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Aberrant venom glands in Amblyoponini (Formicidae, Ponerinae): morphology, ultrastructure and histochemistry

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Abstract

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All ants studied so far have a convoluted gland portion as part of their venom gland. The venom glands of the amblyoponine genera *Amblyopone*, *Mystrium*, *Onychomyrmex* and *Prionopelta*, however, are characterized by the absence of a convoluted gland, which makes this ponerine tribe exceptional among ants. The venom gland of Amblyoponini is similar to that of mutillid wasps, which supports a possible tiphiid ancestral form. Ultrastructurally, the cells of the free tubules do not differ from those of most other stinging ants. A separate series of secretory cells, each with its own end apparatus, lines part of the venom reservoir. These few secretory cells near the orifice of the glandular tubule into the reservoir show an end apparatus with wide extracellular spaces and are similar to those lining the venom reservoir in honeybees. Several muscles run parallel with the longitudinal axis of the reservoir, which results in the curved appearance of replenished reservoirs when these muscles contract.

The absence of a bourreleted convoluted gland in Amblyoponini, and the presence of only a few lipoidal reservoir cells (histochemical results), when compared with histochemistry and ultrastructure of the convoluted gland in other Hymenoptera investigated, leads us to conclude that the Amblyoponini most likely possess a venom which contains only a fraction of lipoids and/or pheromones, hence suggesting the presence of a mainly proteinaceous venom used against their prey. This idea is supported by the fact that several other hymenopteran groups, which are not reported to contain significant amounts of pheromones in their venom glands, also lack the convoluted gland tissue.

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Introduction

Until now, based upon the early descriptions by Forel (1878), two different forms of venom glands are distinguished in ants, the pulvinate (or cushion type with convoluted gland on the reservoir's dorsum, only found in Formicinae producing and spraying formic acid) and the bourreleted type (or bulbous type, in most other ant subfamilies) with a convoluted gland internally located in the reservoir. A major difference between the two groups is that formicines are stingless and uniquely spray their acid, whereas the ants with the second type possess a sting, the development of which depends upon the functional needs of the species. In general, the occurrence of the convoluted gland is a common characteristic of the venom gland in ants.

The venom system of stinging ants has been the subject of several studies (reviewed in Blum and Hermann 1978 a,b). One of the pioneer papers describing the detailed organization of the venom gland in ants is that of Callahan *et al.* (1959), dealing with the fire ant *Solenopsis saevissima* vs. *richteri* (= *S. geminata*). The ultrastructural organization of the venom gland in ants has been discussed only in a restricted number of papers, as the report for *Solenopsis invicta* (Billen 1989) and for *Myrmecia gulosa* (Billen 1990). For a long time, the exact configuration and nature of the convoluted gland has been a subject of controversial reports, Callahan *et al.* (1959), e.g. mention the occurrence of a filter-like structure. A first detailed analysis of the venom system in ponerine ants is given by Schoeters and Billen (1995a) for *Dinoponera australis* and for social wasps (Schoeters and Billen 1995b). Recent morphological studies on Amblyoponini are very rare, except the work of Hashimoto (1996) dealing with skeletomuscular modifications in the abdominal region of Amblyoponini.

Our attention for the venom system of Amblyoponini was first drawn by dissection observations on *Amblyopone reclinata* that revealed the apparent lack of a convoluted gland in this species. Additional histological, histochemical and ultrastructural work has been undertaken in order to confirm this observation and to check whether a similar morphology is found in members of other genera belonging to the tribe Amblyoponini.

Materials and Methods

The following species were investigated: *Amblyopone reclinata* Mayr 1879; *Mystrium camillae* Emery 1889; *Prionopelta kraepelini* Forel 1905, all collected in the Bogor Botanic Gardens, West Java, and *Onychomyrmex hedleyi* Emery 1895, collected in Ravenshoe, NSW, Australia. Foraging worker ants were dissected in insect Ringer solution (Jolly) and then fixed in 2% cold glutaraldehyde (at 4°C in cacodylate buffer (pH 7.3) for 2–16 h) with post-fixation in osmium tetroxide in the same buffer.

A first group of samples, for demonstration of lipoid compounds in the venom gland cells, was dehydrated in a graded ethanol series. After dehydration, samples were embedded in LRWhite at 50°C. Subsequently, the thymol/farnesol procedure according to Wigglesworth (1988) was applied for visualization of lipids and for unmasking lipids.

A second group of samples, for routine light microscopy, was dissected in glutaraldehyde (same blend as mentioned above), with the venom reservoir opened in order to allow better penetration of the cold fixative. After post-fixation in 2% osmium tetroxide in Na-cacodylate buffer, the glands were block-stained in uranyl acetate (in 10% aqueous acetone) and dehydrated in a graded acetone series, followed by embedding in Araldite. Serial semithin sections were stained with methylene blue and thionin.

Thin sections, made with a Reichert Ultracut E microtome were stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer, and examined in a Zeiss EM 900 electron microscope. Samples for SEM analysis were critical point dried in a Balzers CPD 030 critical point drying apparatus.

Results

General morphology

All species investigated have venom glands with two moderately developed secretory tubules that fuse near the reservoir and are characterized by the absence of a convoluted gland (Fig. 1 A-C).

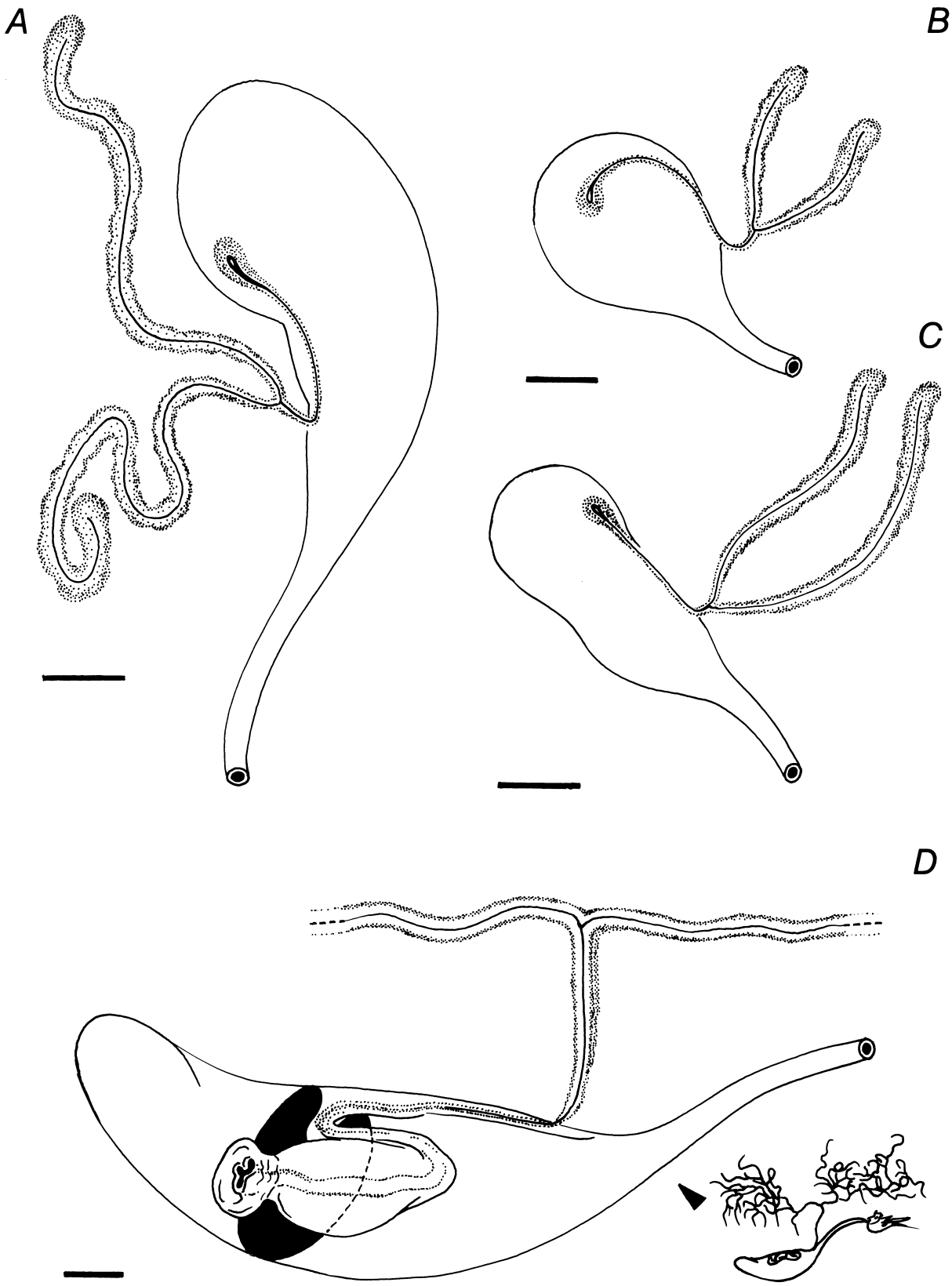
The lack of a convoluted gland means that the duct, that usually opens through the convoluted gland (Fig. 1D), now opens at the apex of the reservoir sac. This was confirmed by histological sections that also illustrate the existence of a few secretory cells lining the venom reservoir (Fig. 3.A). The proximal part of the duct belonging to the free tubule base is embedded between the wall of the reservoir and a set of reservoir muscles (this area is indicated by an arrow in Fig. 2B).

In *Amblyopone reclinata* we found a series of muscles with a parallel course to the longitudinal axis of the reservoir (Fig. 2C, D (B)). This is different from what is found in other ponerines investigated, which possess more randomly orientated muscles surrounding the reservoir. The particular muscular organization in *Amblyopone* is most likely to be responsible for the typical curved shape of the reservoir (Fig. 1A, 2A, B). Another remarkable aspect is that some muscles, especially those near the reservoir apex, curve 180° (Fig. 2C), whereas another group of muscles surrounding the reservoir show an orientation perpendicular to the longitudinal axis of the reservoir (Fig. 2D (A-B)).

Ultrastructure

The cells of the free tubules show similar characteristics as those found in most other stinging ants with a well-developed

Fig. 1.—Comparison between amblyoponine venom glands and platythyreine venom glands. The lumen of the collecting ducts opens apically in the dotted area for accumulation of the venom. In D, this lumen opens through the cuticular lining of the convoluted gland (left).—**A,** *Amblyopone.*—**B,** *Mystrium.*—**C,** *Prionopelta.*—**D,** *Platythyrea.* (inset: shows total tubular apparatus, in addition to cut tubules in D).



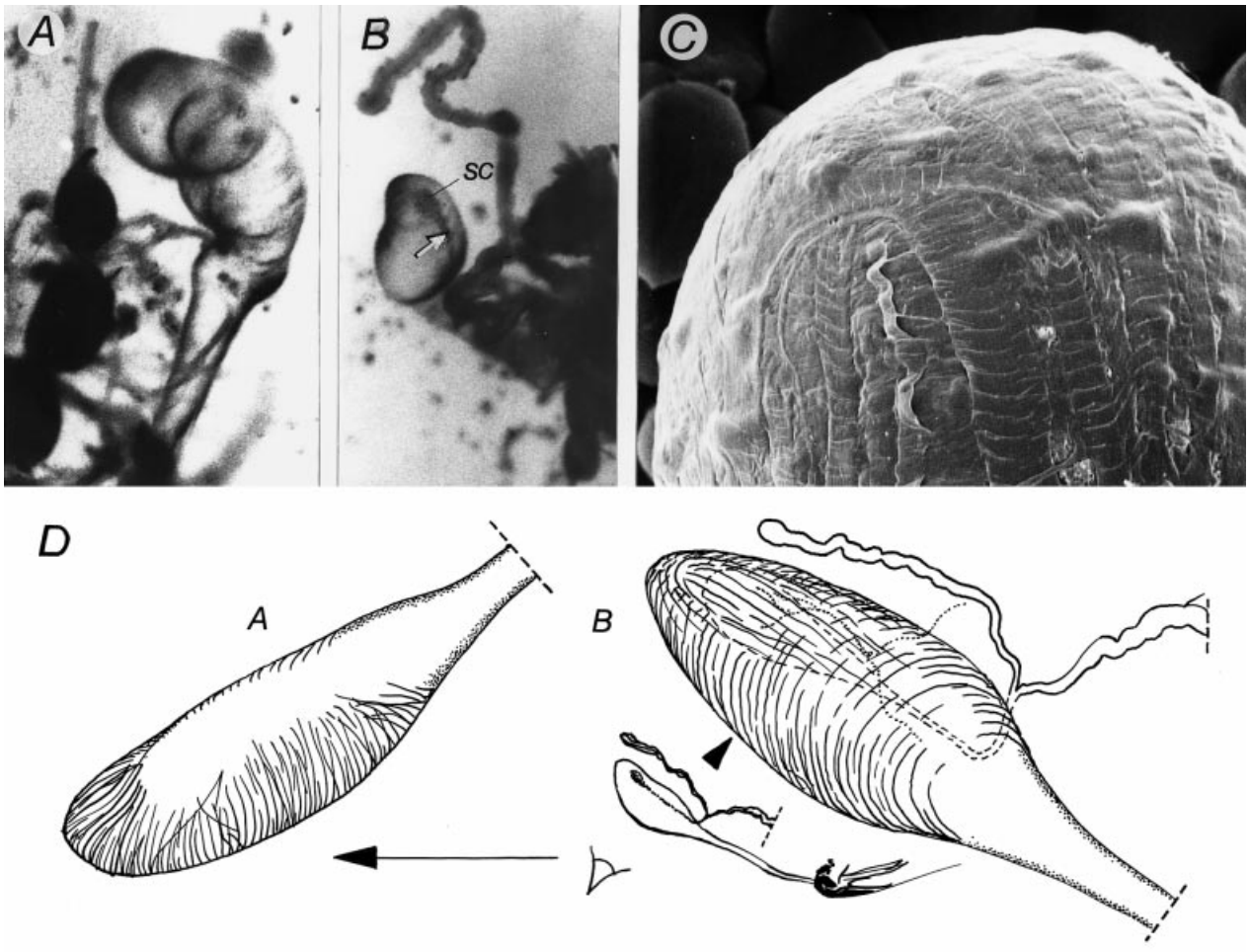
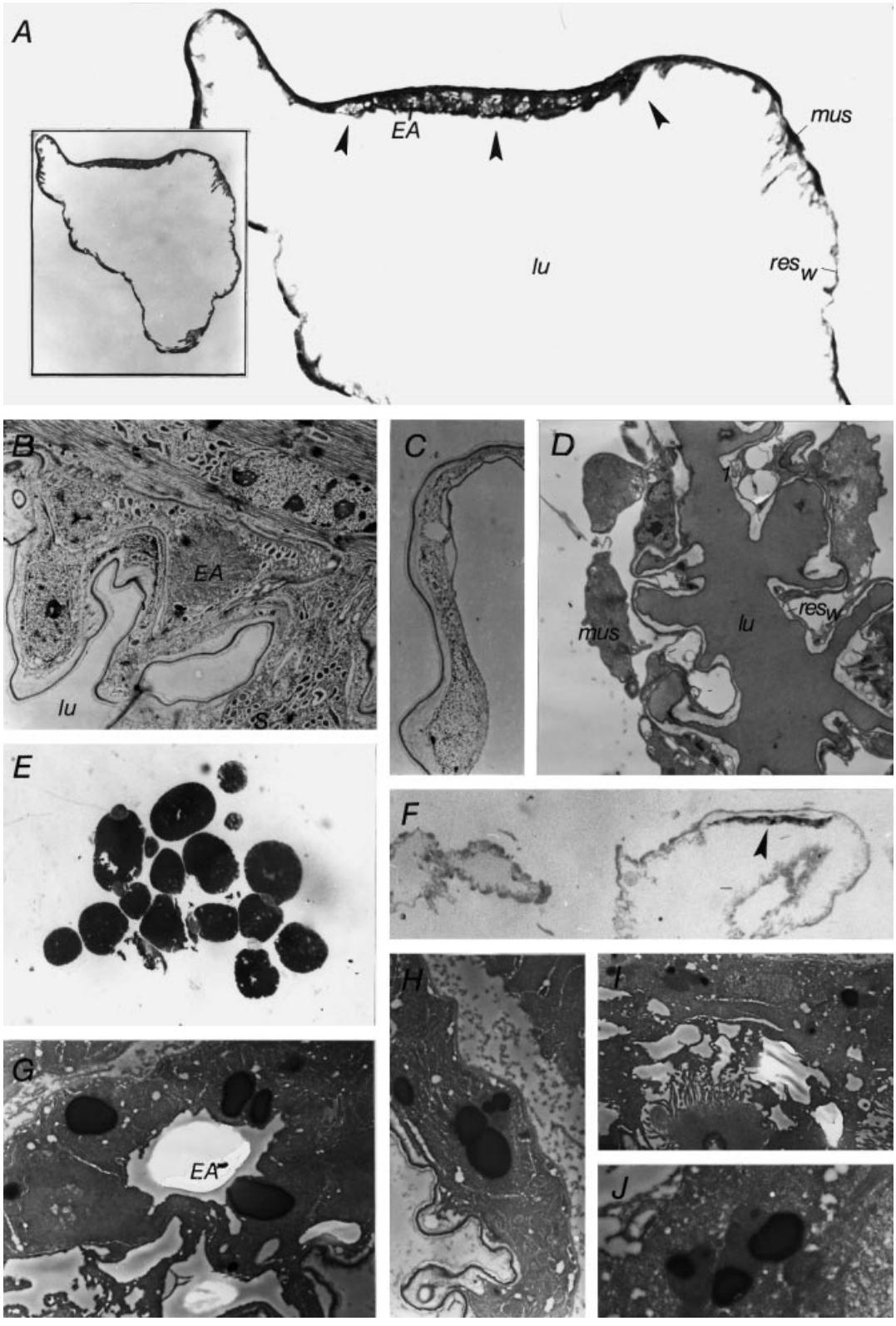


Fig. 2—**A,** Light microscopical view of the venom reservoir in *A. reclinata*, characterized by its transparency and absence of the convoluted gland inside. Scale bar: 100 μm.—**B,** Light microscopical view of the reservoir and its glandular cells in *A. reclinata* (the arrow indicates the nonglandular part embedded within the reservoir musculature and the slightly broader part with a few secretory cells (*sc*) at the right. Note one slender free secretory tubule on top. Scale bar: 100 μm.—**C,** Scanning electron microscopy of the venom reservoir musculature near its apex. Scale bar: 10 μm.—**D,** Ventral (A) and dorsal (B) aspects of reservoir musculature. Scale bar: 100 μm.

granular endoplasmic reticulum. The cell membranes of the secretory cells show obvious invaginations.

The few secretory cells, which line the reservoir, near the orifice of the glandular tubule into the reservoir, show an end apparatus with wide extracellular spaces. In general, the reservoir cells contain numerous mitochondria near the microvilli of their end apparatus (Fig. 3B). These organelles could be involved at that site in enzymatic

Fig. 3—**A,** Transverse semithin section of the reservoir in *Amblyopone reclinata*, showing restricted secretory region (*arrows*). Scale bar: 50 μm. The insert shows relative size of secretory region. *EA* = end apparatus; *lu* = lumen of reservoir; *mus* = muscles; res_w = reservoir wall.—**B,** Ultrastructure of the secretory cells. Scale bar: 2 μm. *EA* = end apparatus; *lu* = lumen of reservoir.—**C,** Ordinary reservoir cells in *A. reclinata* with flattened appearance. Scale bar: 2 μm.—**D,** Ultrastructure of the reservoir wall in *Onychomyrmex*, showing reduced muscles (*mus*); *lu* = lumen of reservoir; res_w = reservoir wall. Scale bar: 2 μm.—**E,** Positive histochemical reaction of the fat body (control) near the gland. The fat body cells showed a very dark staining. Scale bar: 10 μm.—**F,** The histochemical reaction observed for the reservoir secretory cells is strongly positive (semithin section). The results become especially significant when comparison is made with the non-secretory lining of the reservoir wall. Scale bar: 10 μm.—**G,** Electron microscopical confirmation of positive histochemical test. Note large electron dense inclusions near the lumen of the end apparatus. Scale bar: 2 μm.—**H,** Large inclusions near the cuticular lining of the reservoir. Scale bar: 2 μm.—**I,** Smaller lipoid inclusions near the end apparatus. Scale bar: 2 μm.—**J,** Fusion of lipoid inclusions. Scale bar: 2 μm.



conversion of lipoidal compounds into other derivatives. The majority of venom reservoir cells is usually very flat and accompanied by very few muscles, as is shown for *Amblyopone reclinata* (Fig. 3C) and for *Onychomyrmex* (Fig. 3D).

Histochemistry

Our attention was drawn to lipid histochemistry because most other convoluted glands tested so far invariably show a very positive thymol/farnesol and ethyl gallate test (Schoeters, unpublished). We therefore assumed that the few glandular cells lining the reservoir in Amblyoponini could show similar histochemical characteristics. This assumption has gained in importance, since apparently no other (ultra)structural indications were present to suggest the production of other categories of chemicals (e.g. proteins or polysaccharides). The observed positive reaction was compared with the reaction seen in free tubules and the fat body (Fig. 3E) near the gland. In the free tubules we observed just low numbers of lipoidal inclusions, whereas the fat body cells showed a very dark staining (Fig. 3E). The histochemical reaction observed for the reservoir secretory cells is also positive (Fig. 3F), as can be seen from semithin sections. The results become especially significant when comparison is made with the rest of the reservoir wall (Fig. 3F). The positive staining of these cells was additionally confirmed by electron microscopy (Fig. 3 G-J), which showed varying size of lipoid inclusions and variable positions in relation with the end apparatus of the secretory cells.

Discussion

Among the aculeate Hymenoptera, a convoluted venom gland is generally present in Vespoidea, but is absent in Apidae (Bombini, Apini, Meliponini), and Sphecidae. It is equally lacking in Symphyta and Parasitica. In the ants (Formicidae) a convoluted gland occurs in 10 subfamilies examined so far, albeit with structural differences among the various groups. In some cases the venom gland also plays a considerable role in social communication (Maschwitz and Kloft 1971). In all species investigated so far, the secretory parts of the convoluted gland seem to be necessary to contribute to the entire secretory apparatus of the venom system (Schoeters unpublished). Their venoms are used against a diversity of predators and for prey killing or paralysing (Maschwitz and Kloft 1971).

In the ponerine tribes investigated so far, we observed that the winged or bulbous/bourreleted secretory part of the convoluted gland is very well developed. This type of venom gland is most likely to have evolved in response to the functional needs of the insect in relation to its prey (Maschwitz and Kloft 1971).

Nothwithstanding its wide distribution in venom glands of stinging Hymenoptera and in species with secondary modified venom glands, the role of the convoluted gland is not yet understood. Although it is well known that many venoms can induce comparable responses while the substances are biochemically different, a constant character in ponerine venom glands and social wasps is the occurrence of their convoluted gland. From a phylogenetic point of view, the Amblyoponini present a difficult but intriguing picture. Our study shows that the venom gland in Amblyoponini obviously differs from the one in all other ponerines and that it is quite similar to that found in mutillid wasps. Our observation is in agreement with the previously reported tiphiid-like abdominal appearance of Amblyoponini. Another ponerine species that is regarded as being outstanding as a unique member of the ponerine subfamily is *Simopelta oculata* (tribe Ponerini). In that case, however, its unique status within the tribe is based on both behavioural and sting anatomical evidence (Blum and Hermann 1978a), but not on its glandular parts as found for Amblyoponini.

The absence of a convoluted gland in Amblyoponini might be an indication for the lack/or reduced importance of one particular category of compounds in the venom, i.e. lipoids, possibly pheromonal constituents, as suggested by other histochemical work (Schoeters unpublished). The absence of a tissue as possible secretory region for enzymatic activation of venom compounds seems to be unlikely since Amblyoponines also need to have a fully activated venom at their disposal for their specialized prey captures. In this respect, it appears that a convoluted gland is not necessarily substantial for venom activation, but rather should be regarded as an additional functional part of the two-or multichambered character of their venom-producing systems. Some authors have provided an adaptive explanation for the two-chambered nature of defensive glands (e.g. in notodontid caterpillars) that produce formic acid, together with some additives (Wheaterston *et al.*, 1979). They clearly distinguish an inner sac-like chamber for the production of hydrophilic components (formic acid) and a smaller outer chamber for the production of the lipophilic additives. Another two-compartment system is known for bombardeer beetles (Schildknecht and Koob 1969). In this case, the second part of the reservoir delivers the enzymes necessary for the production of their explosive secretory discharges.

In our opinion, it is important to consider the degenerative morphological changes that are usually found in some advanced groups of both Apidae and Formicidae (Robertson 1968). In Apini, that also lack a convoluted gland, the venom gland is used solely for defense. Owen and Bridges (1976) reported on the presence of secretory cells around the proximal third of the venom reservoir of the honeybee worker, *Apis mellifera*. In this respect there is an important link with Amblyoponini. However, honeybees do not possess a secretory tubule embedded in the musculature of the reservoir. In stingless bees, that also lack a convoluted gland, the venom gland is more thoroughly reduced and the

defensive function is displayed in other ways. Contrarily, in primitive Formicidae both offense and defense can be found, as is the case in eumenid, pompilid (see *Pepsis* wasps, in Schoeters *et al*. 1997) and vespid wasps. Members of these groups all possess a well-developed convoluted gland. In myrmicine ants, the venom gland is only used for defense, or serves secondary purposes, such as the production of pheromonal constituents.

Our observations show that a more cautious interpretation is necessary when proposing a generalized model of a venom gland for taxonomic groups at the family level. According to Billen and Taylor (1993), some features of the venom gland are sometimes less constant or diagnostically less reliable as previously reported for some Australian dolichoderine and myrmicine ants at the level of the free tubules, but the general organization of the venom gland, including the presence of a convoluted gland remains the same. In fungus ants belonging to the genus *Atta*, we have shown variations of the free tubule morphology in relation to worker size (Schoeters and Billen 1990).

More caution should thus be paid when interpreting venom gland phylogeny: this is illustrated in the present paper. This is also the first report dealing with the histochemical demonstration of lipid metabolism in the reservoir cells of hymenopteran venom glands.

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