Arthropod Structure & Development 45 (2016) 325–332

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

Functional morphology of the maxillary and propharyngeal glands of *Monomorium pharaonis* (L.)

Sofie Boonen*, Johan Billen

KULeuven, Zoological Institute, Naamsestraat 59 Box 2466, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 16 March 2016
Accepted 18 April 2016
Available online 15 May 2016

Keywords:
Histology
Ultrastructure
Maxillary gland
Propharyngeal gland
Monomorium

ABSTRACT

The maxillary and propharyngeal gland of all 3 castes of *Monomorium pharaonis* were examined with light and electron microscopy. Although both glands possess a pouch in which secretion can be stored temporarily, a proper reservoir is lacking. The paired maxillary gland opens at the base of the maxilla and consists of 4 secretory cells, which are smaller in workers as compared to queens and males. A digestive role is unlikely as the gland is not directly linked to the digestive system and the amount of rER is negligible. The propharyngeal gland consists of 2 clusters of 16 secretory cells, which open in the pharyngeal atrium through a duct. Secretory cells are smallest in males. Two types of endoplasmic vesicles are observed around the end apparatus, suggesting a release of at least 2 substances. High levels of rER indicate the production of digestive enzymes as one of its functions. No differences between mated versus virgin queens were observed for both glands. Further experiments on chemical and behavioural essays can improve our understanding of the role of both glands in the ant colony. Literature on this topic is very inconsistent. We provide a survey to unravel this chaotic nomenclature issue.

1. Introduction

Ants, as other social insects, possess a wide variety of exocrine glands that produce pheromones as well as a variety of substances that are indispensable for the maintenance of the colony's social structure. A substantial number of these glands are situated in the head (Fig. 1). Due to their close association with the mouthparts or digestive tract, most of the cephalic glands were initially regarded as digestive structures (Bausenwein, 1960; Forbes and McFarlane, 1961; Kürschner, 1971; Paulsen, 1969). However, multiple functions have since been revealed. Especially the mandibular and postpharyngeal glands have been the subject of functional research as their considerable size and the presence of a reservoir allows chemical identification and bioassays. Mandibular gland secretions for instance have been shown to release an alarm response in several ant species (Moser et al., 1968; Crewe and Blum, 1970; Brough, 1978; Billen and Morgan, 1998). In honey bees, mandibular glands received considerable attention, because they produce queen pheromones by which queens suppress ovary development in workers (Butler and Simpson, 1958; Butler and Fairey, 1963; Hoover et al., 2003). The postpharyngeal gland is mainly involved in nestmate recognition (Bagnères and Morgan, 1991; Vander Meer and Morel, 1998; for a review see Eelen et al., 2004).

Unlike the mandibular (Boonen et al., 2012) and postpharyngeal gland (Eelen et al., 2004), few studies have been done on the less conspicuous maxillary and propharygeal gland. Furthermore it is difficult to comprehend the existing literature on this matter because of great inconsistency in gland nomenclature. Various authors assign different names to the same gland in different species. In all ant species investigated, the maxillary gland typically consists of secretory cells which open individually at the base of each maxilla through duct cells (Bausenwein, 1960; Emmert, 1968; Kürschner, 1971; Beck, 1972) (Fig. 1). So far, no clear purpose has been assigned to the gland. According to Bausenwein (1960) a digestive role is unlikely as the gland is not directly connected to the digestive system.

The propharyngeal gland, on the other hand, generally has been associated with digestion or trophallaxis (Bausenwein, 1960; Gösswald and Kloft, 1960; Forbes and McFarlane, 1961; Ayre, 1967; Paulsen, 1969; Beck, 1972; Billen and Peusens, 1984; Billen et al., 2013). Its secretory cells are arranged in two clusters located near the infrabuccal cavity. Each secretory cell individually opens in a pharyngeal pouch on either side of the pharynx through an associated duct cell (Bausenwein, 1960; Forbes and McFarlane,

* Corresponding author. Tel.: +32 16 32 45 76.
E-mail addresses: sofie.boonen@bio.kuleuven.be (S. Boonen), johan.billen@bio.kuleuven.be (J. Billen).

http://dx.doi.org/10.1016/j.asd.2016.04.005
1467-8039/

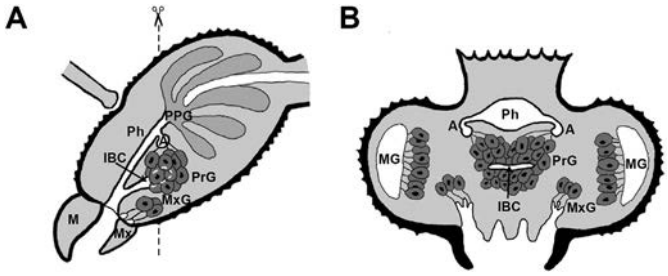

Fig. 1. Schematic drawing of a longitudinal (A) and a cross section (B) through a Pharaoh's ant's head. The scissors icon in (A) marks the position of cross section B. A = atrium, IBC = infrabuccal cavity, M = mandible, MG = mandibular gland, Mx = maxillary stipes, MxG = maxillary gland, Ph = pharynx, PPG = postpharyngeal gland, PrG = propharyngeal gland.

1961; Toledo, 1967; Kürschner, 1971; Phillips and Vinson, 1980; Gama and Cruz Landim, 1982; Amaral and Caetano, 2005; Niculita et al., 2007; Billen et al., 2013) (Fig. 1). The main secretion products consist of proteinaceous substances or digestive enzymes like invertase (Bausenwein, 1960; Ayre, 1967; Paulsen, 1969). Also an involvement in the production of larval nutrition, especially by nursing workers, has already been postulated (Otto, 1958; Beck, 1972).

In this paper, we examine the maxillary and propharyngeal glands histologically in queens, males and workers of the pharaoh's ant, *Monomorium pharaonis*, a highly polygynous and polymorphous species. In temperate regions it is often considered a pest species, because the ants prefer to nest inside heated buildings, where they can cause damage to electronic devices or transmit diseases caused by *Salmonella*, *Pseudomonas*, *Clostridium* and *Staphylococcus* (Beatson, 1972). Glands of individuals of different ages, and in case of queens also a different mating status (mated versus virgin), are analysed by means of light and electron microscopy. Furthermore, some functional implications of the morphological findings are discussed. Finally we aim to clarify inconsistencies in the nomenclature of both glands used by different authors.

2. Material and methods

Queens, males and workers were sampled from lab-reared colonies of *M. pharaonis*. Individuals were reared until they reached the age of interest. Queens were collected immediately after they eclosed and at 4, 7, 10, 14, 21, 42, 70 and 140 days after eclosion. The same age categories were used for workers and males, except for the older ages as males and workers have a much shorter lifespan than queens. On average males die within 2–3 weeks after hatching, so their age categories are limited to 0, 4, 7, 10 and 14 days. Workers can survive for up to 13 weeks after eclosion. Their age categories are therefore restricted to 0, 4, 7, 10, 14, 21 and 70 days. The different age categories were obtained by isolating dark ready-to-hatch worker, male and queen pupae from the rest of the brood. Newly hatched ants were either harvested or kept in separate boxes together with some brood and workers. The boxes were coated with fluon to prevent ants from escaping. Water and food (sugar water, boiled egg yolk, cat food, and pieces of dead flies, mealworms and locusts) were provided *ad libitum*.

To check for possible effects of mating status, virgin as well as mated queens were sampled. Mated individuals were obtained by adding an excess of virgins of the other sex. Generally, copulation begins at 4 days after eclosion. Males can inseminate 2 to 4 gynes, whereas queens can only copulate once (Berndt and Eichler, 1987). The mating status of queens was verified by dissections of their reproductive system. All inseminated queens showed a spermatheca filled with sperm.

When the ants reached the requested age, they were put in the freezer for a few minutes to anaesthetize. Subsequently, their heads were cut transversally behind the eyes and fixed for approximately 10 h in cold 2% glutaraldehyde. Next, the head tissues were buffered at pH 7.3 with 0.05 M sodium cacodylate and 0.15 M saccharose. Afterwards a post-fixation was done in 2% osmium tetroxide followed by dehydration in a graded acetone series and embedding in araldite. Finally, semithin 1 μm sections were cut with a Leica EM UC6 ultramicrotome and stained with methylene blue and thionine. The secretory cell length, secretory cell width, nucleus diameter and the number of ducts of the maxillary gland and the secretory cell length, secretory cell width, nucleus diameter, number of ducts and the atrium diameter of the propharyngeal gland were analysed through an Olympus BX-51 light microscope. The number of ducts was used as estimate for cell number. For the measurements Olympus DpSoft 3.2 was used. At least 3 individuals per caste and per age category were included in the analyses. All measurements are expressed as arithmetic means with standard deviation.

Ultrastructural investigations of 70 nm thin sections were carried out with a Zeiss EM900 electron microscope. These sections were double stained with lead citrate and uranyl acetate. At least 3 sections per caste (worker, male and queen) and age category (eclosing versus 2 weeks old) were observed.

To test for differences between castes, mating status and age categories, general linear models (ANOVA) were performed using R. Age was log(10)-transformed to obtain a best possible fit for the model. Because of a size dimorphism between queens and males on the one hand and workers on the other hand, tests were done using relative measurements that take into account differences in head width. Ten heads of each caste were measured. Worker heads (0.47 ± 0.01 μm) were found 1.27 times narrower as compared to queens and males (0.6 ± 0.01 μm). Relative data were therefore obtained by multiplying data concerning workers with this factor. Only tests with the number of ducts were carried out with absolute quantities.

3. Results

3.1. General morphology, caste differences and age-related changes

The **maxillary gland** has the same morphology in all 3 castes. No differences were observed between mated and virgin queens. It is a rather small, paired gland, situated posteriorly to the infrabuccal cavity. Each gland consists of a cluster of 4 pear-shaped secretory cells located ventrolaterally to the lower left and right tentorial arm (Fig. 2A). Occasionally, secretory cells are found medially to the tentorial arm (Fig. 3A). The maxillary gland belongs to class-3 glands according to the classification of Noirot and Quennedey (1974), in which each secretory cell is accompanied by a duct cell. The duct cells open individually near the articulation membrane that connects the maxillary stipes with the head capsule (Fig. 2B). As some duct cells appear multiple times on the same cross section, the ducts are curved. This curved shape was also observed on longitudinal sections. There is no sign of a reservoir to store secretion products. However, presumably the secretion can be temporarily stored in a pouch formed by a curve in the articulation membrane of the stipes (Fig. 2B,C).

In all 3 castes, the absolute number of maxillary gland duct cells is the same: in each caste on average 3 or 4 ducts were observed. There exist, however, caste-related differences in relative secretory cell width, relative secretory cell length and relative nucleus diameter. All 3 parameters are lower in workers as compared to

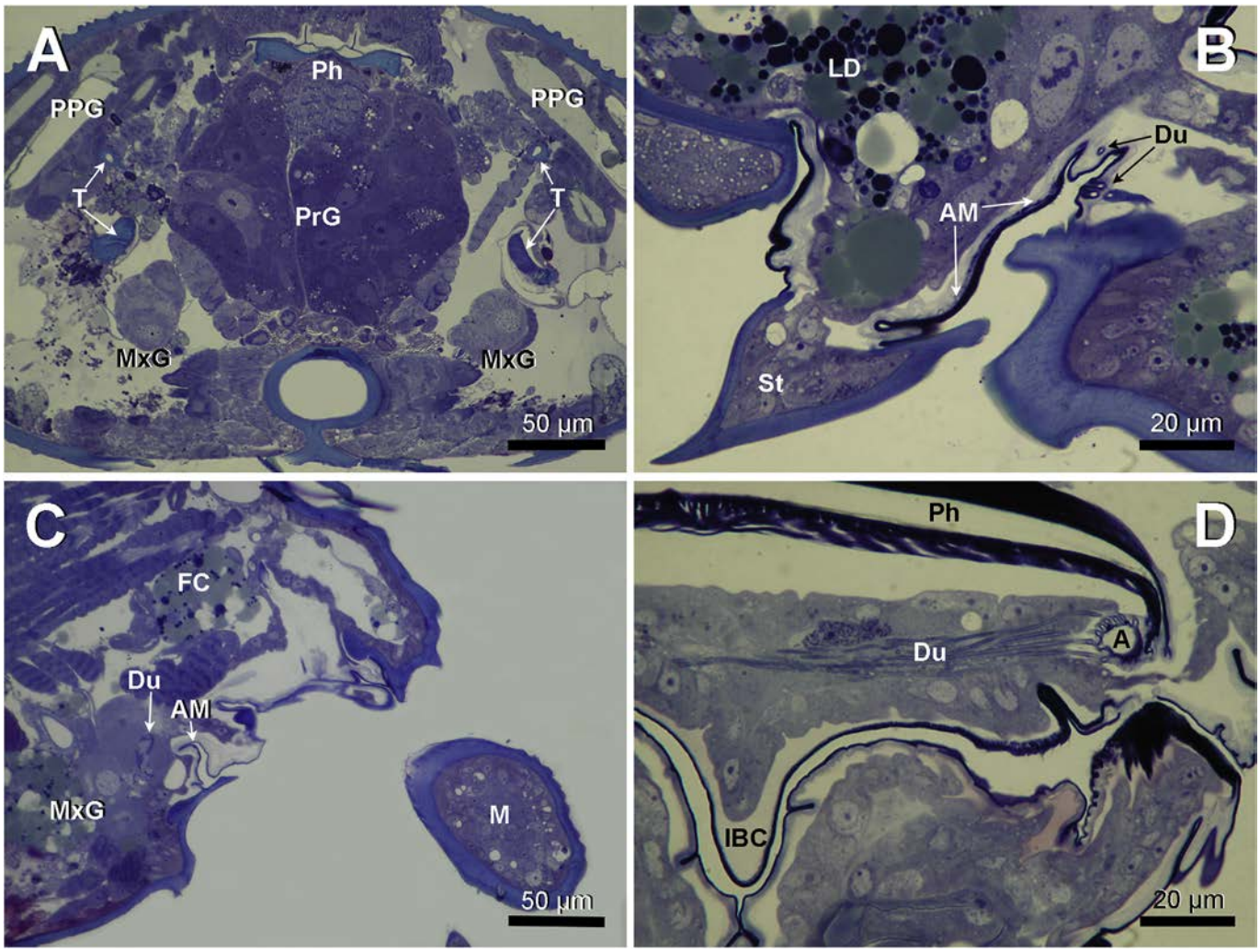

Fig. 2. (A) Cross section through 3 weeks old worker head. (B) Cross section through maxilla of 4 days old queen. Four maxillary gland duct cells open near the articulation membrane. (C) Longitudinal section through cluster of 4 maxillary gland cells of eclosing queens. (D) Cross section through pharynx of eclosing queen. Propharyngeal gland duct cells open in atrium. A = atrium, AM = articulation membrane, Du = duct cells, FC = fat cells, LD = lipid droplets, IBC = infrabuccal cavity, M = mandible, MxG = maxillary gland, Ph = pharynx, PPG = postpharyngeal gland, PrG = propharyngeal gland, St = stipes, T = tentorium.

queens and males (Table 1). No effects of the mating status of queens on secretory cell size or cell number were found.

The paired, class-3 **propharyngeal gland** consists of 2 clusters of 16 pear-shaped secretory cells situated ventrally to the pharynx (Fig. 2A, Fig. 3A–I). The infrabuccal cavity is almost entirely enclosed by the gland's secretory cells (Fig. 3B). Yet, most cells are located posteriorly to the infrabuccal cavity. Each of the secretory cells opens individually in a ventrolateral pharyngeal pouch or atrium through a duct cell. These duct cells form a sieve plate. There is no reservoir but the secretion product is stored in the atrium until it is released in the pharynx. The ducts are straight and open at the mediolateral and posterior end of each atrium (Fig. 2D). This general configuration was observed in queens (mated and virgin), males and workers.

The secretory cells of the propharyngeal gland and their nucleus diameter differ considerably in size between the 3 castes. Secretory cells of queens are significantly longer than those of males and workers. In addition, secretory cells of workers are significantly longer as compared to males. The relative secretory cell width and the nucleus diameter are also smaller in males than in queens and workers. Both parameters do not differ between the two latter castes. The relative atrium diameter is approximately 5 µm in all 3 castes. No differences in absolute number of duct cells were observed between queens, males and workers (Table 1). Additionally, none of the parameters differs between mated and virgin queens.

In eclosing ants, irrespective of caste, the 2 propharygneal gland clusters are separated by numerous fat cells. No clear vesicles are observed light microscopically in this age group (Fig. 3. A, D, G). In 4 and 7 days old ants the fat cells have completely vanished (Fig. 3E, H). A high amount of secretion vesicles appears in ants aged 10 or 14 days (Fig. 3B, F, I). In older ants most vesicles have disappeared (Fig. 3C).

3.2. Ultrastructural observations

Ultrastructural sections of the **maxillary gland** show no difference between the 3 castes (Fig. 4A–F), nor between mated versus unmated individuals. Very few vesicles and rough endoplasmic reticulum are present and only occasionally mitochondria or Golgi apparatus are seen (Fig. 4A–B). Dense microvilli surround the end apparatus in eclosing ants as well as ants aged 14 days (Fig. 4C–F).

Also in the **propharyngeal gland** no mating status-related ultrastructural changes were found. However, some changes in

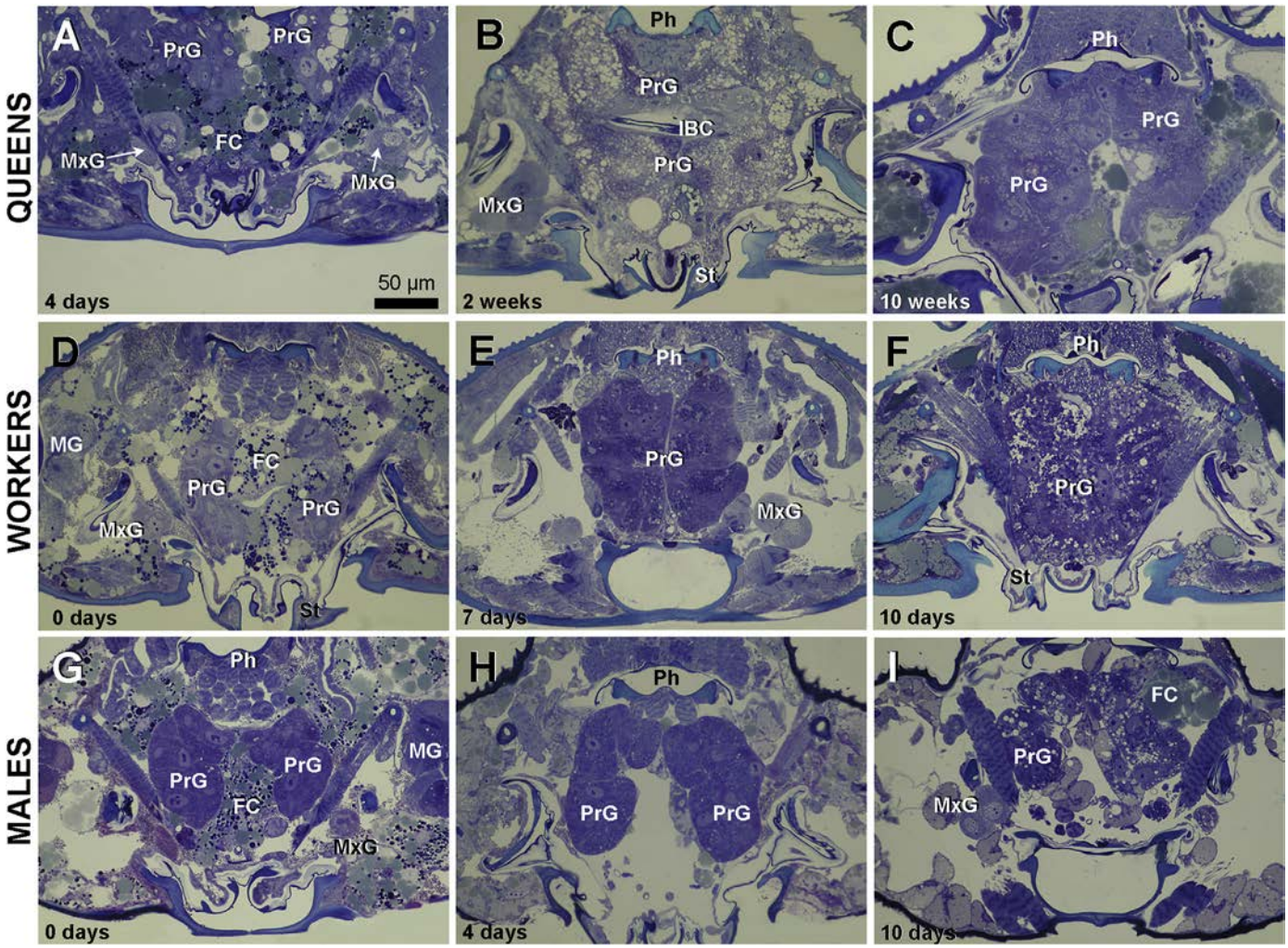

Fig. 3. Cross sections through heads of queens (A–C), workers (D–F) and males (G–I) of different aged ants. FC = fat cells, IBC = infrabuccal cavity, MG = mandibular gland, MxG = maxillary gland, Ph = pharynx, PrG = propharyngeal gland, St = stipes. The scale bar in 3A is valid for all pictures.

Table 1
Summary of measurements and p-values of caste-related differences obtained for the different parameters measured in the maxillary gland and propharyngeal gland in 3 castes of *Monomorium pharaonis* (w = workers, m = males, q = queens, rel. = relative data, abs. = absolute data).

		Average (in µm) ± standard deviation			Caste differences (p-values)		
		Queens	Males	Workers	w vs. q	w vs. m	m vs. q
Maxillary gland	Secretory cell length (rel.)	35.67 ± 1.04 µm	35.58 ± 0.83 µm	33.72 ± 1.03 µm	$<2^{-16***}$	$<2^{-16***}$	0.84
	Secretory cell width (rel.)	28.19 ± 0.71 µm	28.10 ± 0.71 µm	27.52 ± 1.11 µm	1.42^{-5***}	0.00049^{***}	0.56
	Nucleus diameter (rel.)	17.21 ± 0.82 µm	17.06 ± 0.77 µm	16.23 ± 1.21 µm	3.47^{-8***}	5.38^{-6***}	0.52
	Number of duct cells (abs.)	3.59 ± 0.85	3.53 ± 0.82	3.54 ± 0.76	0.82	0.93	0.7
Propharyngeal gland	Secretory cell length (rel.)	40.11 ± 1.15 µm	35.75 ± 0.91 µm	38.04 ± 1.71 µm	7.8^{-16***}	$<2^{-16***}$	$<2^{-16***}$
	Secretory cell width (rel.)	26.04 ± 0.83 µm	24.58 ± 0.95 µm	25.92 ± 1.12 µm	0.50	3.6^{-11***}	2.64^{-15***}
	Nucleus diameter (rel.)	15.47 ± 1.13 µm	14.3 ± 1.1 µm	15.06 ± 1.23 µm	0.067	0.0013^{**}	8.56^{-8***}
	Atrium diameter (rel.)	5.1 ± 0.81 µm	4.85 ± 0.85 µm	4.97 ± 0.66 µm	0.27	0.35	0.29
	Number of duct cells (abs.)	15.9 ± 1.93	16.19 ± 2.53	16.17 ± 1.94	0.72	0.9	0.83

number, electron density and position of vesicles and density of microvilli between castes and age categories are observed. In queens and males the microvilli of eclosing ants are much more loose as compared to 14 days old individuals (Fig. 5A, B, E, F). Microvilli of workers, on the other hand, tend to be dense in each age category (Fig. 5C–D). The vesicles differ considerably in electron density (Fig. 5A–F). Although vesicles are slightly more abundant in the two female castes (Fig. 5A–D), all 3 castes show an increase in the number of vesicles with age (Fig. 5B, D, F). Moreover, the vesicles are more abundant around the end apparatus in 14 day old ants as compared to eclosing ants (Fig. 5A–F). Numerous strains of rough endoplasmatic reticulum are present irrespective of age or caste (Fig. 5A–F).

4. Discussion

In this paper the maxillary glands and propharyngeal glands of the 3 castes of *M. pharaonis* were examined morphologically in order to understand their role in the colony. Due to the ambivalent nomenclature in literature on this topic, however, data

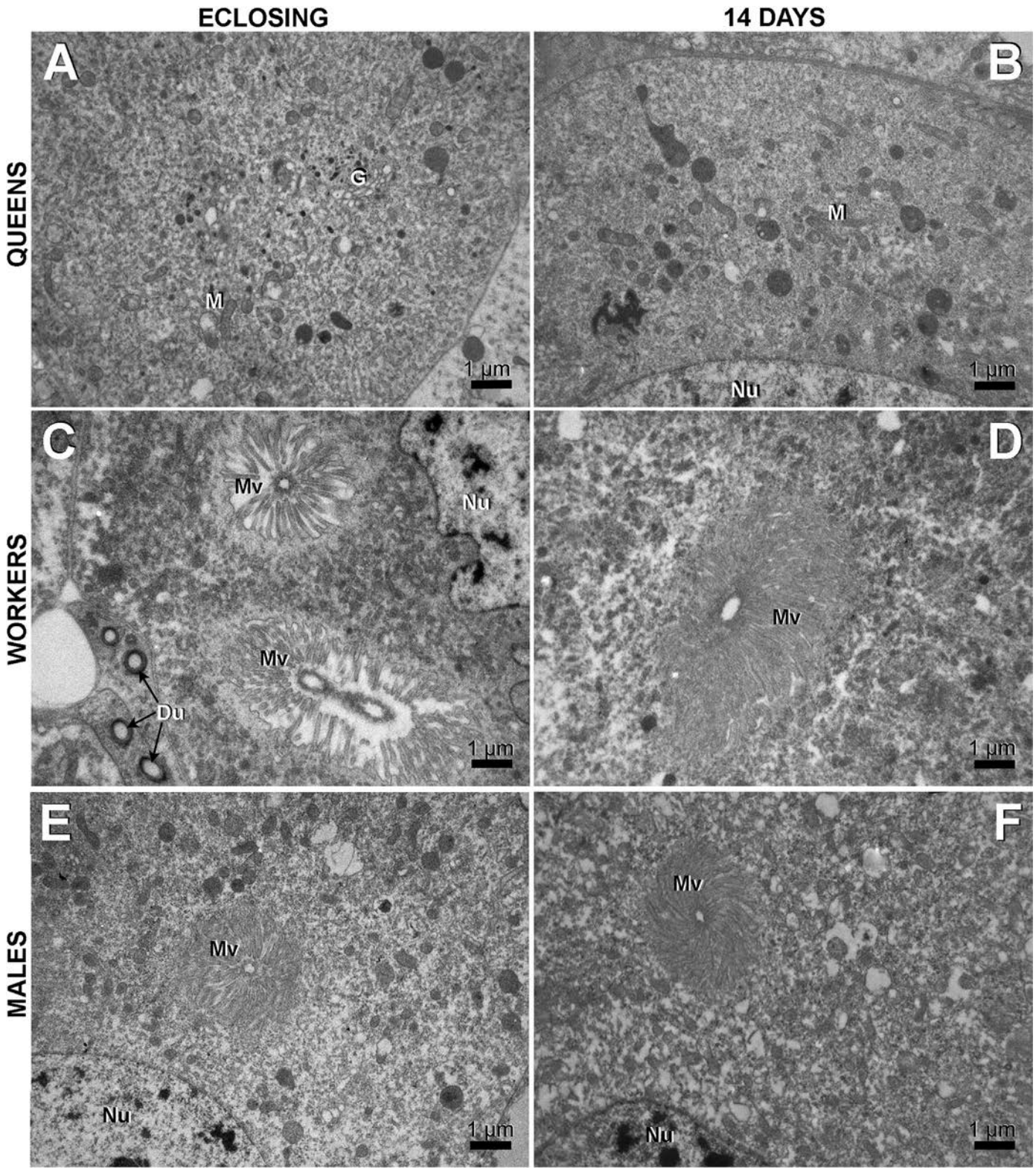

Fig. 4. Electron micrographs of maxillary glands of queens (A–B), workers (C–D) and males (E–F). Left: eclosing, right: 14 days old. Du = duct cells, G = Golgi apparatus, M = mitochondria, Mv = microvilli, Nu = nucleus, rER = rough endoplasmic reticulum.

comparison is complicated. Therefore we unravelled all inconsistencies (Table 2).

Previously, gland morphology was often based on the position of the gland in the head. Since interspecific differences in gland position are common, this is not a straightforward criterium. A classification method which relies on the site where secretion is released, results in a more straightforward nomenclature. Although Emmert (1966) in the 60's already named the gland according to the site where secretion is discharged, other authors did not follow his logic. The name 'maxillary gland' in

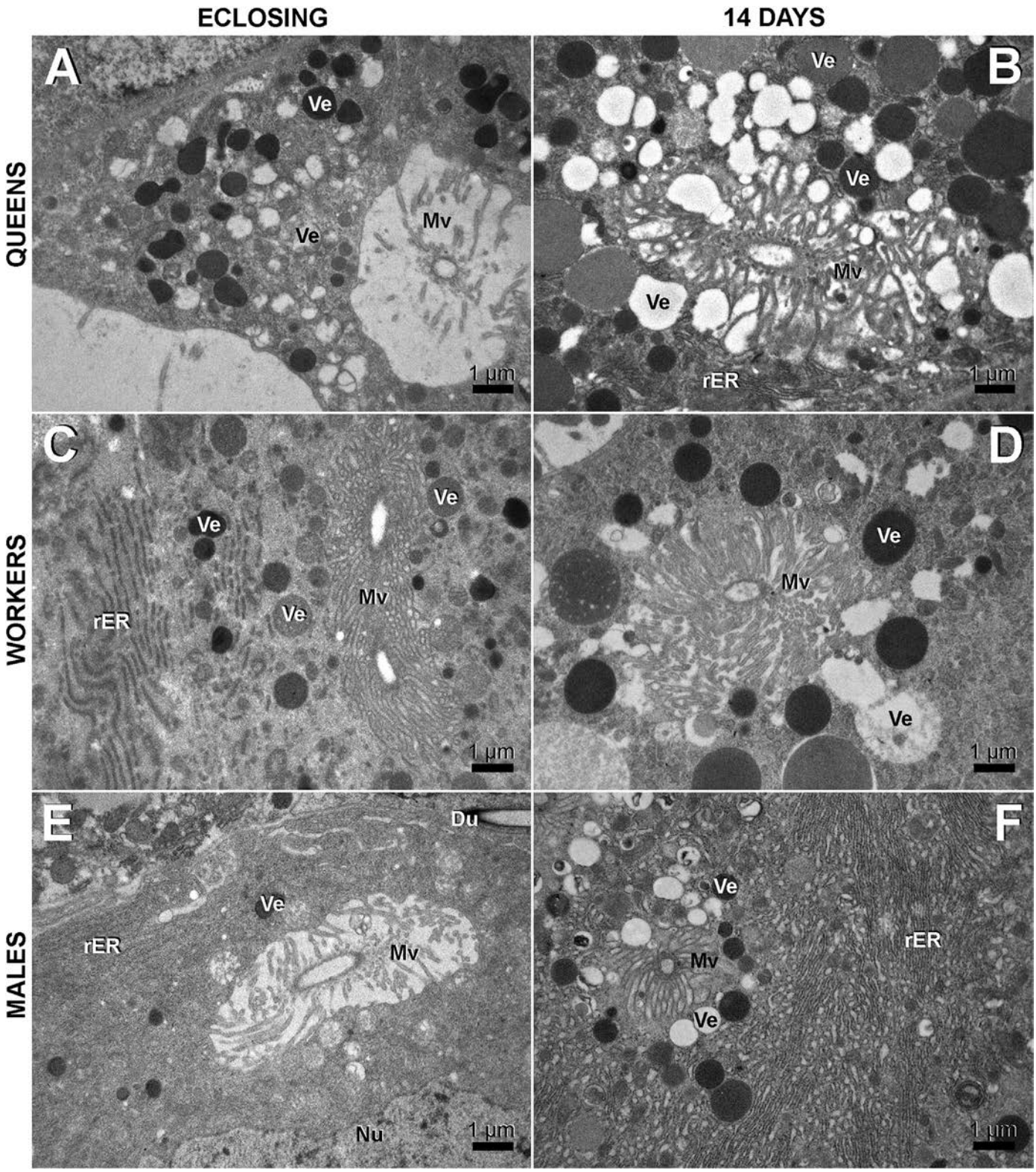

Fig. 5. Electron micrographs of propharyngeal glands of queens (A–B), workers (C–D) and males (E–F). Left: eclosing, right: 14 days old. Du = duct cells, Mv = microvilli, Nu = nucleus, rER = rough endoplasmic reticulum, Ve = vesicles.

this paper corresponds with the location where its duct cells open: at the base of the maxilla. The secretion products of the propharyngeal gland on the other hand are released in the anterior region of the pharynx.

In his morphological survey of head glands of *Formica rufa*, Bausenwein (1960) claims that the maxillary gland consists of 2 clusters of secretory cells, which open individually in a pharyngeal curve through duct cells. Based on the opening site of the ducts, it is

Table 2
Overview of gland terminology used among different authors.

Paper	Species	Terminology used	Actual gland
Confusing gland terminology			
Bausenwein (1960)	*Formica rufa*	Maxillary gland	Propharyngeal gland
Forbes and McFarlane (1961)	*Camponotus pennsylvanicus*	Maxillary gland	Propharyngeal gland
Kürschner (1971)	*Formica pratensis*	Maxillary gland	Propharyngeal gland
Phillips and Vinson (1980)	*Solenopsis invicta*	Maxillary gland	Propharyngeal gland
Toledo (1967)	*Atta sexdens*	Maxillary gland	Propharyngeal gland
Amaral and Caetano (2005)	*Atta sexdens rubropilosa*	Hypopharyngeal gland	Propharyngeal gland
Baiocco and da Cunha (1999)	*Monomorium pharaonis*	Hypopharygneal gland	Propharyngeal gland
Gama and Cruz Landim (1982)	18 species of subfamilies Ponerinae, Dorylinae, Myrmicinae, Pseudomyrmecinae, Dolichoderinae, Formicinae	Maxillary gland or hypopharyngeal gland	Propharyngeal gland
Gama (1985)	*Camponotus rufipes*	Hypopharyngeal gland	Propharyngeal gland
Bausenwein (1960)	*Formica rufa*	Maxilla basis gland	Maxillary gland
Beck (1972)	*Polyergus rufescens* and *Raptiformica sanguinea*	Maxilla basis gland	Maxillary gland
Otto (1958)	*Formica polyctena*	Tongue gland	Maxillary gland
Kürschner (1971)	*Formica pratensis*	Tongue gland	Maxillary gland
Meinert (1861)	*Myrmica sabuleti*	Throat gland	Maxillary gland
Straightforward gland terminology			
Emmert (1966)	*Formica pratensis* and *Apis mellifica*	Maxillary gland	Maxillary gland
Beck (1972)	*Polyergus rufescens* and *Raptiformica sanguinea*	Propharyngeal gland	Propharyngeal gland
Niculita et al. (2007)	*Lasius niger*	Propharyngeal gland	Propharyngeal gland
Billen et al. (2013)	*Protanilla wallacei*	Propharyngeal gland	Propharyngeal gland
Billen and Al-Khalifa (2015)	*Brachyponera sennaarensis*	Propharyngeal gland	Propharyngeal gland

clear that he actually refers to the propharyngeal gland. The same is true for Forbes and McFarlane (1961). Their description of the 'maxillary gland' in *Camponotus pennsylvanicus* clearly refers to the propharyngeal gland. It consists of 2 groups of secretory cells on either side of the pharynx, near the infrabuccal cavity. Each secretory cell has its own duct cell, which join into a main duct. The main ducts continue forward and open in the posterior lateral margins of the buccal tube. Toledo (1967), Kürschner (1971) and Phillips and Vinson (1980) also use the term 'maxillary gland' instead of 'propharyngeal gland'. In their morphological survey of head glands of 18 species of poneroid and myrmecoid ants, Gama and Cruz Landim (1982) even use maxillary gland and hypopharyngeal gland as synonyms, when in fact they describe the propharyngeal gland. Amaral and Caetano (2005) also use 'hypopharyngeal gland' instead of 'propharyngeal gland'. Hypopharyngeal glands produce royal jelly in honey bee workers (Halberstadt, 1980). Although some authors claim a homology with the propharyngeal gland of ants (Otto, 1958; Beams et al., 1959), this is not generally accepted. So far, only Emmert (1966), Beck (1972), Niculita et al. (2007), Billen et al. (2013) and Billen and Al-Khalifa (2015) used a straightforward terminology which is related to the site of secretion release.

The same confusion rises in literature concerning maxillary glands. Names like 'maxilla basis gland' (Bausenwein, 1960; Beck, 1972), 'tongue gland' (Otto, 1958; Kürschner, 1971) or even 'throat gland' (Meinert, 1861) have been used. The maxillary gland of *M. pharaonis* is a paired class-3 gland consisting of 4 secretory cells located at the base of each maxilla. Each secretory cell is accompanied by a single, curve-shaped duct cell. These ducts open in a pouch formed by a curve in the articulation membrane of the maxillary stipes. Kürschner (1971) and Beck (1972) describe a similar configuration in *Formica pratensis*, *Polyergus rufescens* and *Raptiformica sanguinea*. Presumably the secretion products are stored in the pouch before they are released to the outside (Kürschner, 1971; Beck, 1972).

Compared with other species, the maxillary gland of *Monomorium* is rather small. In *Formica rufa* Bausenwein (1960) counted 2 clusters of 50 secretory cells. *P. rufescens and R. sanguinea* on the other hand possess 8 (queens and males) to 16 (workers) secretory cells on average (Beck, 1972). We did not find caste-dependent differences in secretory cell number, yet cell sizes varied significantly between castes. Opposite to Beck (1972), secretory cells were smallest in workers, followed by males and queens. Although secretory cells tend to be larger in queens, further behavioural tests and chemical analyses are required to rule out caste-specific functions of the maxillary gland. As we did not find any effect of the mating status of queens, it is unlikely that the maxillary gland serves a reproductive purpose.

Due to the large variation in secretory cell number among species and inconsistent trends in caste-specific cell sizes it is hard to draw conclusions on the function of the maxillary gland. Its small size and low abundance of endoplasmic reticulum or other cytoplasmic organelles point to a fairly minor secretion activity and therefore presumably also a minor role in *Monomorium* colonies. An increased microvillar densification in the end apparatus in queens and males indicates a high glandular activity 2 weeks after eclosion. As the gland appears in all 3 castes, without major morphological changes according to age or mating status, we assume it serves a general purpose. A digestive function can be excluded since the gland is not directly linked to the digestive system (Bausenwein, 1960) and the concentration of rER is neglectable. Therefore it seems more likely that the maxillary gland is involved in lubricating the maxillae.

As previously found in other ant species, the propharyngeal gland of *M. pharaonis* is composed of 2 clusters of secretory cells individually connected to a ventrolateral pharyngeal atrium through duct cells (Bausenwein, 1960; Forbes and McFarlane, 1961; Toledo, 1967; Kürschner, 1971; Beck, 1972; Phillips and Vinson, 1980; Amaral and Caetano, 2005; Billen et al., 2013). These ducts show no conspicuous curves. When secretion products are released in the pharyngeal pouch, they pass through a sieve plate (Forbes and McFarlane, 1961; Gama and Cruz Landim, 1982; Billen and Al-Khalifa, 2015). The pharyngeal atrium probably serves as a storage chamber until its content is discharged into the pharynx (Amaral and Caetano, 2005; Billen et al., 2013; Billen and Al-Khalifa, 2015). The propharyngeal gland fits the description by Noirot and Quennedey (1974) of a paired class-3 gland.

Each cluster consists of 16 pear-shaped secretory cells. Similar to the maxillary gland the number of secretory cells varies considerably between species. On average 180 to 300 secretory units per cluster were observed in *Formica rufa* (Bausenwein, 1960), 200 in

Formica pratensis (Kürschner, 1971), 30 in *Brachyponera sennaarensis* (Billen and Al-Khalifa, 2015) and only 6–8 in *Protanilla wallacei* (Billen et al., 2013). Also within colonies differences in secretory cell numbers and cell sizes are common. All ant species studied so far show a caste-specific trend in cell size and/or cell number which is in favour of the queens (Bausenwein, 1960; Forbes and McFarlane, 1961; Phillips and Vinson, 1980; Amaral and Caetano, 2005; Billen et al., 2013). The total number of secretory cells in Pharaoh's ants is equal in all 3 castes. In terms of size, however, queens tend to possess larger secretory units as compared to workers and males. Whether propharyngeal glands serve a more prominent role in queens, however, cannot be excluded, as we did not compare the amount of secretion products or the chemical composition between castes. Since the mating status of queens did not affect any of the morphological parameters, involvement in reproduction is unlikely.

Within a single caste the secretory cell size of the propharyngeal gland is fixed across ants of different age categories. On the contrary, some clear changes in fat cells and vesicles are visible. Numerous fat cells and loose microvilli in most castes indicate a low glandular activity in eclosing ants. In older ants fat cells have disappeared and numerous vesicles are closely associated with the end apparatus, suggesting high levels of secretion. Various dark as well as pale vesicles are present (Bausenwein, 1960; Forbes and McFarlane, 1961; Kürschner, 1971; Beck, 1972; Billen and Al-Khalifa, 2015). The difference in electron density is probably due to the production of several kinds of secretion products. The lack of vesicles in ants aged 21 weeks or older indicates a decrease in glandular activity.

Two main functions have already been ascribed to the propharyngeal gland. Based on the observation that *Formica polyctena* ants which spend most of their time in the nest, have larger gland cells as compared to ants outside the nest, Otto (1958) postulated an involvement of the propharyngeal gland in larval feeding. This role in brood care has been further supported by other authors (Gösswald and Kloft, 1960; Forbes and McFarlane, 1961; Beck, 1972). A second hypothesis relates to a digestive function. Ayre (1967) and Paulsen (1969) reported the digestive enzyme invertase in the propharyngeal gland secretion. This idea is supported by the occurrence of abundant rER in the cytoplasm (Gama, 1985; Billen et al., 2013; Billen and Al-Khalifa, 2015), which we also observed in *Monomorium*. As the gland is connected with the pharynx, the release of digestive enzymes probably is one of its main tasks. Whether also pheromones are produced could not be confirmed. Therefore, chemical analyses and behavioural experiments are necessary to reveal the main purpose of this gland in social insect colonies.

Acknowledgements

This research was supported by FWO grant G.0699.08. We thank An Vandoren for assisting with tissue sectioning, Prof. G. Buczkowski for kindly supplying *M. pharaonis* colonies, and two anonymous reviewers for constructive comments.

References

Amaral, J.B., Caetano, F.H., 2005. The hypopharyngeal gland of leaf-cutting ants (*Atta sexdens rubropilosa*) (Hymenoptera: Formicidae). Sociobiology 46, 1–9.
Ayre, G.L., 1967. The relationship between food and digestive enzymes in five species of ants (Hymenoptera: Formicidae). Can. Entomol. 99, 408–411.
Bagnères, A.G., Morgan, E.D., 1991. The postpharyngeal glands and the cuticle of the Formicidae contain the same characteristic hydrocarbons. Experientia 47, 106–111.
Baiocco, L., da Cunha, M.S., 1999. Histologia das glândulas exócrinas em rainhas de *Monomorium pharaonis* (Linnaeus, 1758) (Hymenoptera, Formicidae). Nat. (Rio Claro) 17, 139–152.
Bausenwein, F., 1960. Untersüchungen über sekretorische Drüsen des Kopf- und Brustabschnittes in der *Formica rufa*-Gruppe. Acta Soc. Entomol. Czech 57, 31–57.
Beams, H.W., Tahmisian, T.N., Anderson, E., Devine, R.L., 1959. An electron microscope study on the pharyngeal glands of the honeybee. J. Ultrastr Res. 3, 155–170.
Beatson, S.H., 1972. Pharaoh's ants as pathogen vectors in hospitals. Lancet 19, 425–427.
Beck, V.H., 1972. Vergleichende histologische Untersuchungen an *Polyergus rufescens* Latr. und *Raptiformica sanguinea* Latr. Insect Soc. 19, 301–342.
Berndt, K.P., Eichler, W., 1987. Die Pharaoameise, *Monomorium pharaonis* (L.) (Hym., Myrmicidae). Mitt Mus. Natur. Berl. 63, 3–168.
Billen, J., Morgan, E.D., 1998. Pheromone communication in social insects – sources and secretions. In: Vander Meer, R.K., Breed, M.D., Espelie, K.E., Winston, M.L. (Eds.), Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites. Westview Press, Oxford, pp. 3–33.
Billen, J., Peusens, G., 1984. Ultrastructure de la glande propharyngienne chez les fourmis formicines (Hymenoptera Formicidae). Actes Coll. Insect Soc. 1, 121–129.
Billen, J., Bauweleers, E., Hashim, R., Ito, F., 2013. Survey of the exocrine system in *Protanilla wallacei* (Hymenoptera, Formicidae). Arthropod Struct. Dev. 42, 173–183.
Billen, J., Al-Khalifa, M.S., 2015. Morphology and ultrastructure of the pro-and postpharyngeal glands in workers of *Brachyponera sennaarensis*. Sociobiology 62, 276–281.
Boonen, S., Eelen, D., Børgesen, L., Billen, J., 2012. Functional morphology of the mandibular gland of queens of the ant *Monomorium pharaonis* (L.). Acta Zool. 94, 373–381.
Brough, J.E., 1978. The multifunctional role of the mandibular gland secretion of an Australian desert ant *Calomyrmex* (Hymenoptera, Formicidae). Z Tierpsych 46, 279–297.
Butler, C.G., Simpson, J., 1958. The source of the queen substance of the honey bee (*Apis mellifera* L.). Proc. R. Entomol. Soc. Lon 33, 120–122.
Butler, C.G., Fairey, E.M., 1963. The role of the queen in preventing oogenesis in worker honey bees. J. Apic. Res. 2, 14–18.
Crewe, R.M., Blum, M.S., 1970. Alarm pheromones in the genus *Myrmica* (Hymenoptera: Formicidae). J. Comp. Physiol. 70, 363–373.
Eelen, D., Børgesen, L., Billen, J., 2004. Functional morphology of the postpharyngeal gland of queens and workers of the ant *Monomorium pharaonis* (L.). Acta Zool. 87, 101–111.
Emmert, W., 1966. Die Morphogenese sekretorischer Drüsen des Kopf-und Thoraxbereichs von Formica pratensis Retz und Apis mellifica L. (Ins. Hym.) (im Verlauf der Postembryonalentwicklung. Diss. Würzburg).
Emmert, W., 1968. Die Postembryonalentwicklung sekretorischer Kopfdrüsen von *Formica pratensis* Retz. und *Apis mellifica* L. (Ins., Hym.). Z Morphol. Tiere 63, 1–62.
Forbes, J., McFarlane, A.M., 1961. The comparative anatomy of digestive glands in the female castes and the male of *Camponotus pennsylvanicus* Degeer (Formicidae, Hymenoptera). J. N. Y. Entomol. Soc. 69, 92–103.
Gama, V., 1985. O sistema salivar de *Camponotus rufipes* (Fabricius, 1775) (Hymenoptera: Formicidae). Rev. Bras. Biol. 45, 317–359.
Gama, V., Cruz Landim, C., 1982. Da estudo comparative das glândulas do sistema salivar de formigas (Hymenoptera, Formicidae). Naturalia 7, 145–165.
Gösswald, K., Kloft, W., 1960. Neuere Untersuchungen über die sozialen Wechselbeziehungen im Ameisenvolk, durchgeführt mit Radioisotopen. Zool. Beitr 5, 519–556.
Halberstadt, K., 1980. Elektrophoretische Untersuchungen zur Sekretionstätigkeit der Hypopharynxdrüse der Honigbiene (*Apis mellifera* L). Insect Soc. 27, 61–77.
Hoover, S.E.R., Keeling, C.I., Winston, M.L., Slessor, K.N., 2003. The effect of queen pheromones on worker honey bee ovary development. Naturwissenschaften 90, 477–480.
Kürschner, I., 1971. Zur Anatomie von *Formica pratensis* Retzius, Morphologische Untersuchungen der sekretorischen Kopfdrüsen (Postpharynxdrüse, Maxillardrüse, Mandibulardrüse, Zungendrüse) und der am Kopf ausmündenden Labialdrüse. Beitr Entomol. 21, 191–210.
Meinert, F., 1861. Bidrag til de danske Myrers Naturhistorie. Kongl Dankse Vidensk. Selsk. Skr. 5, 273–341.
Moser, J.C., Brownlee, R.C., Silverstein, R., 1968. Alarm pheromones of the ant *Atta texana*. J. Insect Physiol. 14, 529–530.
Niculita, H., Billen, J., Keller, L., 2007. Comparative morphology of cephalic exocrine glands among castes of the black ant *Lasius niger*. Arthropod Struct. Dev. 36, 135–141.
Noirot, C., Quennedey, A., 1974. Fine structure of insect epidermal glands. Annu. Rev. Entomol. 19, 61–80.
Otto, D., 1958. Uber die Homologieverhältnisse der Pharynx-und Maxillardrüsen bei Formicidae und Apidae (Hymenoptera). Zool. Anz 161, 216–226.
Paulsen, R., 1969. Zur Funktion der Propharynx-, Postpharynx- und Labialdrüsen von *Formica polyctena* Foerst. (Hymenoptera, Formicidae). Diss. Würzbg. 1–90.
Phillips, S.A., Vinson, S.B., 1980. Comparative morphology of glands associated with the head among castes of the red imported fire ant, *Solenopsis invicta* Buren. J. Ga. Entomol. Soc. 15, 215–226.
Toledo, L.F.A., 1967. Histo-anatomia de glândulas de *Atta sexdens rubropilosa* Forel (Hymenoptera). Arq. Inst. Biol. 34, 321–329.
Vander Meer, R.K., Morel, L., 1998. Nestmate recognition in ants. In: Vander Meer, R.K., Breed, M.D., Espelie, K.E., Winston, M.L. (Eds.), Pheromone Communication in Social Insects. Ants, Wasps, Bees and Termites. Westview Press, Boulder, pp. 79–103.