Pergamon

Int. J. Insect Morphol. & Embryol., Vol. 27, No. 2, pp. 121–128, 1998

Printed in Great Britain
0020-7322/98 $19.00+0.00

PII: S0020-7322(97)00026-3

DISTRIBUTION AND COMPARATIVE MORPHOLOGY OF THE CLOACAL GLAND IN ANTS (HYMENOPTERA: FORMICIDAE)

Tom Wenseleers[1], Eric Schoeters[1], Johan Billen[1]* and Rüdiger Wehner[2]

[1]Zoological Institute, University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium; [2]Zoologisches Institut, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

(*Received* 20 *February* 1997; *accepted* 18 *July* 1997)

Abstract—The cloacal gland is a paired exocrine structure, which has so far been described only in the formicine species, *Camponotus ephippium* and *Cataglyphis savignyi* (Hymenoptera : Formicidae). The gland is formed by 2 clusters of bicellular units with slender duct cells, releasing the glandular secretion through the cloacal membrane. In the present work, a number of ant species, largely of the Formicinae subfamily, have been surveyed for the presence of a cloacal gland. The gland is present in nearly all formicines screened, albeit with a variable development. *Cataglyphis*, one of the genera with a very prominent cloacal gland, was chosen for a more detailed comparative study. At the ultrastructural level, secretory cells were observed having a well-developed smooth endoplasmic reticulum and Golgi apparatus, typical for pheromone-producing glandular cells. The gland is also present in all dolichoderines screened, but in none of the species of the Aneuretinae, Myrmeciinae, Myrmicinae, Nothomyrmeciinae, or Pseudomyrmecinae investigated. This provides tentative evidence that the cloacal gland is a synapomorphy of the Formicinae and Dolichoderinae, giving support for their hypothesized sister group relationship. Up to now, the function of the cloacal gland remains largely enigmatic. © 1998 Elsevier Science Ltd. All rights reserved.

Index descriptors (in addition to those in the title): ultrastructure; exocrine glands; sternal glands; phylogeny; scanning electron microscopy.

INTRODUCTION

Exocrine glands are known to perform a variety of functions, ranging from the elaboration of saliva to antibiotics, or from lubricants to defensive substances. The highly complex social system in Hymenoptera almost completely relies on pheromone-based communication (reviewed by Hölldobler and Wilson, 1990). As a result, a massive and very complex exocrine system has evolved in social insects. In ants, for example, at least 39 different exocrine glands have been described so far (Billen, 1994). Several of these represent a "standard exocrine set" (Fig. 1), whereas others are thought to have only a limited distribution. Among the latter is the cloacal gland, first described by Hölldobler (1982) in *Camponotus ephippium*, and subsequently also found in *Cataglyphis savignyi* (Billen, 1989). The gland consists of 2 clusters of secretory cells located underneath the 7th abdominal sternite, which release their secretion into the cloacal chamber through slender duct cells. Little is known about its function, although it was found to be involved in recruitment in *C. ephippium* (Hölldobler, 1982) and in territorial marking in *Cataglyphis niger* (Wenseleers *et al.*, in preparation).

The aim of the present contribution is to bring a comparative and fine structural examination of the cloacal gland in 8 species of the genus *Cataglyphis*, in which we found the gland to be very pronounced. Moreover, data on their phylogenetic position within the genus are available (Agosti, 1990), which are necessary to assess the extent of phylogenetic inertia. Additionally, we examined species of the Formicinae and Dolichoderinae sister subfamilies (Baroni Urbani *et al.*, 1992) and the Aneuretinae, Myrmeciinae, Myrmicinae, Nothomyrmeciinae, and Pseudomyrmecinae outgroup subfamilies, for the presence of cloacal glands.

*Author to whom correspondence should be addressed. Tel: +32 16 32 39 75; Fax: +32 16 32 45 75; E-mail: johan.billen@bio.kuleuven.ac.be.

MATERIALS AND METHODS

Collection of material

Workers of the following ant species were collected and examined for the presence of cloacal glands: Aneuretinae: *Aneuretus simoni* (Gilimale, Sri Lanka), Dolichoderinae: *Azteca alfari* (Manaus, Brasil), *Dolichoderus doriae* (Mongarlowe, Australia), *Dolichoderus quadripunctatus* (Strasbourg, France), *Dolichoderus* sp. (W. Malaysia), *Iridomyrmex purpureus* (Canberra, Australia), *Leptomyrmex erythrocephalus* (Mongarlowe, Australia), Formicinae: *Acropyga myops* (W. Nelligen, Australia), *Cataglyphis bicolor* (Maharès, Tunisia), *Cataglyphis bombycinus* (Metlaoui-El Kriz, Tunisia), *Cataglyphis cursor* (Banyuls, France), *Cataglyphis fortis* (Maharès, Tunisia), *Cataglyphis mauritanicus* (Grombalia, Tunisia), *Cataglyphis niger* (Tel Aviv, Israel), *Cataglyphis savignyi* (Chebika, Tunisia), *Cataglyphis viaticus* (Soliman, Tunisia), *Lasius fuliginosus* (Schoten, Belgium), *Formica rufa* (Zonhoven, Belgium), *Formica sanguinea* (Zonhoven, Belgium), *Melophorus* sp. (Canberra, Australia), *Oecophylla longinoda* (Gazi, Kenya), *Oecophylla smaragdina* (Colombo, Sri Lanka), *Paratrechina minutula* (Misty Mountain, Australia), *Plagiolepis pygmaea* (Calvi, France), *Polyrhachis schistacea* (Ukunda, Kenya), *Prolasius brunneus* (Captains Flat, Australia), *Proformica* sp. (Barcelona, Spain), Myrmeciinae: *Myrmecia pilosula* (Canberra, Australia), Myrmicinae: *Aphaenogaster spinosa* (Calvi, France), *Atta sexdens* (Viçosa, Brasil), *Crematogaster scutellaris* (Barcelona, Spain), *Myrmica sabuleti* (Zonhoven, Belgium),

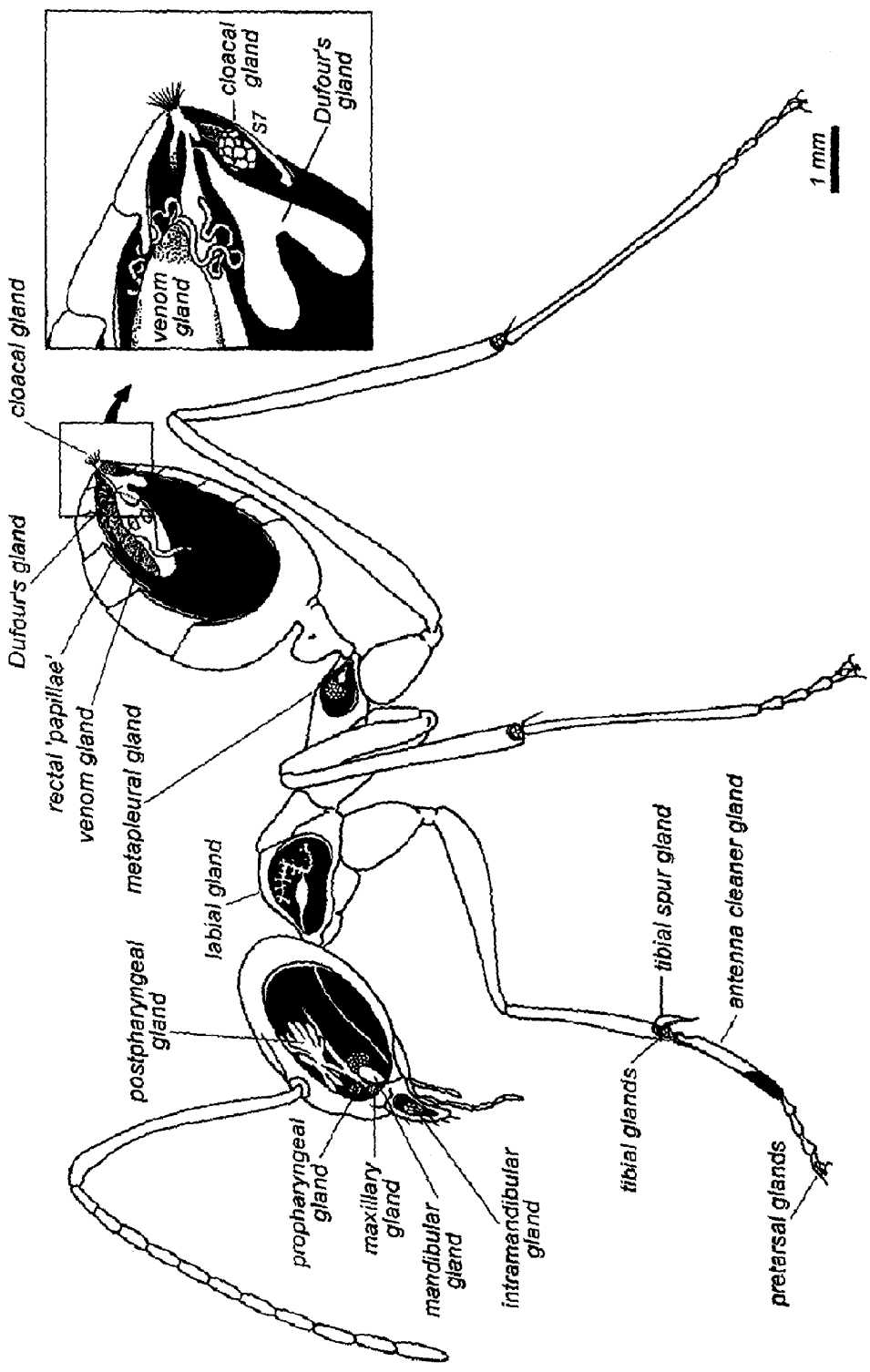

Fig. 1. Schematic drawing of a *Cataglyphis* worker, showing the location of the various exocrine glands. The inset shows an enlargement of the abdominal tip, with the position of the cloacal gland indicated.

Solenopsis invicta (Gainesville, Florida, U.S.A.), Nothomyrmeciinae: *Nothomyrmecia macrops* (Poochera, Australia), Pseudomyrmecinae: *Pseudomyrmex* sp. (San Juan, Mexico), *Tetraponera punctulata* (Laing, New Guinea).

Histology and ultrastructure

For size measurements, the glands were carefully dissected and the surrounding tissues removed. For all *Cataglyphis* species included, the diameter of each glandular cluster and standard head width (HW) of 6 randomly sampled workers were measured, using a Wild M5 stereo microscope and a Vidas® image analysis system. For interspecies comparisons, cloacal gland development was calculated in terms of relative development, i.e., as cloacal gland diameter relative to standard head width (see Table 1).

Glands for histology and ultrastructure were fixed in 2% cold glutaraldehyde, buffered at pH 7.3 with 0.05 M sodium cacodylate and 0.15 M saccharose added, and postfixed in 2% osmium tetroxide. After dehydration in a graded acetone series and embedding in Araldite, sectioning was done using a Reichert Ultracut E microtome. Serial semithin sections allowed estimates of the number of secretory cells per glandular cluster. After double-staining in an LKB 2168 Ultrostainer, thin sections were examined in a Zeiss EM900 microscope. For scanning electron microscopy, glands were dehydrated in an ethanol series after postfixation, critical point dried and viewed in a Philips SEM515 microscope.

RESULTS

Comparative morphology and ultrastructure in the genus Cataglyphis

The comparative morphological work, with focus on *Cataglyphis*, reveals the presence of a prominent cloacal gland in the workers of all species studied (Table 1). The gland consists of 2 clusters of secretory cells (Fig. 2(A)), each cell with an associated duct cell that carries the secretory products to the cloacal chamber through a narrow cuticular duct (Fig. 2(B, C)). Both within each species and among the different species, a positive correlation was found between gland diameter and worker size (based upon HW) (within-species regression: $R^2 = 0.62$, $n = 6$, $P < 0.001$; between-species regression: $R^2 = 0.66$; $P < 0.01$). Therefore, interspecies comparisons of cloacal gland development are always based on relative development, i.e., cloacal gland diameter relative to standard head width. The following order of increasing relative size could be observed: 1. *cursor*, 2. (*bombycinus*, *viaticus*), 3. *fortis*, 4. *bicolor*, 5. *mauritanicus*, 6. (*savignyi*, *niger*) (Table 1). The numbers of secretory cells per cluster show a relatively similar pattern, ranging from approximately 10 in a minor worker of *C. bombycinus* to approximately 40 in a major worker of *C. savignyi*. The inclusion of the closely related genus *Proformica* (Agosti, 1991, 1994), used as an outgroup, indicates that a weakly developed cloacal gland is possibly the ancestral state in *Cataglyphis* (Table 1).

The general morphology of the cloacal gland of the *Cataglyphis* species investigated corresponds to the glandular organization with type-3 secretory cells, as described by Noirot and Quennedey (1974, 1991). Each glandular unit is thus formed by a bicellular system consisting of a secretory cell associated with a slender duct cell (Fig. 2(C) and Fig. 3(A)). The spherical secretory cells are approximately 10 μm long, and possess an eccentrically located nucleus. A thin 10 nm thick basement membrane surrounds the plasmalemma of the entire secretory cell, except near the apical part where it is in contact with the duct cell (Fig. 3(A)). The apical membrane of the secretory cells forms a long and sinuous central canal lined with microvilli and encloses a perforated cuticular ductule. The combination of this ductule and its surrounding microvillar sheath is known as the intracellular end apparatus (Fig. 3(A, E)). Microvillar length varies from 0.9 to 1.7 μm. Apically, the microvilli show an electron-dense zone. The cuticular ductule consists of a basal procuticle (thickness 350 nm) and an apical epicuticle (thickness 30 nm). The former is in turn composed of a basal electron-lucent endocuticle (thickness 150–200 nm) and an apical fibrillar exocuticle (thickness 150 nm; diameter of fibrillae 20 nm) (Fig. 3(E)). The ductular lumen shows an invariable diameter of 0.3 μm. The intracellular ductule continues its course as a non-

Table 1. Comparative development of the cloacal gland in the genus *Cataglyphis* and the outgroup genus *Proformica*

Species group	Species	Mean gland diameter ± SD (μm)	Number of secretory cells	Relative gland development ± SD*
albicans group	*Cataglyphis fortis*	100 ± 20	10–20	6.5 ± 2.0
altisquamis group	*C. mauritanicus*	120 ± 30	15–25	8.5 ± 1.5
bicolor group	*C. bicolor*	140 ± 30	25–30	7.5 ± 0.5
	C. niger	160 ± 40	30–40	9.0 ± 1.0
	C. savignyi	160 ± 40	30–40	9.0 ± 1.0
	C. viaticus	100 ± 30	10–20	6.0 ± 1.0
bombycinus group	*C. bombycinus* workers	90 ± 20	10–15	6.0 ± 0.5
	C. bombycinus soldiers	150 ± 10	10–15	5.0 ± 0.5†
cursor group	*C. cursor*	80 ± 20	10–20	5.5 ± 1.0
	Proformica sp.	50 ± 20	3–8	5.0 ± 0.5

*Relative gland development is calculated here as gland diameter divided by standard head width multiplied by 100 (in all cases, $n = 6$). With the exception of *C. fortis*, the intraspecific variation (i.e., the given SD squared) is always smaller than the interspecific variation (2.3).

†The disproportionally large head of the soldiers probably overcorrects for worker size.

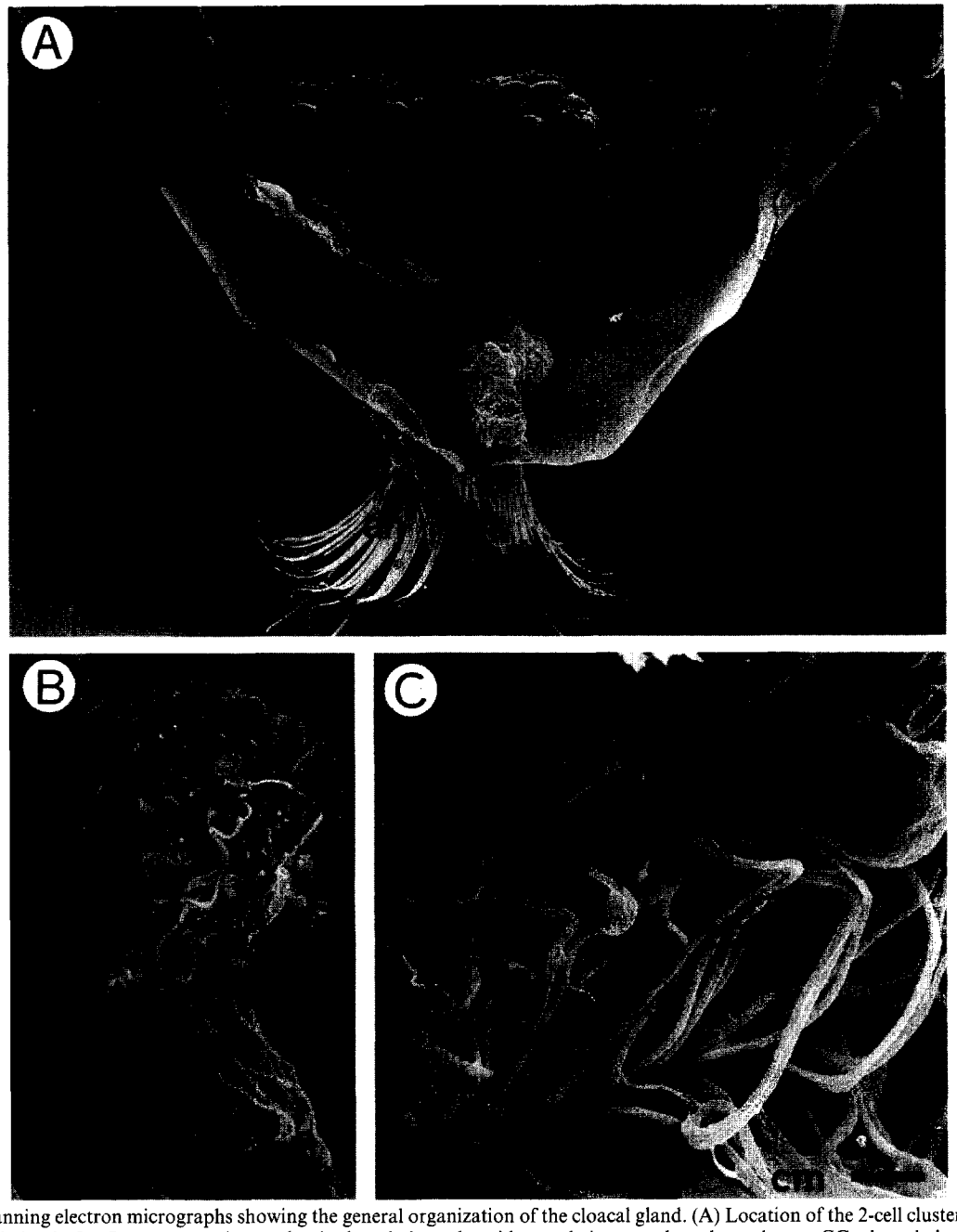

Fig. 2. Scanning electron micrographs showing the general organization of the cloacal gland. (A) Location of the 2-cell clusters forming the cloacal gland of a *C. savignyi* major worker in dorsal view. ah, acidopore hairs; cm, cloacal membrane; CG, cloacal gland; S7, 7th sternite. Bar = 100 μm. (B) Cluster of secretory cells with elongated duct cells in a *C. bicolor* major worker. DC, duct cells; SC, secretory cells. Bar = 20 μm. (C) Detail of duct cells in *C. savignyi* with thickening where nuclei are situated. cm, cloacal membrane; DC, duct cells; N, nuclei of duct cells; SC, secretory cells. Bar = 10 μm.

perforated cuticular duct inside the duct cell. The cuticle of this duct is twice as thick as that of the ductule (Fig. 3(A)).

The granular endoplasmic reticulum of the secretory cell is strongly reduced in all species, and ribosomes are encountered only as polysomes, not as free ribosomes. The tubular smooth endoplasmic reticulum and Golgi apparatus are much better developed, although development was variable both among different individuals as among different secretory cells of the same individual. Sometimes, the cytoplasm of the secretory cells reveals a weakly developed tubular smooth endoplasmic reticulum and Golgi apparatus (diameter 0.8 μm), with only few small mitochondria with an electron-dense matrix. In these cases, the microvilli of the end apparatus are always tightly hexagonally packed, without any noticeable distortion otherwise caused by the accumulation of secretion. These are all indications of a low secretory activity. In some specimens though, a higher activity could be inferred from a distortion of the microvillar arrangement of the end apparatus through the accumulation of electron-lucent secretion. Actual transport of

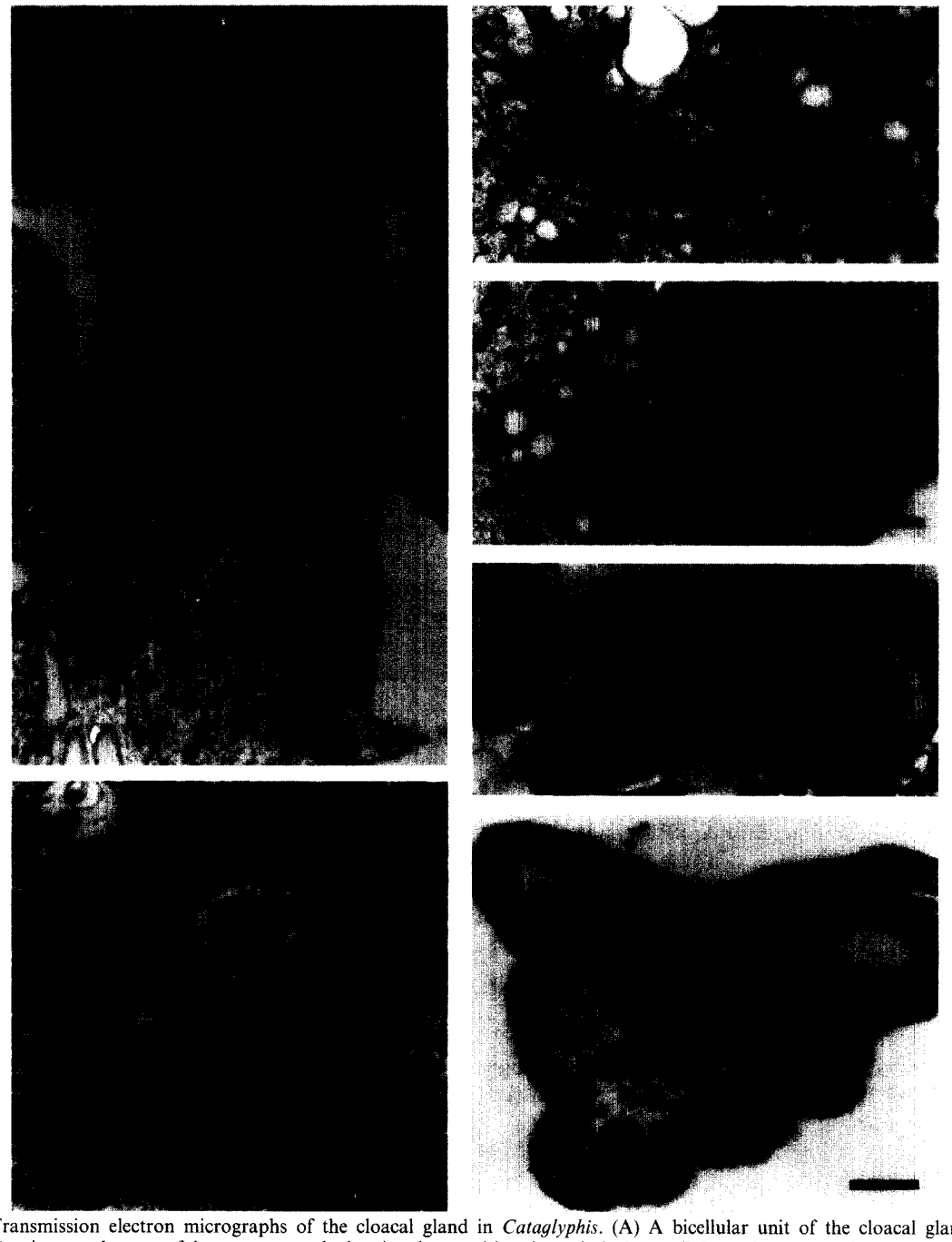

Fig. 3. Transmission electron micrographs of the cloacal gland in *Cataglyphis*. (A) A bicellular unit of the cloacal gland of *C. mauritanicus* in an early stage of the secretory cycle showing the transition through the intracellular end-apparatus between the secretory cell and the duct cell. Note the compact hexagonal arrangement of the microvilli of the end-apparatus and the absence of secretory vesicles. DC, duct cell; SC, secretory cell; N, nucleus; mv, microvilli. Bar = 2 μm. (B) Secretory cell of the cloacal gland of *C. fortis* in a later stage of the secretory cycle showing a well-developed Golgi apparatus, mitochondria with an electron-lucent matrix and a multitude of secretory vesicles. GA, Golgi apparatus; sv, secretory vesicles. Bar = 0.1 μm. (C) Secondary lysosomes forming in a secretory cell of the cloacal gland of *C. mauritanicus*. SL, secondary lysosomes. Bar = 1 μm. (D) Multilamellar bodies and lipid vesicles in a secretory cell (*C. fortis*). MLB, multilamellar body. Bar = 1 μm. (E) A multilamellar body being secreted apically through the microvillar border of the end apparatus into the extracellular space (*C. mauritanicus*). MLB, multilamellar body. Bar = 1 μm. (F) Duct cell of the cloacal gland showing three sections of the cuticular duct within the same cell (*C. viaticus*). Bar = 1 μm.

secretory material (mainly consisting of electron-lucent vesicles and multilamellar bodies) towards the extracellular space in between the ductule and the microvillar sheath (thus acting as a temporary storage site) was also observed (Fig. 3(E)). Moreover, obvious changes could be noted in the cytoplasm of the secretory cell: an increase in number of pinocytotic vesicles at the basal part of the cell, an increase in size of both the Golgi apparatus and the smooth endoplasmic reticulum, frequent occurrence of electron-lucent secretory vacuoles (Fig. 3(B)) and some mitochondria gaining an electron-lucent matrix. Pinocytotic vesicles (diameter 0.25 μm) are sometimes seen

fusing with vesicles originating from the Golgi apparatus, resulting in the subsequent formation of primary and secondary lysosomes (Fig. 3(C)). The latter are primarily made up of multilamellar bodies (Fig. 3(D)), consisting of a central electron-lucent core containing some electron-dense granules and surrounded by multiple membrane layers.

The duct cells possess an irregularly shaped, spindle-like nucleus (approx. 7×3 μm). The cytoplasm is usually more electron-lucent than that of the secretory cell (Fig. 3(A)). Some free ribosomes occur, together with small mitochondria (diameter 0.1 μm, length max. 0.5 μm) containing an electron-dense matrix. A basement membrane (thickness 80 nm) lines the entire duct cell and is continuous with that of the secretory cell. Sometimes, multiple sections through cuticular ducts could be seen within the same duct cell (Fig. 3(F)).

Distribution of the cloacal gland in ants

A systematic survey for the presence of a cloacal gland in the (Formicinae + Dolichoderinae + Aneuretinae) clade reveals that it occurs not only in the majority of Formicinae screened, but also in all Dolichoderine species included, the hypothesized sister group of the Formicinae (Table 2). Among the Formicinae, the 2 species of *Oecophylla* were the only exceptions in which no cloacal gland could be found. A cloacal gland was absent in *A. simoni*, the sole member comprising the subfamily of the Aneuretinae, and together with the Formicinae and Dolichoderinae making up an unresolved trichotomy (Baroni Urbani *et al.*, 1992). Because so little was formerly known about the distribution of the cloacal gland in ants, we took the conservative step of including a limited number of members of the (Myrmeciinae + Myrmicinae + Nothomyrmeciinae + Pseudomyrmecinae) "sister branch" subfamilies (Baroni Urbani *et al.*, 1992) for use as outgroups. In none of these was a cloacal gland found.

DISCUSSION

Although a relationship between worker size and size of the gland, and a certain amount of interspecies variability in the relative size of the gland could be detected, ultrastructurally the cloacal gland revealed a relatively uniform pattern in all *Cataglyphis*, with a well-developed smooth endoplasmic reticulum and Golgi apparatus, which is typical of pheromone-producing glandular cells. The relationship between worker size class and exocrine gland size has repeatedly been used to gain insight into the functional aspect of glands, especially for those which cannot easily be studied experimentally (e.g., Phillips and Vinson, 1980; Wilson, 1980; Schoeters and Billen, 1990; Soroker *et al.*, 1995). The relationship between worker size and size of the cloacal gland in all *Cataglyphis* species we investigated was approximately isometric. Given the limited sample size, the function of the gland could not therefore be shown to be associated with any specific worker size class.

A greater than average development might also be associated with task specialization (in *Cataglyphis* probably coinciding with age; Bonavita-Cougourdan and Morel, 1984; Mayade and Suzzoni, 1990), as indicated by a recent morphological study of the cloacal gland in *C. niger* (Wenseleers *et al.*, 1996). The foragers of this species were shown to have a cloacal gland of larger relative diameter than the intranidal workers, which is consistent with the observed function in territorial marking in this species (Wenseleers *et al.*, in preparation). This in turn agrees with the present ultrastructural study; a well-developed smooth endoplasmic reticulum and Golgi apparatus is indicative of the elaboration of a non-proteinaceous secretion, most probably of lipophilic and possibly of pheromonal nature. Indeed, electron-lucent vesicles and conspicuous multilamellar bodies were observed in association with the microvillar border of the end apparatus, and both are known to be related to the elaboration of a pheromonal secretion (Hefetz and Orion, 1982; Billen, 1991). The obvious intercellular and inter-individual differences in secretory activity observed, might indicate the presence of a secretory cycle (Bazire-Bénazet and Zylberberg, 1979). More work on individuals of known age would, however, be required to elaborate on this. In any case, the intercellular differences in secretory activity imply an asynchronous secretory activity, which is relatively rare in hymenopteran tegumentary glands (Bazire-Bénazet and Zylberberg, 1979; Delfino *et al.*, 1983).

Another interesting observation at the ultrastructural level concerned the multiple sections through cuticular ducts seen within the same duct cell. This is most readily explained by a highly sinuous organization, because in the ontogeny of exocrine glands with type-III secretory units only one duct cell for each secretory cell is formed from a ciliar precursor (Sreng, 1979). Moreover, our scanning electron micrographs clearly show that in all cases only one duct cell is associated with each secretory cell.

Comparison of relative development of the cloacal gland in *Cataglyphis* (Tables 1 and 2) indicates that phylogenetic inertia at the generic level is relatively low, e.g., in *Cataglyphis*, *C. mauritanicus* has a very prominent cloacal gland, whereas *C. cursor*, belonging to a sister species group (Wehner, 1983; Agosti, 1990; Keegans *et al.*, 1992; Wehner *et al.*, 1995), has the least-developed cloacal gland (Table 1). Also within the *bicolor* species group, variation in the relative development of the gland is almost as large as that observed in the entire genus (Table 1). At the generic level, the observed pattern agrees more closely with known phylogenetic relationships. In the *Formica* genus group, a gradual increase can be noted from *Formica* up to *Proformica* and *Cataglyphis* (Table 2), completely coinciding with their phylogenetic position (Emery, 1912; Agosti, 1990). Therefore, relative development of the cloacal gland can be expected to confer

Table 2. Occurrence and development of a cloacal gland in the (Formicinae + Dolichoderinae + Aneuretinae) branch of ant subfamilies, including the (Myrmeciinae + Myrmicinae + Nothomyrmeciinae + Pseudomyrmecinae) "sister branch" outgroup subfamilies (see Baroni Urbani *et al.*, 1992)

Subfamily genus group	Species	Cloacal gland	Number of secretory cells
Aneuretinae	*Aneuretus simoni*	No	–
Dolichoderinae	*Azteca alfari*	Yes	10–15
	Dolichoderus doriae	Yes	25–35
	D. quadripunctatus	Yes	12–20
	Dolichoderus sp.	Yes	15–25
	Iridomyrmex purpureus	Yes	5–10
	Leptomyrmex erythrocephalus	Yes	10–15
Formicinae			
Formica g.g.	*Camponotus ephippium*	Yes*	Not reported
	Cataglyphis spp.	Yes	10–40
	Formica rufa	Yes	2–3
	F. sanguinea	Yes	2–3
	Melophorus sp.	Yes	30–40
	Polyrhachis schistacea	Yes	7–10
	Proformica sp.	Yes	3–8
Lasius g.g.	*Acropyga myops*	Yes	4–7
	Lasius fuliginosus	Yes	2–3
	Prolasius brunneus	Yes	3–8
Pseudolasius g.g.	*Paratrechina minutula*	Yes	10–15
	Plagiolepis pygmaea	Yes	10–15
Oecophylla g.g.	*Oecophylla longinoda*	No†	–
	Oecophylla smaragdina	No†	–
Myrmeciinae	*Myrmecia pilosula*	No	–
Myrmicinae	*Aphaenogaster spinosa*	No	–
	Atta sexdens	No	–
	Crematogaster scutellaris	No	–
	Myrmica sabuleti	No	–
	Solenopsis invicta	No	–
Nothomyrmeciinae	*Nothomyrmecia macrops*	No	–
Pseudomyrmecinae	*Pseudomyrmex* sp.	No	–
	Tetraponera punctulata	No	–

*The species in which a cloacal gland was first discovered (Hölldobler, 1982). The size of the gland and the number of secretory cells were not reported.

†The sternal gland of *Oecophylla longinoda* (Hölldobler and Wilson, 1977) and *O. smaragdina* (Billen, unpublished data) is not homologous to the cloacal gland; for explanations see text.

useful information on the importance of the gland in the different species (e.g., in chemical communication), only when compared to a related species of the same genus.

The systematic survey for the presence of a cloacal gland in the (Formicinae + Dolichoderinae + Aneuretinae) branch of the ant subfamilies reveals that a cloacal gland is much more widespread than was previously thought (Billen, 1993). It occurs in all Formicinae investigated except 2 *Oecophylla* species, and in all Dolichoderinae screened. This clearly illustrates that care should be taken not to confuse a true limited distribution with poor sample size. In *A. simoni*, the sole member of the related Aneuretinae, as well as in all species of the outgroup subfamilies, a cloacal gland is absent. Consequently, the most parsimonious conclusion is that the presence of a cloacal gland (or presence in the majority of species) is a synapomorphy of the Formicinae and Dolichoderinae subfamilies, yielding support for their hypothesized sister group relationship (Shattuck, 1992). In the Formicinae, 2 species of *Oecophylla*, in which no cloacal gland could be found, were the only exceptions. The sternal gland of *O. longinoda* (Hölldobler and Wilson, 1977) and *O. smaragdina* (Billen, unpublished data) cannot be regarded as homologous to the cloacal gland because of its different morphology with duct cells releasing their secretion directly through the integument at the anterior side of the 7th sternite — clearly reflecting a different ontogeny. This implies that the cloacal gland is either replaced there by an analogous exocrine gland with a similar pheromonal function-recruitment in both *C. ephippium* (Hölldobler, 1982) and *O. longinoda* (Hölldobler and Wilson, 1978), or that a cloacal gland never evolved in the primitive *Oecophylla* genus group (Agosti, 1991), and that the presence of a cloacal gland would be an autapomorphy of the Dolichoderinae and the Formicinae minus the *Oecophylla* group. Nevertheless, we think the latter scenario is less plausible than that presented above because it would involve 2 separate evolutionary origins of a structure indistinguishable on morphological grounds.

Summarizing up to this point, the presence of a cloacal gland seems to have a clear potential in the assessment

of phylogenetic relationships at higher taxonomic levels. In addition to the metatibial gland, Dufour's gland, Pavan's gland, and the sting bulb gland, this is the 5th exocrine gland with such potential usefulness in ant phylogeny (see references in Baroni Urbani *et al.*, 1992).

The function of the cloacal gland remains largely enigmatic. In *C. ephippium* (Hölldobler, 1982) the gland is known to be involved in recruitment. In *C. niger* (Wenseleers *et al.*, in preparation) and possibly also in *C. cursor* (Mayade *et al.*, 1993), it is involved in territorial marking. Nothing is known about its function in all other species known to have a prominent cloacal gland. The discovery of a cloacal gland in a number of dolichoderines could prove to be interesting, because alternative interpretations of published reports on the function of Pavan's gland are now possible. The glandular epithelium of this gland is also associated with the 7th sternite, and could easily be confounded with the cloacal gland in bioassays. A detailed reexamination of some of the reports of Pavan's gland as the exclusive source of trail-substances (first reported by Wilson and Pavan, 1959; reviewed by Billen and Morgan, 1998) are therefore necessary. In this way, morphological research of the social insects' exocrine system will always offer new challenges to future ethological research.

Acknowledgements—We greatly acknowledge D. Corstjens for skilful section preparation and thank J. Cillis for his assistance in scanning electron microscopy. For help in providing and/or identifying material for this work, we are very grateful to C. Baroni Urbani, J. M. Cherrett, C. Collingwood, X. Espadaler, A. Hefetz, A. Lenoir, and J. van Boven. We also thank D. Agosti, G. Delfino, X. Espadaler, R. Minckley, Ch. Noirot and E. Smets for stimulating discussions and useful comments. E.S. acknowledges the support of a postdoctoral research grant of the Fund for Scientific Research, Flanders (FWO-Vlaanderen), and R.W. the support of the Swiss National Science Foundation, grant no. 31-30063.90.

REFERENCES

Agosti, D. (1990) Review and reclassification of *Cataglyphis* (Hymenoptera Formicidae). *Journal of Natural History* **24**, 1457–1505.

Agosti, D. (1991) Revision of the oriental ant genus *Cladomyrma* with an outline of the higher classification of the Formicinae. *Systematic Entomology* **16**, 293–310.

Agosti, D. (1994) The phylogeny of the ant tribe Formicini with the description of a new genus. *Systematic Entomology* **19**, 93–117.

Baroni Urbani, C., Bolton, B. and Ward, P. S. (1992) The internal phylogeny of ants (Hymenoptera : Formicidae). *Systematic Entomology* **17**, 301–329.

Bazire-Bénazet, M. and Zylberberg, L. (1979) An integumentary gland secreting a territorial pheromone in *Atta* sp.: detailed structure and histochemistry. *Journal of Insect Physiology* **25**, 751–765.

Billen, J. (1989) Morphology of the cloacal gland in the ant *Cataglyphis savignyi*. *Actes des Colloques Insectes Sociaux* **5**, 301–306.

Billen, J. (1991) Ultrastructural organization of the exocrine glands in ants. *Ethology, Ecology and Evolution* **1**, 67–73.

Billen, J. (1993) Morphology of the exocrine system in ants. In: *Proceedings of the Colloquia on Social Insects*, ed. V. E. Kipyatkov, pp. 1–15. Socium, St. Petersburg.

Billen, J. (1994) Morphology of exocrine glands in social insects: an update 100 years after Ch. Janet. In: *Les Insectes Sociaux*, eds. A. Lenoir, G. Arnold and M. Lepage, p. 214. Publications Université Paris Nord, Paris.

Billen, J. and Morgan, E. D. (1998) Pheromone communication in social insects — sources and secretions. In: *Communication in Social Insects: Ants, Wasps, Bees, and Termites*, eds. M. Breed, R. K. Vander Meer, K. Espelie and M. Winston, pp. 3–33. Westview Press, Boulder, CO.

Bonavita-Cougourdan, A. and Morel, L. (1984) Polyethism in social interactions in ants. *Behavioural Processes* **11**, 425–433.

Delfino, G., Piccioli, M. T. M. and Calloni, C. (1983) Ultrastructure of the venom glands in *Polistes gallicus* (L.) (*Hymenoptera Vespidae*). *Monitore Zoologico Italiano* **17**, 263–277.

Emery, C. (1912) Die Wanderung der Steppen- und Wüstenameisen von Zentral-Asien nach Süd-Europa und Nord-Afrika. *Zoologisches Jahrbuch (Suppl.)* **15**, 95–104.

Hefetz, A. and Orion, T. (1982) Pheromones of ants of Israel: I. The alarm-defence system of some larger Formicinae. *Israeli Journal of Entomology* **16**, 87–97.

Hölldobler, B. (1982) The cloacal gland, a new pheromone gland in ants. *Naturwissenschaften* **69**, 186–187.

Hölldobler, B. and Wilson, E. O. (1977) Colony-specific territorial pheromone in the African weaver ant *Oecophylla longinoda* (Latreille). *Proceedings of the National Academy of Sciences of the U.S.A.* **74**, 2072–2075.

Hölldobler, B. and Wilson, E. O. (1978) The multiple recruitment systems of the African weaver ant *Oecophylla longinoda* (Latreille). *Proceedings of the National Academy of Sciences of the U.S.A.* **74**, 2072–2075.

Hölldobler, B. and Wilson, E. O. (1990) *The Ants*. Harvard University Press, Cambridge, Massachusetts.

Keegans, S. J., Morgan, E. D., Agosti, D. and Wehner, R. (1992) What do glands tell us about species? A chemical case study of *Cataglyphis* ants. *Biochemistry and Systematic Ecology* **20**, 559–572.

Mayade, S. and Suzzoni, J.-P. (1990) Le polyéthisme chez *Cataglyphis cursor*. *Actes des Colloques Insectes Sociaux* **6**, 123–130.

Mayade, S., Cammaerts, M.-C., Suzzoni, J.-P. (1993) Home range marking and territorial marking in *Cataglyphis cursor* (Hymenoptera : Formicidae). *Behavioural Processes* **30**, 131–142.

Noirot, C. and Quennedey, A. (1974) Fine structure of insect epidermal glands. *Annual Review of Entomology* **19**, 61–80.

Noirot, C. and Quennedey, A. (1991) Glands, gland cells, glandular units: some comments on terminology and classification. *Annales de la Société Entomologique de France* **27**, 123–128.

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. *Journal of the Georgia Entomological Society* **15**, 215–226.

Schoeters, E. and Billen, J. (1990) Morphology of the venom gland in relation to worker size in leaf-cutting ants (Formicidae Attini). *Actes des Colloques Insectes Sociaux* **6**, 249–252.

Shattuck, S. O. (1992) Higher classification of the ant subfamilies Aneuretinae Dolichoderinae and Formicinae (Hymenoptera : Formicidae). *Systematic Entomology* **17**, 199–206.

Soroker, V., Hefetz, A., Cojocaru, M., Billen, J., Franke, S. and Francke, W. (1995) Structural and chemical ontogeny of the postpharyngeal gland in the desert ant *Cataglyphis niger*. *Physiological Entomology* **20**, 323–329.

Sreng, L. (1979) Ultrastructure et chimie de la sécrétion des glandes tergales du mâle de *Blatella germanica* (L.) (Dictyoptera : Blattellidae). *International Journal of Insect Morphology and Embryology* **8**, 213–227.

Wehner, R. (1983) Taxonomie, Funktionsmorphologie und Zoogeographie der saharischen Wüstenameise *Cataglyphis fortis* (Forel, 1902) stat. nov. (Insecta : Hymenoptera : Formicidae). *Senckenbergiana Biologia* **64**, 89–132.

Wehner, R., Wehner, S. and Agosti, D. (1995) Patterns of the biogeographic distribution within the *bicolor* species group of the North African desert ant, *Cataglyphis* Foerster, 1850. *Senckenbergiana Biologia* **74**, 163–191.

Wenseleers, T., Schoeters, E. and Billen, J. (1996) Morphologie et ultrastructure de la glande cloacale chez *Cataglyphis niger*. *Actes des Colloques Insectes Sociaux* **10**, 189–194.

Wilson, E. O. (1980) Caste and division of labor in leaf-cutting ants (Hymenoptera : Formicidae : *Atta*). I. The overall pattern in *A. sexdens*. *Behavioural Ecology and Sociobiology* **7**, 143–156.

Wilson, E. O. and Pavan, M. (1959) Glandular sources and specificity of some chemical releasers of social behavior in Dolichoderine ants. *Psyche* **66**, 70–76.