Comparative reproductive biology of pre-, imaginal, and neotenic castes of the Asian termite *Coptotermes gestroi* (Blattaria, Isoptera, Rhinotermitidae)

Author for correspondence:
Ana Maria Costa-Leonardo,
Email: ana.costa-leonardo@unesp.br

Ana Maria Costa-Leonardo 🄳, Vanelize Janei 🄳 and Iago Bueno da Silva 🄳

Laboratório de Cupins, Departamento de Biologia Geral e Aplicada, Instituto de Biociências, UNESP – Univ Estadual Paulista, Av. 24A, No. 1515, 13506-900, Rio Claro, SP, Brazil

Abstract

The Asian subterranean termite *Coptotermes gestroi* is a worldwide structural pest, although its reproductive biology has been poorly investigated due to a cryptic habit and occurrence of polycalic nests. In this study, we investigated ovarian development and oogenesis in different-aged females of *C. gestroi*: fourth-instar nymphs, non-functional neotenics, alates, and functional queens. We show that the ovaries develop gradually according to their age and functionality, as younger individuals possess immature oocytes, whereas alates and functional queens always undergo vitellogenesis. Oocytes were classified into previtellogenic (stages I, II, and III) or vitellogenic (stages IV, V, and VI). Ovary development varied among non-functional neotenics, and a rapid differentiation and/or the presence of primary reproductives are believed to influence such a maturation. Immature oocyte stages were shared between fourth-instar nymphs and neotenics. These characteristics, together with other neotenic features (wing buds, body pigmentation, and eye color), should be evaluated in detail aiming to clarify which nymphal instars differentiate into secondary reproductives. Oogenesis was not uniform among alate females, and cross-sectional area of terminal oocytes was significantly smaller in alates when compared to functional queens, suggesting different degrees of maturation in swarming individuals. Functional queens always had mature terminal oocytes (stage VI). Ovariole number and oocyte maturation in *C. gestroi* relies on several factors and may therefore differ among individuals of the same caste. Future studies should take into account these reproductive features to evaluate how they impact colony development.

Introduction

The structure of termite colonies is based on a reproductive division of labor between reproductive and sterile individuals, in which the former monopolizes the reproduction inside the nests (Korb, 2007). According to Wilson (1985), this division of labor leads to a task specialization that induces a greater efficiency of the colony and therefore benefits the whole society. Termite colonies undergo three distinct growth stages: juvenile, mature, and senile, and only the mature ones produce alate reproductives, a sexual caste responsible for the dispersal of the species through the establishment of new colonies (Noirot, 1969). Society survival and its success during the growth stages depend on the performance of collective tasks, as well as self-regulatory mechanisms, such as caste ratio and development (Thorne, 1997; Chouvenc and Su, 2014).

The subterranean termite *Coptotermes gestroi* is an important pest species, due to its ability to cause structural damage to infrastructure (Costa-Leonardo, 2002). It is native to Southeast Asia and has been introduced into many countries due to anthropogenic activities (Jenkins *et al.*, 2007). Thus, *C. gestroi* has been recorded in tropical and subtropical regions, and at least 24 countries (Li *et al.*, 2013). Moreover, human maritime activity has facilitated the arrival of this termite in Europe (Ghesini *et al.*, 2011). Global warming and reproductive strategies of this termite species may also contribute to its expansion and success in invasive areas (Costa-Leonardo and Arab, 2004; Robinet and Roques, 2010). However, since the introduction of *C. gestroi* into these regions, little progress has been made regarding its reproductive biology (Costa-Leonardo and Arab, 2004).

C. gestroi exhibits a bifurcated caste differentiation, comprising an early divergence between the apterous and sexual lines. Second-instar larvae originate the sexual line, composed of six wing-budded nymphal instars (N1–N6), which ultimately differentiate into alate reproductives (Barsotti and Costa-Leonardo, 2005). Social organization in termites is strongly influenced by caste differentiation, including the ability to develop neotenic reproductives. Within Rhinotermitidae, the occurrence of replacement reproductives seems to be a pattern, although their origin varies according to the genus (Myles, 1999).

Reproductive sampling is quite difficult in field colonies of *C. gestroi*, since they display a cryptic habit and may have polycalic nests. Therefore, most sampled nests are satellites and do not contain reproductive castes (Lelis, 1999). A mature colony of *C. gestroi* may possess an imaginal physogastric queen with variable mass and egg-laying rate. Nevertheless, the development of neotenic reproductives has also been reported for this species, although their fertility ranges from non-functional individuals to physogastric queens (Lelis, 1999; Costa-Leonardo *et al.*, 2004; Costa-Leonardo and Arab, 2004; Chouvenc *et al.*, 2015a). A suitable way to access the knowledge about termite reproductive biology is through the rearing and monitoring of laboratory colonies. Such a strategy has been used for sampling different-age reproductives and monitoring colony development in subterranean termites (Costa-Leonardo and Barsotti, 1998, 2001; Costa-Leonardo *et al.*, 2004; Chouvenc *et al.*, 2015a, 2015b; Chouvenc, 2019).

In Southeast Brazil, buildings infested by *C. gestroi* were first reported in 1923; since then, this species has been an important structural and economic pest (Martins *et al.*, 2010). However, its reproductive biology has been poorly investigated (Laranjo *et al.*, 2019), especially regarding nymph, alate, and neotenic differentiation, and ovarian development during the lifecycle of the females. Termite females possess ovaries constituted of panoistic ovarioles, which are found in primitive insect groups, and are characterized by the absence of specialized nurse cells (Büning, 1994). Each ovariole is composed of an anterior terminal filament followed by a germarium, containing germinal cells, and a vitellarium, in which yolk deposition takes place (Grassé, 1982; Grandi, 1991; Morini and Costa-Leonardo, 1992).

According to Raina *et al.* (2003), queens of the congeneric *Coptotermes formosanus* went through a first oviposition cycle during the first 60–70 days. Such an oviposition is followed by a 4-month period of inactivity, in which the ovaries decrease in size with the absence of developing oocytes. In the congeneric species *C. gestroi*, however, information concerning ovary development in different-aged females is not well understood. Thus, the current study deals with a comparative analysis on the ovaries of *C. gestroi* females, as we hypothesize that their development and maturation are influenced by female age, reproductive status, and external factors such as colony population and presence of sexual partners. As a complementary information to the reproductive biology of *C. gestroi*, we discuss the likely origin and functionality of neotenics in this species.

Material and methods

Establishment of incipient colonies

Alate reproductives of *C. gestroi* (Wasmann, 1896) were collected during the swarming season in the city of Rio Claro, SP, Brazil (22° 23′ S, 47° 31′ W) and sexed under a Zeiss Stemi V6 stereomicroscope. Alate females and males were paired and placed in 6 cm Petri dishes containing decayed and moistened *Pinus* sp. sawdust. All incipient colonies were kept in the dark at 25 ± 3 °C.

Termites

We used only female individuals: fourth-instar nymphs, nonfunctional nymphoid neotenics, alates, and different-aged queens of *C. gestroi* collected in the city of Rio Claro, SP, Brazil. Nymphs were obtained from foraging sites using corrugated paper, while non-functional neotenic females were sampled from a field nest. Neotenics were identified according to their yellowish-pigmented body and sclerotized wing buds. Their non-functional classification was made due to the absence of spermatozoa in the spermatheca and mature terminal oocytes. Alate females were collected from swarming flights, whereas functional queens (80-d, 2- and 4-year-old) were obtained from incipient colonies (described above) kept under laboratory conditions at the São Paulo State University (UNESP), Rio Claro, SP, Brazil. According to their development, colonies were transferred to larger plastic chambers before sampling. A likely 10+-year-old queen, collected from a field nest, was also included in the experiment. The age of the latter queen was estimated based on its conspicuous physogastry.

Analysis of the ovaries in different-aged females of *C. gestroi*

Total mounting

Abdomens of nymphs, alate females, and 2-year-old queens were dissected under a Zeiss Stemi V6 stereomicroscope, and their ovaries were isolated. Then, they were individually placed on histological slides, stained with acetic orcein or methylene blue solutions (with exception of ovaries from 80-d-old queens), and photographed under Leica ICC50 photomicroscope and Zeiss Stemi V6 stereomicroscope. Three females of each age were selected, except for 2-year-old queens, for which four samples were dissected.

Histology

Abdomens of nymphs, alate females and queens were fixed in FAA (absolute alcohol, glacial acetic acid, 40% formaldehyde, in the ratio 3:1:1) or 4% paraformaldehyde for 24 h. At least three fourth-instar nymphs, three non-functional neotenics, six alates, and four 4-year-old queens were used. Posteriorly, the samples were dehydrated in a series of ethanol (70, 80, 90, and 95%), transferred to a Leica® historesin solution, and kept in the refrigerator for seven days. The abdomens were then embedded in special molds, which were filled with historesin (JB-4 Polaron Instruments/Bio Rad) plus catalyzer for polymerization. Sections of 2–4 μm thick were performed using a Leica RM 2145 microtome and stained with hematoxylin-eosin and toluidine blue. At least three non-functional neotenic females were fixed in FAA, included in paraffin and sectioned with 6–7 μm thickness and their sections stained according to the described above. All sections containing oocytes and the spermatheca were analyzed and photographed under Leica ICC50 photomicroscope, using the software LAS v4.0. Developmental stages of the oocytes were classified according to Grandi *et al.* (1988).

Oocyte morphometry

Aiming to evaluate the cross-sectional area of the terminal oocytes in alate females and functional queens, histological sections of the ovaries of alates and 4-year-old queens were photographed at a magnification of 400x. Posteriorly, terminal oocytes were outlined using the free-hand tool of the software ImageJ (available at http://imagej.nih.gov/ij/), which automatically provided the enclosed cross-sectional area of each oocyte. Data were compared using a *t* test with the software RStudio version 1.3.1093.

Results

Analysis of ovaries in different-aged females of C. gestroi

General morphology and location

Similar to other insect females, the reproductive apparatus of *C. gestroi* females consists of two ovaries and two lateral oviducts that merge in a common oviduct, which ends in a genital chamber. The paired ovaries of the females of this species are disposed dorsolaterally in the abdomen, each one composed of several panoistic ovarioles, long and twisted, which are surrounded by many tracheas. These ovarioles leave the lateral oviducts and extend to the insertion region, located in the dorsal wall of the thorax. This arrangement of ovarioles can be observed in a histological section of a non-functional neotenic female (fig. 1a). The ovarioles connected with the lateral oviduct are arranged in a linear sequence (fig. 1b). In each ovariole, oocytes develop and mature in an anteroposterior gradient, with terminal oocytes located at the base of these ovarioles (fig. 1c). The sampled non-functional neotenics were about 6 mm long, showed a non-physogastric and yellowish body, and originated from nymphs which were ascertained to the presence of wing buds. These nymphoid females had from light brown to brown eyes, many body hairs, and genital plates similar to those present in alate females (fig. 2a–c). Individuals were classified as non-functional due to the absence of stored spermatozoa and mature terminal oocytes.

Total mounting and histology of the ovaries

Fourth-instar nymphs, neotenics, alate females, 80-d, and 2-year-old queens of *C. gestroi* showed variation in the number of ovarioles and oocyte development (fig. 3a–f, Table 1). Also, ovaries grow asymmetrically and therefore do not have the same number of ovarioles. Based on histological features of the ovaries, these structures show well-differentiated regions from the apex to the base: a terminal filament, devoid of germinal cells, which is followed by a short region occupied by oogonia and prefollicular cells, the germarium. Nevertheless, the most part of the ovariole is composed of cells arranged in a linear disposition, the vitellarium. The results showed remarkable morphological differences among the oocytes located in the different parts of the vitellarium, with three previtellogenic (I, II, and III) and three vitellogenic stages (IV, V, and VI). Such stages are described as follows: Stage I oocytes are characterized by the rounded shape, occurrence of a central germinal vesicle with a distinct nucleolus, and are enfolded by a single layer of flattened follicular cells. Stage II oocytes are larger than stage I, with ovoid shape, and are enfolded by cuboidal follicular cells (fig. 4a–c). Stage III oocytes are remarkably ovoid in shape, with a conspicuous germinal vesicle and central nucleolus. Moreover, the follicular epithelium is composed of columnar cells (fig. 4d).

Yolk deposition takes place only in stage IV oocytes, although in a small quantity. In this stage, the oocytes are ovoid and are also enfolded by columnar follicular cells (fig. 4e). Stage V oocytes display a greater amount of deposited yolk and epithelium of cuboidal follicular cells (fig. 4f). Stage VI oocytes, also called mature terminal oocytes, are the largest when compared to any other stage, characterized by a rich deposition of yolk granules and presence of flattened follicular cells enfolding them (fig. 4g). A detailed schematic representation of these developmental stages is shown in fig. 5. The cytoplasm of the previtellogenic oocytes (stages I, II, and III) are basophilic and, due to the yolk deposition, oocytes increase in size and their cytoplasm becomes gradually eosinophilic (stages IV, V, and VI).

Fourth-instar nymphs. In these individuals, there were from four to five ovarioles, all of them strongly compacted and composed only of previtellogenic oocytes, which probably belonged to stages I and II (fig. 6a–d).

Non-functional neotenics. In non-functional neotenics, ovaries were composed of about 10 ovarioles (fig. 1a–c). The oocyte
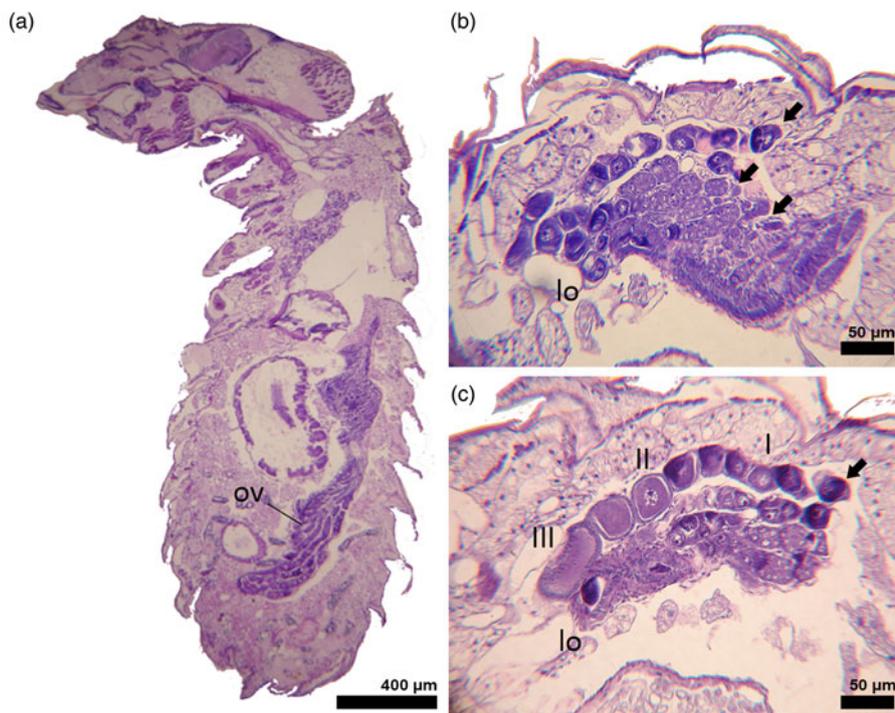

Figure 1. Histological sections of a non-functional neotenic female of *C. gestroi*. (a) Longitudinal section of the body indicating the dorso-lateral disposition of the ovary inside the insect body. (b) Note the arrangement of ovarioles (arrows) leaving the lateral oviduct. (c) Detail of the structural organization of an ovariole (arrow) showing three different stages of previtellogenic oocytes (stages I, II and III). Stage III oocyte is ovoid and basal. lateral oviduct = lo, ov = ovary.
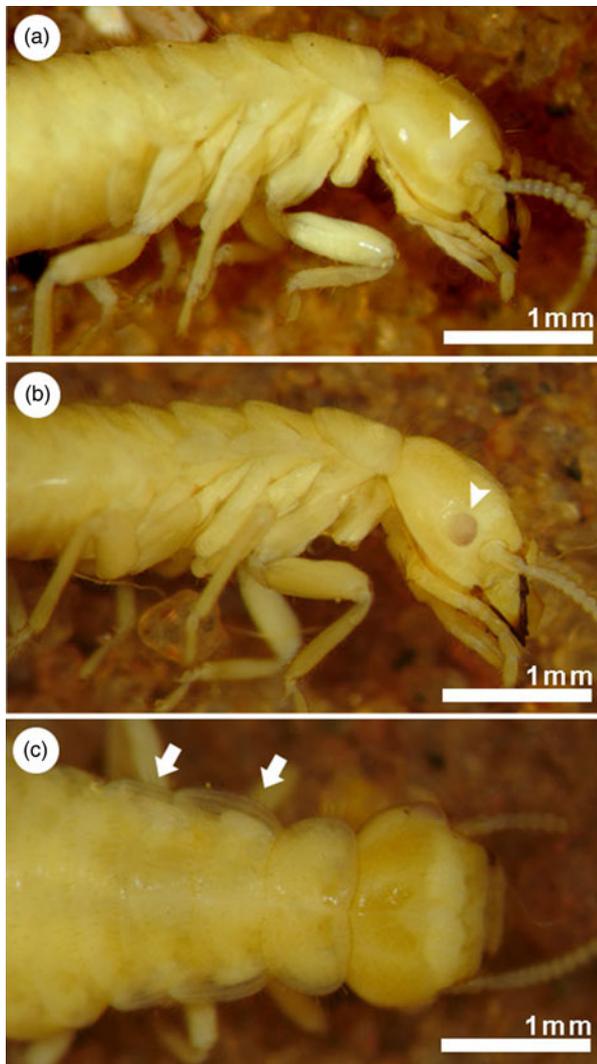
Figure 2. External morphology of non-functional neotenic females of *C. gestroi*. (a) View of a neotenic female with light brown eyes (arrowhead), mandibles with sclerotized edges and wingbuds in the thorax. (b) Another neotenic female with similar morphological features, but exhibiting brown eyes (arrowhead). (c) Detail of the wingbuds (arrows) in a neotenic female.

development in some neotenics, however, resembles that from nymphs, since only previtellogenic oocytes were observed (fig. 1a–c). However, other non-functional neotenics showed oocytes at late developmental stages, but without reaching a terminal stage, similar to some alates (fig. 7a, b).

Alate females. Ovariole number ranged from four to five per ovary (fig. 3a). Oocyte maturation in alates varied among individuals. Some females exhibited only vitellogenic oocytes at stage V, similar to those observed in neotenics (fig. 7c, d), whereas other alates showed stage VI terminal oocytes (fig. 8a, b), resembling those from functional queens (fig. 8c, d).

80-d-old queens. During the period of 80 days after colony establishment, the population of the incipient colonies was composed only by larvae, although eggs were also observed. Ovaries of 80-d-old queens presented a conspicuous development, since an increasing number of ovarioles, ranging from five to six, and

vitellogenic oocytes (including mature terminal ones) were reported (fig. 3b).

2-year-old queens. The highest number of ovarioles ($n = 35$) occurred among these queens, which also reflected their mass and colony population (fig. 3c–f). For example, fig. 3d shows the ovarioles containing several terminal oocytes from the heaviest sampled 2-year-old queen (10.3 mg), whose colony contained the highest number of individuals, with 592 workers. Additionally, larger terminal oocytes were observed in these queens, characterized by a remarkable deposition of the chorion (fig. 3f).

Histology of the spermatheca
The spermatheca from different-aged females contained a secretion in the spermathecal lumen of alates and non-functional neotenics, even though spermatozoa were absent (fig. 9a–d). However, spermatheca of 4-year-old queens was more developed than those of alates and neotenics, and contained numerous spherical spermatozoa in the lumen (fig. 9e, f).

Oocyte morphometry
Based on measures performed using ImageJ, the cross-sectional area of terminal oocytes was significantly different between alates and functional queens (4-year-old individuals), being 43.52 ± 2.91 mm^3 for alates and 73.95 ± 7.27 mm^3 for queens ($t = 3.8816$, df $= 6.567$, $P < 0.01$, t test).

Discussion

For insects in general, oogenesis is a crucial process and is influenced by several factors such as nutrition and season (Wheeler, 1996). Termites have some reproductive peculiarities, which contribute to the success of their colonies, such as queen fecundity associated with longevity and replacement of reproductives (Noirot, 1990). However, there is a lack of studies monitoring queens during their reproductive life inside the colonies, predominantly due to the cryptic habits of termites.

The current study followed several changes in the gonads, since the ovaries showed variation in the ovariole number and development among different-aged females of *C. gestroi* (Table 1). Similar to *Reticulitermes speratus* and *Hodotermopsis sjostedti*, in which nymphs do not possess vitellogenic oocytes (Maekawa *et al.*, 2010; Oguchi *et al.*, 2016), fourth-instar nymphs of *C. gestroi* displayed shrunk ovaries composed only of previtellogenic oocytes. Oogenesis in non-functional neotenic females varied, as the ovaries of some sampling females (fig. 1a–c) resembled those of fourth-instar nymphs, while others went through vitellogenesis (fig. 7a, b). Similarly, their non-functionality was especially due to the absence of spermatozoa in the spermatheca and mature terminal oocytes. According to Albino and Costa-Leonardo (2011), nymphs are present all year round in nests of *C. gestroi*, and fourth-instar individuals are the most common sampled nymphs. Assuming the high occurrence of fourth-instar nymphs in *C. gestroi* and their similar ovarian development to those of some non-functional neotenics, we encourage future morphometric assessments to clarify which nymphal instars may differentiate into nymphoid neotenics in this species. Neotenic females are likely to quickly develop their reproductive system as an adaptation to breed inside the nest, since unlike alates, they do not invest in flight apparatus and do not swarm (Oguchi *et al.*, 2016). It would explain the higher
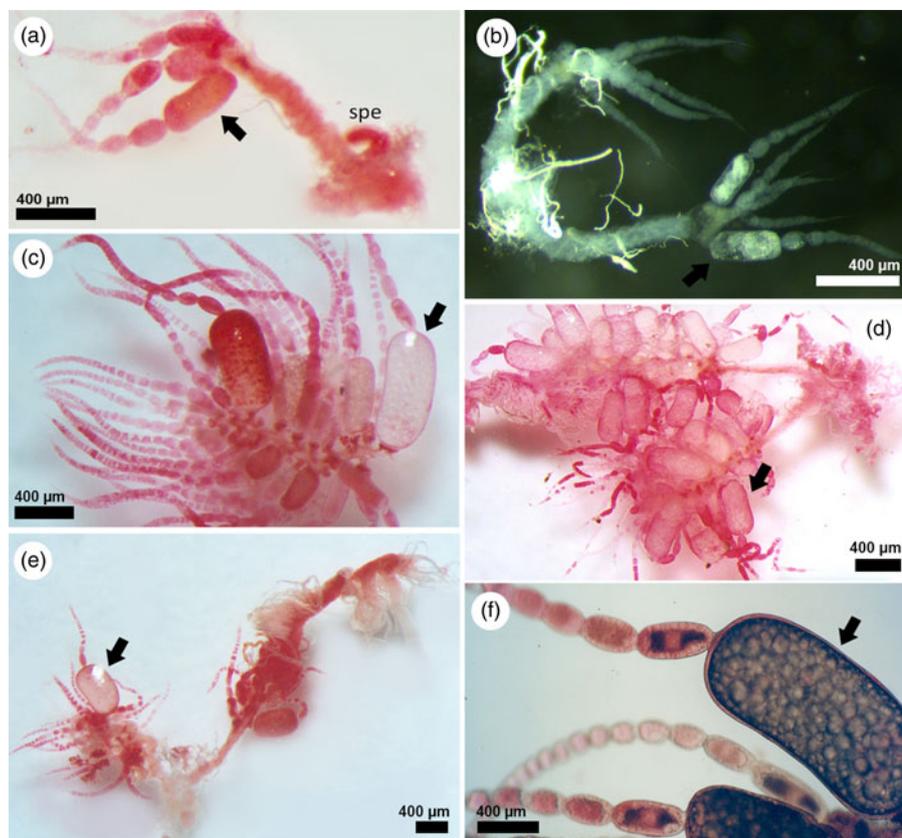
Figure 3. Total mounting of the ovaries in different-aged females of *C. gestroi*. (a) Ovary of an alate female. Note the ovarioles and small curved spermatheca. (b) Ovaries of an 80d-old queen, showing developing oocytes. (c–e) Ovary from 2-year-old functional queens. Note the well-developed oocytes of the heaviest sampled queen (10.3 mg) in Fig. (d, f) Detail of a large terminal oocyte and immature oocytes from a 2-year-old functional queen. Arrows indicate terminal oocytes. Stain: acetic orcein. spe = spermatheca.

number of ovarioles in our sampled neotenics when compared to nymphs, even though they were not reproducing. Non-functional neotenics which went through vitellogenesis may have differentiated from later instar nymphs, but it also deserves further investigations.

Alate females of *C. gestroi* displayed vitellogenic oocytes in later stages (Table 1). However, when compared to functional queens (4-year-old), their terminal oocytes were significantly smaller. It may be explained due to the presence of both stages V and VI oocytes as terminal ones among alate females, whereas only stage VI was identified as terminal one for functional queens. Furthermore, the presence of stage VI oocytes in alate females agrees with reports on *R. speratus* (Maekawa *et al.*, 2010). On the other hand, oogenesis in *R. flavipes* and *H. sjostedti* went through vitellogenesis, but terminal mature oocytes were absent (Dean and Gold, 2004; Oguchi *et al.*, 2016). It suggests that ovary activation and development vary among alates after they leave the natal nest, since this event stimulates female maturation (Greenberg and Stuart, 1979). In some cases, alates may express similar rates of vitellogenin when compared to young queens (Husseneder *et al.*, 2012), what would explain the occurrence of mature terminal oocytes.

During oogenesis, previtellogenic oocytes display features of increased metabolic activity. Moreover, they grow in size due to the incorporation of different compounds from the hemolymph through endocytic events (Grandi and Chicca, 1999). Additionally, the follicular epithelium also undergoes morphological changes, influenced by the increasing number of organelles

Table 1. Number of ovarioles and developmental stages of oocytes in different-aged females of *Coptotermes gestroi*

| | | Developmental stage of oocytes | | | | | |
| | | Previtellogenic | | | Vitellogenic | | |
Females	Ovarioles per ovary (maximum)	I	II	III	IV	V	VI
Fourth-instar nymph	5	+	+	−	−	−	−
Non-functional neotenics	~10	+	+	+	±	±	−
Alates	5	+	+	+	+	+	±
80-d-old queens	6	+	+	+	+	+	+
2-year-old queens	35	+	+	+	+	+	+

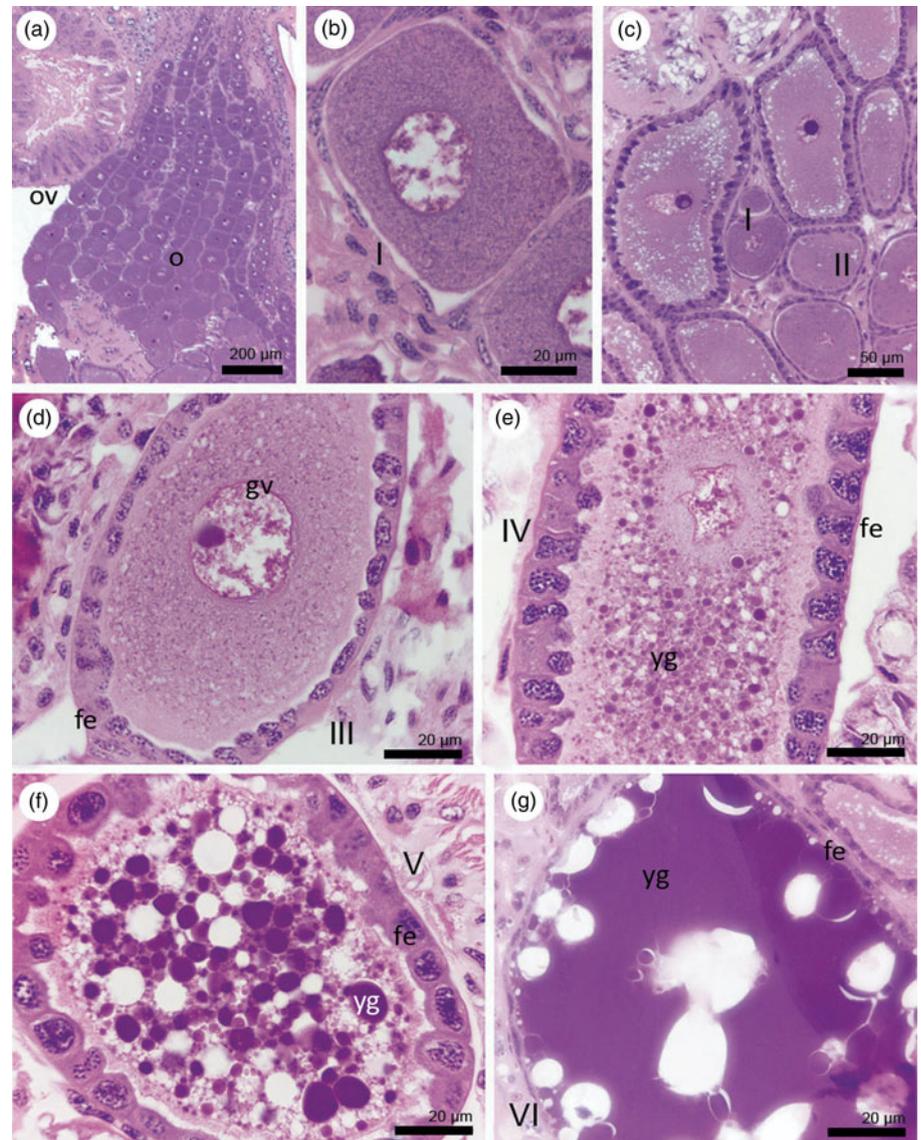± Occurrence or absence varied among individuals of the same caste.

Figure 4. Oogenesis in functional queens of *C. gestroi*, detailing each developmental stage. (a) General view of the anterior part of ovarioles of a 4-year-old queen. (b) Detail of a previtellogenic oocyte (stage I) of a 10+-year-old queen. Note the flattened follicular epithelium. (c) Previtellogenic (stages I, II, and III) of a 4-year-old queen. (d) Detail of a previtellogenic oocyte of stage III with a conspicuous germinal vesicle and nucleolus. (e) Detail of a vitellogenic oocyte of stage IV with small yolk granules and columnar follicular epithelium. (ep). (f) Detail of a stage V vitellogenic oocyte with many yolk granules and cuboidal follicular epithelium. (g) Detail of a vitellogenic oocyte of stage VI (mature terminal oocyte) with flat follicular epithelium and richness of yolk. Stain: Hematoxylin-eosin. fe = follicular epithelium, gv = germinal vesicle, o = ovarioles, ov = ovary, yg = yolk granule.

(Dossi and Cônsoli, 2014), especially those related to synthesis of precursors of the tunica propria (Grandi and Chicca, 1999). Throughout this period, vitellogenin synthesis is influenced by JH titers, which also induce the process of patency of the follicular cells, allowing the assimilation of vitellogenin and other compounds by the oocytes (Dossi and Cônsoli, 2014; Santos *et al.*, 2019). Moreover, follicular cells present high synthesis of products which may be incorporated into yolk or facilitate the endocytic uptake of vitellogenin (van Antwerpen and Law, 1993; Grandi and Chicca, 1999). This is a likely reason for the high development of the follicular epithelium, especially during stage IV of vitellogenesis observed in alates and functional queens of *C. gestroi*.

Termite queens always exhibited ovarioles containing vitellogenic oocytes, including mature terminal ones (Table 1), which showed epithelium composed of flattened follicular cells and reached the maximum size among all oocyte stages. The absence of degenerative ovarioles suggests an active vitellogenesis and therefore egg production. Dean and Gold (2004) also observed terminal mature oocytes only in 10-d-old queens and tertiary reproductives (neotenics) of *Reticulitermes flavipes*. It was

suggested that the presence of a male and/or food quality promotes ovary maturation in females (Brent and Traniello, 2001*a*, 2002), which could also explain the development of these structures in queens of *C. gestroi* and *R. flavipes* after pairing. Moreover, workforce can enhance queen fecundity, since it performs colony labor and therefore increases the energy reserves of the reproductives (Brent and Traniello, 2001*b*). It agrees with our observations on colony population and ovary development in 2-yearr-old queens.

Late vitellogenesis is characterized by an intense protein synthesis and transport of compounds associated with the production of vitelline membrane and chorion deposition, a two-layered structure which covers the mature oocyte (Grandi, 1988, 1990; Grandi and Chicca, 1999). It is important to highlight the ovary maturation in 80-d-old queens. According to Raina *et al.* (2003), after the first oviposition cycle (about 60–70 days after colony foundation), queens of *C. formosanus* suffer an ovarian atrophy, with absence of pre- and vitellogenic oocytes. In our 80-d-old samples of *C. gestroi*, ovaries are still developed and composed of vitellogenic oocytes, including terminal ones, after the referred period. Thus, reproductive strategies and fecundity
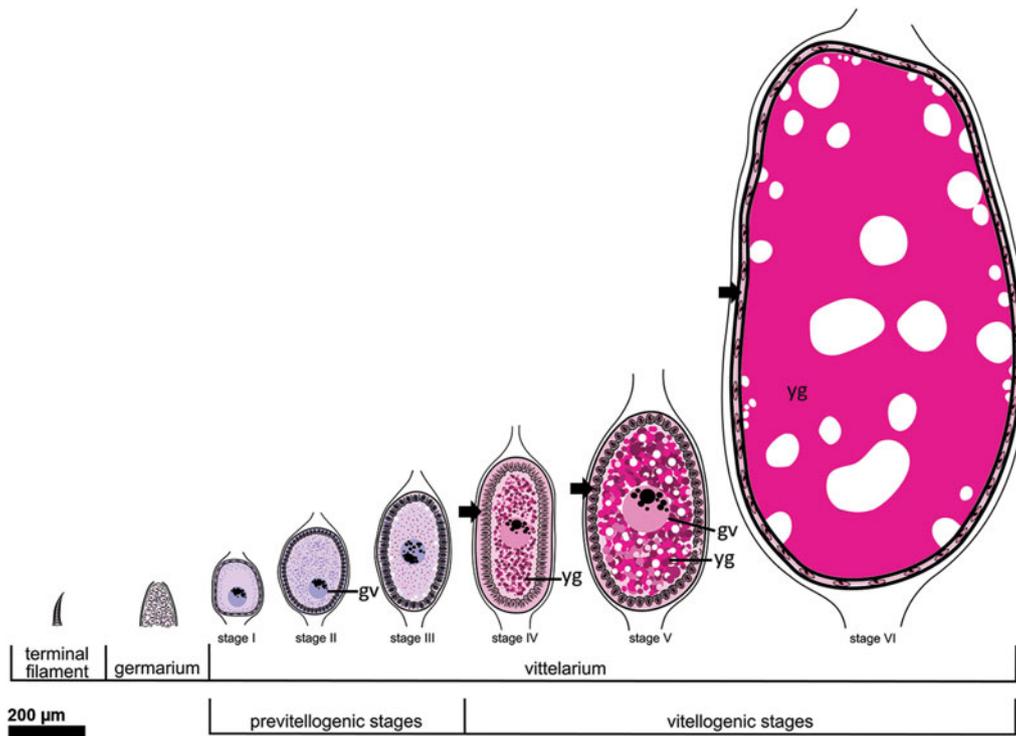
Figure 5. Diagrammatic representation of the oogenesis in a functional queen of *C. gestroi*, with emphasis on the developmental stages of the oocytes. Arrows indicate the follicular epithelium. gv = germinal vesicle, yg = yolk granule.
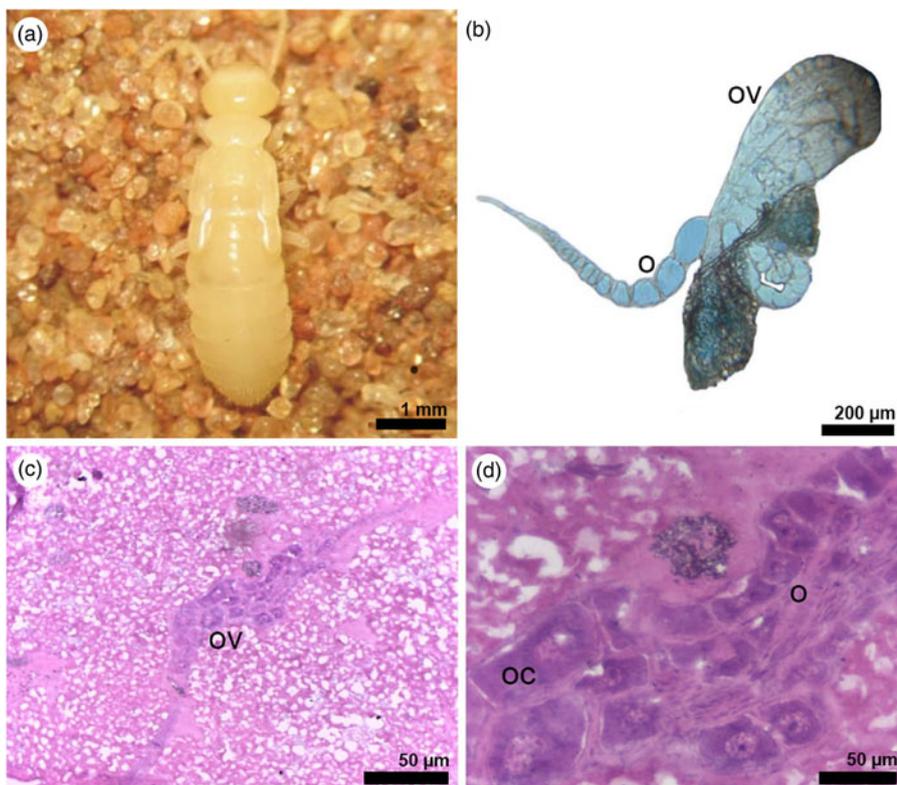

Figure 6. Ovary development and oogenesis in fourth-instar nymphs of *C. gestroi*. (a) External view of a fourth-instar nymph. Note the wingbuds of the meso- and metathorax. (b) Total mounting of an ovary of fourth-instar nymph, highlighting the ovarioles. (c, d) Longitudinal sections of an ovary, composed only of previtellogenic oocytes. This structure is shrunk and oocytes are likely to belong to stages I and II. Stain: Hematoxylin-eosin. o = ovariole, oc = oocyte, ov = ovary.
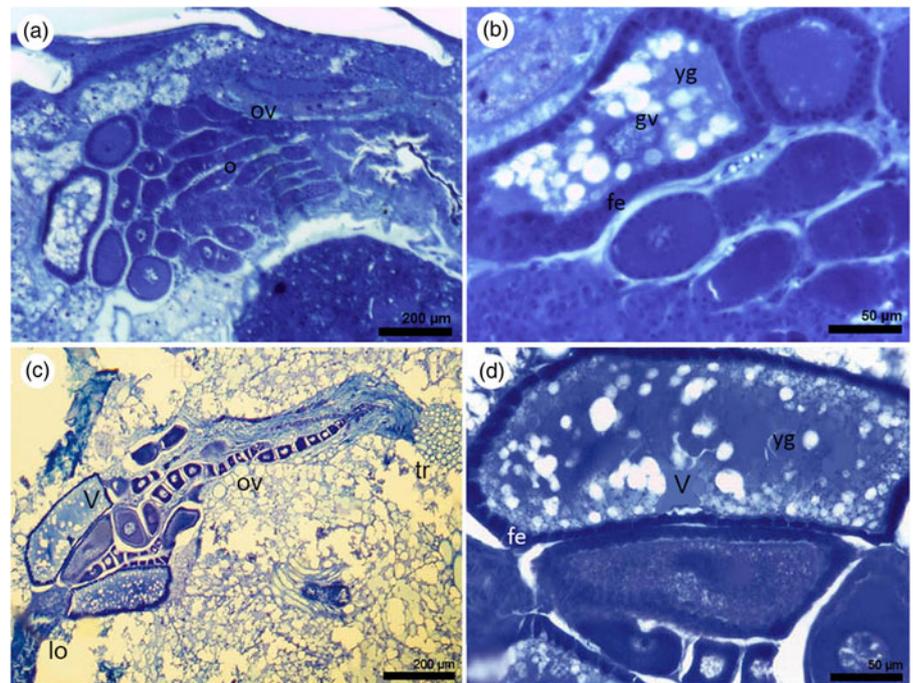
Figure 7. Comparative oogenesis in non-functional neotenics and alates of *C. gestroi*. (a, b) Ovary of a non-functional neotenic containing several ovarioles, which contains previtellogenic and vitellogenic oocytes. (c, d) Ovary and ovarioles of an alate female. Note that the terminal oocyte belongs to the stage V. Stain: Toluidine blue. fe = follicular epithelium, gv = germinal vesicle, lo = lateral oviduct, o = ovarioles, ov = ovary, tr = tracheae, V = stage V terminal oocyte, yg = yolk granule.

may vary between these congeneric species, and the likely reasons may include the environmental conditions and location where termites were sampled and paired.

Termite colonies and their reproductive dynamics rely on several conditions. Oogenesis in different-aged females of *C. gestroi* varies considerably, with ovaries developing progressively as females age and turn into swarming and functional reproductives. Among non-functional neotenics, ovarian development was not uniform, as some of them do not go through vitellogenesis. If this variation is a result of different-nymphal instars developing into nymphoid neotenics, it is still unknown. Moreover, the social context (e.g., sociotomy and presence of the royal pair) might influence neotenic maturation. Alate females may show stage V or VI oocytes as terminal ones, which reflects different degrees of ovary activation during swarming events. Functional queens always displayed mature terminal oocytes, including 80-d-old individuals. Future analyses should clarify how ovary maturation among individuals of the same caste and other reproductive features impact colony development.
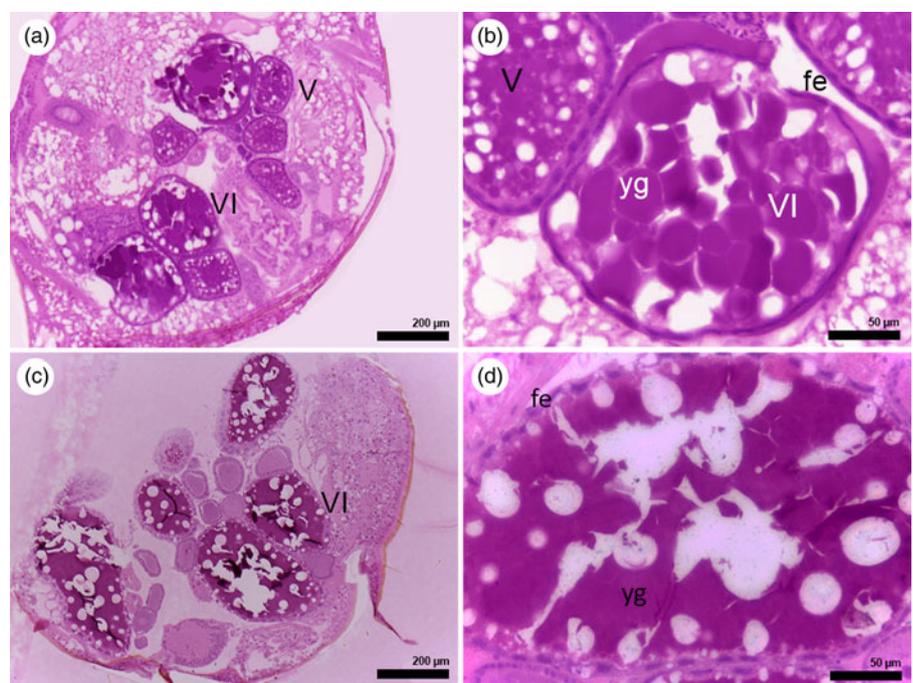

Figure 8. Vitellogenesis in alates and functional queens (4-year old) of *C. gestroi*. (a, b) General view and detail of vitellogenic oocytes of an alate female, respectively. Note the occurrence of mature terminal oocytes (stage VI). (c, d) General view and detail of several mature terminal oocytes of a 4-year-old queen, respectively. Stain: Hematoxylin-eosin. fe = follicular epithelium, V = stage V oocyte, VI = stage VI terminal oocyte, yg = yolk granule.
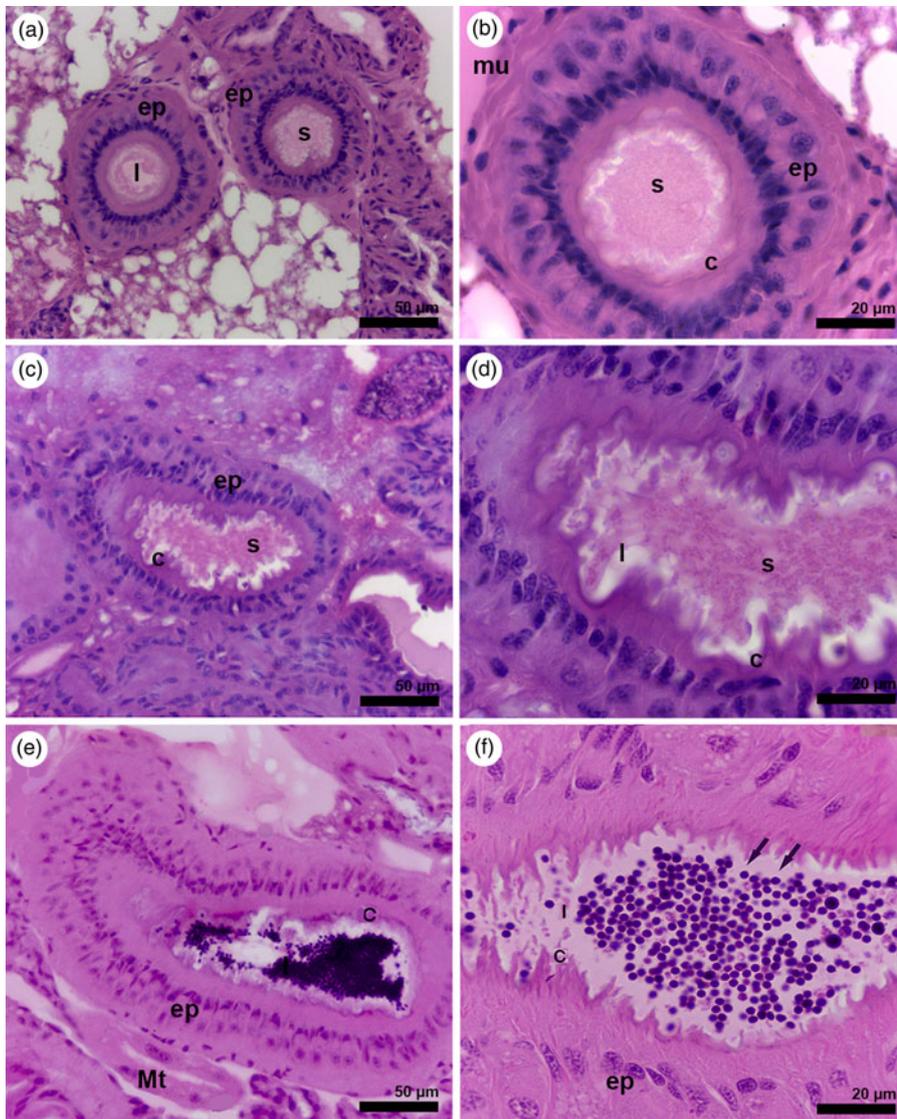
Figure 9. Morphological features of the spermatheca among different-aged females of *C. gestroi*. (a, b). Alate. (c, d) Non-functional neotenic. Note the stored secretion in the lumen of the spermatheca in both females. (e, f) 4-year-old functional queen, showing a developed epithelium and the spermathecal lumen filled with rounded spermatozoa (black arrows). Stain: Hematoxylin-eosin. c = cuticle, ep = epithelium, l = lumen, Mt = Malpighian tubule, mu = musculature, s = secretion.

References

Albino E and Costa-Leonardo AM (2011) Nymphs in foraging populations of *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Animal Biology* **61**, 427–439.

Barsotti RC and Costa-Leonardo AM (2005) The caste system of *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Sociobiology* **46**, 87–103.

Brent CS and Traniello JF (2001*a*) Influence of sex-specific stimuli on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*. *Physiological Entomology* **26**, 239–247.

Brent CS and Traniello JF (2001*b*) Social influence of larvae on ovarian maturation in primary and secondary reproductives of the dampwood termite *Zootermopsis angusticollis*. *Physiological Entomology* **26**, 78–85.

Brent CS and Traniello JF (2002) Effect of enhanced dietary nitrogen on reproductive maturation of the termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). *Environmental Entomology* **31**, 313–318.

Büning J (1994) The ovary of Ectognatha, the Insecta s.str. In Büning J (ed.), *The Insect Ovary: Ultrastructure, Previtellogenic Growth and Evolution.* London: Chapman & Hall, pp. 281–299.

Chouvenc T (2019) The relative importance of queen and king initial weights in termite colony foundation success. *Insectes Sociaux* **66**, 177–184.

Chouvenc T and Su N-Y (2014) Colony age-dependent pathway in caste development of *Coptotermes formosanus* Shiraki. *Insectes Sociaux* **61**, 171–182.

Chouvenc T, Mullins AJ and Su N-Y (2015*a*) Rare production of nymphs in an Asian subterranean termite (Isoptera: Rhinotermitidae) incipient colony. *Florida Entomologist* **98**, 972–973.

Chouvenc T, Basille M and Su N-Y (2015*b*) The production of soldiers and the maintenance of caste proportions delay the growth of termite incipient colonies. *Insectes Sociaux* **62**, 23–29.

Costa-Leonardo AM (ed.) (2002) *Cupins-Praga: Morfologia, Biologia e Controle.* Rio Claro: Divisa Editora, p. 128.

Costa-Leonardo AM and Barsotti RC (1998) Swarming and incipient colonies of *Coptotermes havilandi* (Isoptera, Rhinotermitidae). *Sociobiology* **31**, 131–142.

Costa-Leonardo AM and Barsotti RC (2001) Growth patterns of incipient colonies of *Coptotermes havilandi* (Isoptera, Rhinotermitidae) initiated in the laboratory from swarming alates. *Sociobiology* **37**, 551–561.

Costa-Leonardo AM and Arab A (2004) Reproductive strategy of *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Sociobiology* **44**, 123–125.

Costa-Leonardo AM, Arab A and Casarin FE (2004) Neotenic formation in laboratory colonies of the termite *Coptotermes gestroi* after orphaning. *Journal of Insect Science* **4**, 10.

Dean SR and Gold RE (2004) Sex ratios and development of the reproductive system in castes of *Reticulitermes flavipes* (Kollar) (Isoptera:

Rhinotermitidae). *Annals of the Entomological Society of America* **97**, 147–152.

Dossi FCA and Cônsoli FL (2014) Gross morphology and ultrastructure of the female reproductive system of *Diaphorina citri* (Hemiptera: Liviidae). *Zoologia* **31**, 162–169.

Ghesini S, Puglia G and Marini M (2011) First report of *Coptotermes gestroi* in Italy and Europe. *Bulletin of Insectology* **64**, 53–54.

Grandi G (1988) Oogenesis in *Kalotermes flavicollis* (Fabr.) (Isoptera: Kalotermitidae). II-prefollicular and follicular cell ultrastructure during oogenesis in female supplementary reproductives. *Bollettino di zoologia* **55**, 279–292.

Grandi G (1990) Oogenesis in *Kalotermes flavicollis* (Fabr.) (Isoptera, Kalotermitidae) III. Choriogenesis and corpus luteum formation in female supplementary reproductive. *Bollettino di zoologia* **57**, 97–107.

Grandi G (1991) Oogenesis in immature stages and sterile castes of *Kalotermes flavicollis* (Fabr.) (Isoptera Kalotermitidae). *Ethology Ecology & Evolution* **3**, 29–32.

Grandi G and Chicca M (1999) Oogenesis in supplementary reproductives of *Reticulitermes lucifugus* Rossi (Isoptera Rhinotermitidae): an ultrastructural study. *Invertebrate Reproduction & Development* **35**, 65–79.

Grandi G, Barbieri R and Colombo G (1988) Oogenesis in *Kalotermes flavicollis* (Fabr.) (Isoptera: Kalotermitidae). I. Differentiation and maturation of oocytes in female supplementary reproductives. *Bolletino di Zoologia* **55**, 103–122.

Grassé PP (1982) *Termitologia*. Vol. **1**, Paris: Masson, p. 676.

Greenberg SLW and Stuart AM (1979) The influence of group size on ovarian development in adult and neotenic reproductives of the termite *Zootermopsis angusticollis* (Hagen)(Hodotermitidae). *International Journal of Invertebrate Reproduction* **1**, 99–108.

Husseneder C, McGregor C, Lang RP, Collier R and Delatte J (2012) Transcriptome profiling of female alates and egg-laying queens of the Formosan subterranean termite. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* **7**, 14–27.

Jenkins TM, Jones SC, Lee C-Y, Forschler BT, Chen Z, Lopez-Martinez G, Gallagher NT, Brown G, Neal M, Thistleton B and Kleinschmidt S (2007) Phylogeography illuminates maternal origins of exotic *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Molecular Phylogenetics and Evolution* **42**, 612–621.

Korb J (2007) Workers of a drywood termite do not work. *Frontiers in Zoology* **4**, 1–7.

Laranjo LT, Haifig I and Costa-Leonardo AM (2019) A glycoproteinaceous secretion in the seminal vesicles of the termite *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Insects* **10**, 428.

Lelis AT (1999) Primeiro registro no Brasil de rainha de substituição de *Coptotermes havilandi* e sua implicação no controle desse cupim. *Vetores Pragas* **4**, 19–23.

Li H-F, Fujisaki I and Su N-Y (2013) Predicting habitat suitability of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) with species distribution models. *Journal of Economic Entomology* **106**, 311–321.

Maekawa K, Ishitani K, Gotoh H, Cornette R and Miura T (2010) Juvenile hormone titre and vitellogenin gene expression related to ovarian development in primary reproductives compared with nymphs and nymphoid reproductives of the termite *Reticulitermes speratus*. *Physiological Entomology* **35**, 52–58.

Martins C, Fontes LR, Bueno OC and Martins VG (2010) *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in Brazil: possible origins inferred by mitochondrial cytochrome oxidase II gene sequences. *Genome* **53**, 651–657.

Morini MSC and Costa-Leonardo AM (1992) The functional ovarioles in female imagoes of *Cornitermes cumulans* (Isoptera, Termitidae) at the swarming time. *Revista Brasileira de Entomologia* **36**, 709–715.

Myles TG (1999) Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology* **33**, 1–43.

Noirot C (1969) Formation of castes in the higher termites. In Krishna K and Weesner FM (eds), *Biology of Termites*, Vol. **1**. New York, NY, USA: Academic Press, pp. 311–350.

Noirot C (1990) Sexual castes and reproductive strategies in termites. In Engels W (ed.), *Social Insects - an Evolutionary Approach to Caste and Reproduction*. Heidelberg: Springer Verlag, pp. 5–35.

Oguchi K, Shimoji H, Hayashi Y and Miura T (2016) Reproductive organ development along the caste differentiation pathways in the dampwood termite *Hodotermopsis sjostedti*. *Insectes Sociaux* **63**, 519–529.

Raina A, Park YI and Florane C (2003) Behavior and reproductive biology of the primary reproductives of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *Sociobiology* **41**, 37.

Robinet C and Roques A (2010) Direct impacts of recent climate warming on insect populations. *Integrative Zoology* **5**, 132–142.

Santos CG, Humann FC and Hartfelder K (2019) Juvenile hormone signaling in insect oogenesis. *Current Opinion in Insect Science* **31**, 43–48.

Thorne BL (1997) Evolution of eusociality in termites. *Annual Review of Ecology and Systematics* **28**, 27–54.

Van Antwerpen R and Law JH (1993) Lipophorin lipase from the yolk of *Manduca sexta* eggs: identification and partial characterization. *Archives of Insect Biochemistry and Physiology* **20**, 1–12.

Wheeler D (1996) The role of nourishment in oogenesis. *Annual Review of Entomology* **41**, 407–431.

Wilson EO (1985) The sociogenesis of insect colonies. *Science (New York, N.Y.)* **228**, 1489–1495.