out the sexual dimorphic characteristic, the anal glands openings and the crural tubercles are shown. a= anus, cr = crural glands, gp = genital pore.

***Figure 2.*** *Diagrams of the reproductive genital tract along the different reproductive activity stages.*



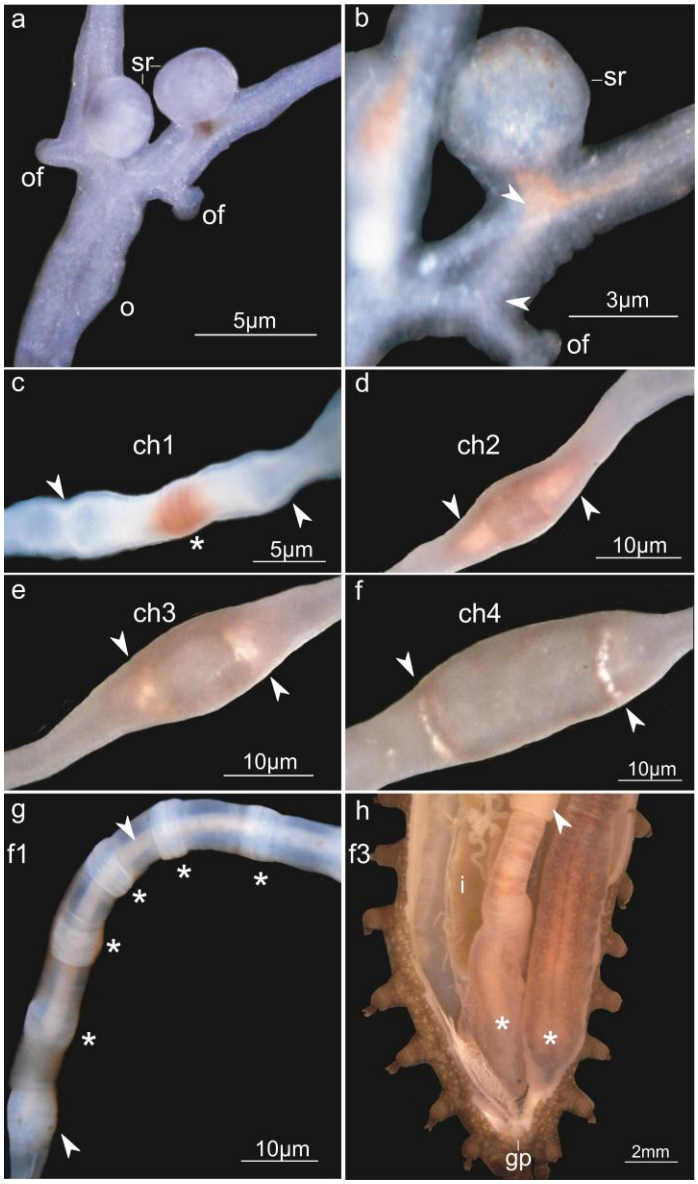

a) Virgin juvenile, the uterus is empty. b) Young-mature female, its ascendant uterus present embryonic chambers with embryos in different developmental processes. c) Old-mature female, its uterus is completely filled with embryonic chambers in the ascendant uterus and fetuses in the descendant uterus. Notice the pigmentation process in the fetuses in the left descendant uterus and the external appearance of the uterus in the right uterus. o= ovary, of= ovarian funnel, sr= seminal receptacle, u= uterus, au= ascendant uterus, du= descendant uterus gp= genital pore, vg= vagina, ch1-ch5= embryonic chambers, f1-f3= fetuses.

***Figure 3.*** *Genital system in series of postnatal reproductive stages of* Macroperipatus *aff.* geayi.



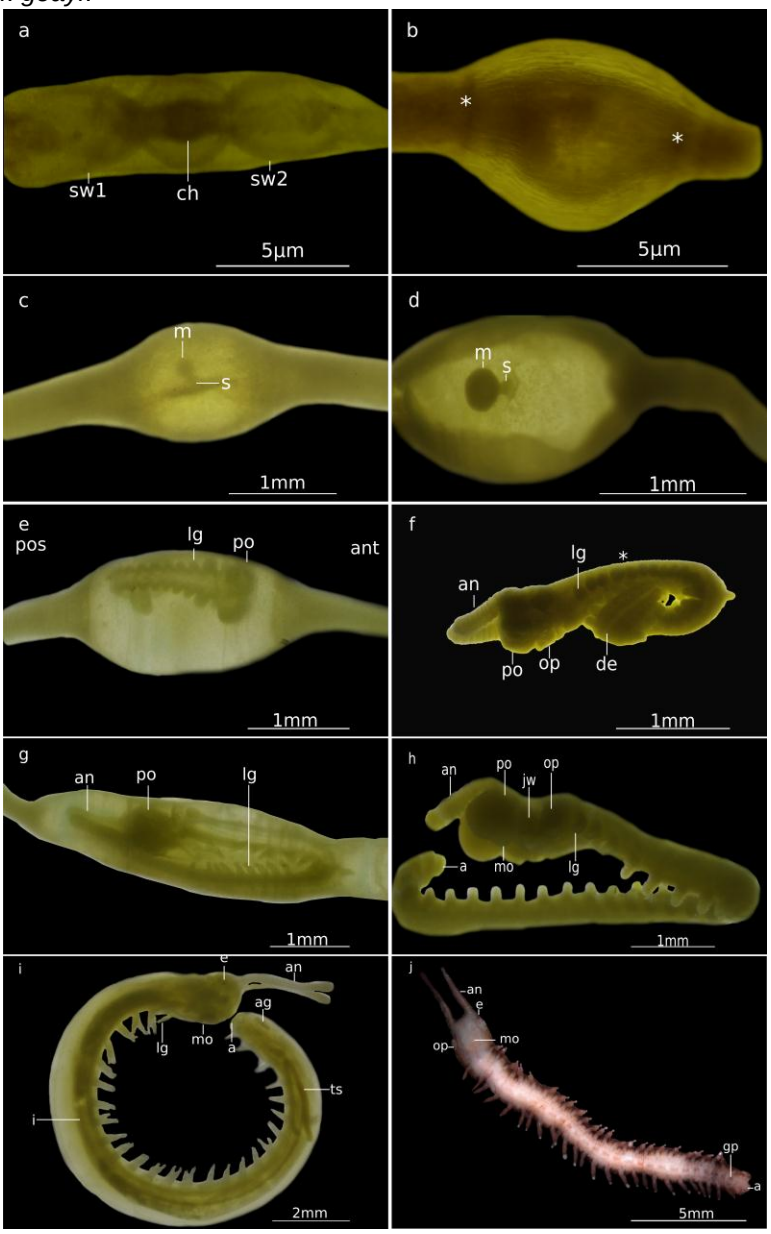

a) The genital tract of a juvenile, the intense color is given by Lugol staining. b) Genital tract of a young mature female, the arrow heads show the number of chambers along the ascendant uterus. ca) Old mature female genital system. cb) Close up of the genital tract, arrow heads indicate the chambers in the ascendant uterus, the descendant uterus is filled up with unpigmented fetuses (*) and pigmented fetuses. a= anus, h= head, i= intestine, gp= genital pore, o= ovary, of= ovarian funnel, ul= left uterus, ur= right uterus.

**Figure 4.** Old mature female genital tract of *Macroperipatus* aff. *geayi*.




a) Overview of the ovary, ovarian funnel, seminal receptacle and a short portion of the uterus. b) Close-section of the ovarian funnel and seminal receptacle, arrow heads indicate the ducts connecting both organs. c) First chamber in the ascendant uterus, arrow heads pointed swellings in both sides of the red-striated chamber (*). d) Second chamber, arrow heads show the closed tips in the chamber. e) Third chamber in the ascendant uterus, observe the thin tips pointed by arrow heads. f) Last chamber in the ascendant uterus, arrow heads show the tips of both sides are reduced. g) Unpigmented fetus in the bent of the ascendant uterus, notice the vestigial placentation zones in the form of rings (*), arrow heads show the digestive system and the head of the fetus. h) Posterior part of the genital system, the asterisks show two pigmented fetuses near to the genital pore, the arrow head shows a constriction of the uterus. o= ovary, of= ovarian funnel, sr= seminal receptacle, ch1-ch4= embryonic chambers.

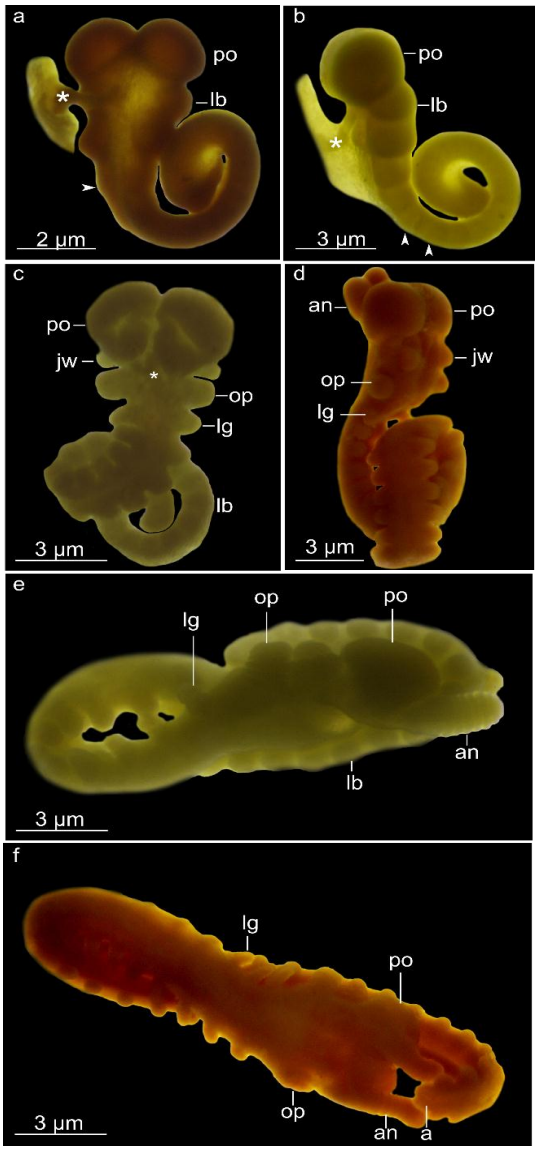
**Figure 5.** Embryonic chambers, embryos and fetuses stages in the uterus of old mature females of *Macroperipatus* aff. *geayi*.




a) First embryonic chamber in the uterus, swellings are in both ends of the forming chamber. b) Second embryonic chamber, the previous swellings are replaced for constrictions in both ends (*). c) Visible vesicle stalked inside the third embryonic chamber d) stalked vesicle. e) Coiled stage inside the embryonic chamber within the fourth embryonic chamber f) coiled embryo with a striation pattern (*) in the body and antenna, the anterior region (ant) points to the ovary while the posterior (pos) points to the head of the female. g) Flexed embryo within the embryonic chamber, notice that the embryos fills the chamber almost completely. h) Flexed stage, the jaws are moving to the mouth and the oral papillae, legs striation pattern is also noticeable. i) Unpigmented fetus, internal organs like intestine, testes and anal glands are clearly visible, the mouth is completely formed and all the legs bear claws. j) Pigmented fetus with the adult coloration pattern. a= anus, an= antenna, ag= anal glands, de= dorsal ectoderm, e= eye, ch= chamber, gp= genital pore, i= intestine, jw= jaws lg= legs, mo= mouth, po= preoral segment, op= oral papillae, ts= testes.

# ANEXOS

**Anexo A**.Photographs showing the changes in the form of the embryos during segment formation.




a) Early coiled embryo showing some limb buds and segment folds (arrow), the embryo is attached to the wall of the embryonic chamber by a stalk (asterisk). b) Embryo with more limb buds and segment folds (arrows) along the whole body. c) Embryo showing the segments in the anterior region in a paddle-shape form and differentiated jaws and oral papillae. d) Embryo showing the jaws near to mouth, behind the preoral segments the antennae appears as buds, anterior segments are differentiated in legs. e) Embryo with a more developed antennae that exhibit the characteristic striated pattern. f) Flexed embryo with segments completely formed, oral papillae and the anterior legs present the striated pattern. a= anus, an= antenna, jw= jaws, lb = limb buds, lg= legs, op= oral papillae, po= preoral segments, arrows= show segment folds, asterisks= stalk.