

Uncovering head gland diversity in neotropical Polistinae wasps (Hymenoptera, Vespidae): Comparative analysis and description of new glands



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ABSTRACT

Exocrine glands are involved in several wasp colony activities; however, the number of known glands in the Vespidae is rather low when compared to other social insect groups. The aim of this study is to survey the head of Neotropical social wasps and to provide a detailed comparative study of the glands found in the Polistinae. A total of 33 species distributed over 13 genera were studied with serial histological sections of the head, excluding the labiomaxillary complex. Additionally, the exoskeleton was explored using scanning electron microscopy looking for associated modifications. A total of eleven exocrine glands were observed, five are structures recorded for the first time for the Hymenoptera, three are new records for the Polistinae and three are previously known organs. The glands studied are: ocellar gland I, ocellar gland II, periocular gland, subantennal gland, hypopharyngeal gland, clypeal gland, posterobasal genal gland, ectal mandibular gland, mesal mandibular gland, intramandibular gland I, and intramandibular gland II. The widespread distribution of most of these glands suggests an origin prior to the evolution of the Polistinae. Our results highlight the importance of detailed morphological studies to unveil the significance of chemical communication in one of the most characteristic groups of social animals.

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1. Introduction

Glands are fundamental structures for different aspects of social insect life, as they provide chemical signaling substances for defense, mating, trail tracking/marking, egg laying regulation, nest construction, social structuring, and general communication (Hölldobler and Wilson, 1990; Billen et al., 1998; Billen, 2006, 2009). The study of Hymenoptera glands dates back to at least the 17th century with famous names such as Swammerdam, Reaumur, and Dufour among others (Heselhaus, 1922; Billen and Wilson, 2008). Later, by the end of the 19th century a new era in gland research was established by outstanding publications such as these of Janet (Billen and Wilson, 2008). In the Vespidae, the works by Bordas (1907) are exceptional for their detailed description (Heselhaus, 1922). The study of social insect glands has continued

since and Billen (2006) listed a total of 105 glands known in the small body of the Hymenoptera. However, this number is not evenly distributed in the different taxa. While 68% of the known glands have been reported in ants, only 13% have been found in social wasps. A total of eight cephalic glands have been reported for the Vespidae as follows (Downing, 1991): the ectal mandibular gland, the mesal mandibular gland, the hypopharyngeal gland, the labial palps gland, the labiomaxillary gland, the maxillo-hypopharyngeal gland, the sublingual gland, and the endostipital gland. The first three are present on the head capsule while the other five are found on the mouthparts.

Differences in their general biology have been proposed as a key factor to explain these data. In addition, and perhaps as a consequence of the historical span from the first studies to current times, there has been a wide variety of standards for morphological description and naming of exocrine glands. The Richards' gland is a good example of this problem: its definition varied through the years, and after a comparative analysis, it was found that more than one type of glandular structure is present in association with the fifth sternite of the Polistinae and that the definition provided by

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Heselhaus (1922) should be closely followed in order to avoid confusion (Samacá et al., 2013).

Gland discovering is an ongoing process with new structures proposed almost every year (Billen, 2006), to the point where glands that have long been proposed as traits of a taxon and whose functions are related to the specific biology of that group, are found in distant species challenging the status quo and indicating the plasticity of these structures (Herzner et al., 2007). Comparative studies are quite important as these improve our understanding of the phylogenetic history of a gland and provide highlights on their function (Smith et al., 2001; Rocha and Caetano, 2003; Britto and Caetano, 2006; Samacá et al., 2013).

Heselhaus (1922) was among the first researchers to extensively study the glands in social wasps, and later Landolt and Akre (1979) provided an updated and more detailed revision of the group, listing a total of 14 glands. Eight of these are found in the head: ectal mandibular gland, mesal mandibular gland, hypopharyngeal gland, labial palps gland, labio-maxillar gland, maxillo-hypopharyngeal gland, sublingual gland, and endostipital gland. A decade later, Downing (1991) published another review on Vespidae glands including functional information of some of these organs. Studies of the head glands were published by other authors such as Britto et al. (2004) and Britto and Caetano (2008), describing the structure and functionality of the hypopharyngeal gland, while Delfino et al. (1979) and Turillazzi (2012) described glands in the antennae of male Stenogastrinae suggesting that these may be involved in mating behaviors.

The pioneer paper on exocrine glands by Noirot and Quennedey (1974) distinguishes three kinds of glands, of which only class 1 and class 3 are commonly occurring and the initially described class 2 glands were later homologized with oenocytes (Noirot and Quennedey, 1991). Class 1 glands have an epithelial arrangement, and appear either as a differentiation of the tegumental epidermis, or form the lining of an internalized reservoir sac. Class 3 glands are made up by bicellular units, each unit consisting of a large secretory cell and its accompanying duct cell. The duct cells continue inside the secretory cells as the 'end apparatus', which is a secretion-draining device that is formed by a central cuticular lining surrounded by a sheath of microvilli. Similar to class 1 glands, the class 3 units can also open directly through the tegument where the duct openings appear as pores with a diameter of 0.5–1 μm , or open into an internalized reservoir sac.

The current work describes and compares the morphology of glands located in the head of 33 species of social wasps. These species represent 13 genera belonging to the major clades of neotropical Polistinae. A total of eleven glands found in the head of vespids are studied, of which three are known organs, three are new reports for the subfamily and five are reported for the first time for any social hymenopteran. Their widespread taxonomic distribution, including the genera *Polistes* and *Mischocyttarus*, suggests that most of these glands appeared earlier to the origin of the Polistinae.

2. Materials & methods

A total of 33 species belonging to 13 genera of the neotropical Polistinae were included. Species were identified using different taxonomic sources (Richards, 1978; Cooper, 2000; Pickett and Wenzel, 2007; Andena et al., 2007, 2009a, 2009b; Andena and Carpenter, 2012; Cely and Sarmiento, 2011). Between one and seven foraging workers per species were studied (Suppl. Table 1). Tissues were prepared according to Samacá et al. (2013) as follows: The posterior part of the head vertex was removed to allow an efficient fixative penetration. Heads were submerged in 2%

glutaraldehyde (in 50 mmol L⁻¹ sodium cacodylate buffer) for about 12 h at 4 °C. Samples were then transferred to fresh buffer and posteriorly dehydrated in a graded acetone series and embedded in Araldite. Semithin serial sections of 2 μm thickness were obtained with a Leica EM UC6 ultramicrotome. Staining was achieved with methylene blue and thionin.

Sections were observed through a Zeiss Jenaval optical microscope. Pictures were taken with a Canon D90 digital camera. Gold-coated heads were studied for skeletal modifications using a FEI Quanta 200 scanning electron microscope at an acceleration voltage between 25 and 30 kV.

Given that we rely only on structural traits for gland definition, we follow several criteria to avoid confusion of proposed glands with other tissues. Thus, to define a gland we consider cell characteristics, clear spatial delimitation, and cell class composition (Herzner et al., 2007; Samacá et al., 2013). Class 1 glandular cells were identified by their larger size having a cylindrical or cubic shape, and by their larger nucleus. By comparison, non-glandular epithelium is squamous and the nuclei of its cells are flattened (Fig. 1A–B). Class 1 glands were always surrounded by epithelial tissue with evident contrasting characteristics. Additionally, as stated before, we only sampled foragers, as they are older individuals. This is important for the identification of class 1 glands as newly hatched workers, that only occur inside the nest, often have a fairly thick epithelium because of its involvement in cuticle formation. By using foragers, we can exclude epithelium thickness to be related to cuticle formation, as this has come to an end at their age. These criteria for class 1 glands meet other studies where epithelial glands have been clearly identified (Gobin et al., 2003; Billen, 2009). Class 3 glandular cells were recognized by their larger size, their end apparatus and usually round shape. Their ducts were also diagnostic for identification. Their nucleus is large and nucleoli are distinct, while secretory vesicles are often visible.

The following information was recorded for each gland: location, cell type according to the classification provided by Noirot and Quennedey (1974), Billen (2009), and Britto et al. (2004), maximum dorsoventral or anteroposterior extension (i.e. gland height), and cell diameter for class 3 glands. Descriptions were generated using DELTA 1.04 software (Dallwitz et al., 1999). Delta allows to record standardized descriptions of characters and produces a document integrating all the information from individual species. Characters were standardized and coded following Sereno (2007).

Cell height for class 1 glands was recorded as the average of ten measurements taken from different sections where the gland could be observed. Cell diameter for class 3 gland cells was the average of the measurements taken for the largest cells from ten sections taken at random.

To make cell counts for class 3 glands, we proceeded as follows: The largest diameter found in the gland cells of a particular gland was divided by 2 μm , being the thickness of the sections, to yield the number of consecutive sections required to sample a completely new set of cells. For example, for cells 10/20 μm in diameter, the counts from every fifth/tenth section would be summed to estimate the total number of cells in the gland. The cumulative number of gland cells, counted on the different sections of a particular class 3 gland, looking at an interval equivalent to the average cell diameter of that gland, thus gives the total cell number of that particular gland. The nomenclature of head gland structures follows Huber et al. (2006) and Silveira and Araújo dos Santos (2011). Glands from labium, glossa, paraglossa, and antennae were not studied as we focus on the mean head capsule. We track the distribution of the glands on the phylogenetic hypothesis proposed by Wenzel and Carpenter (1994).

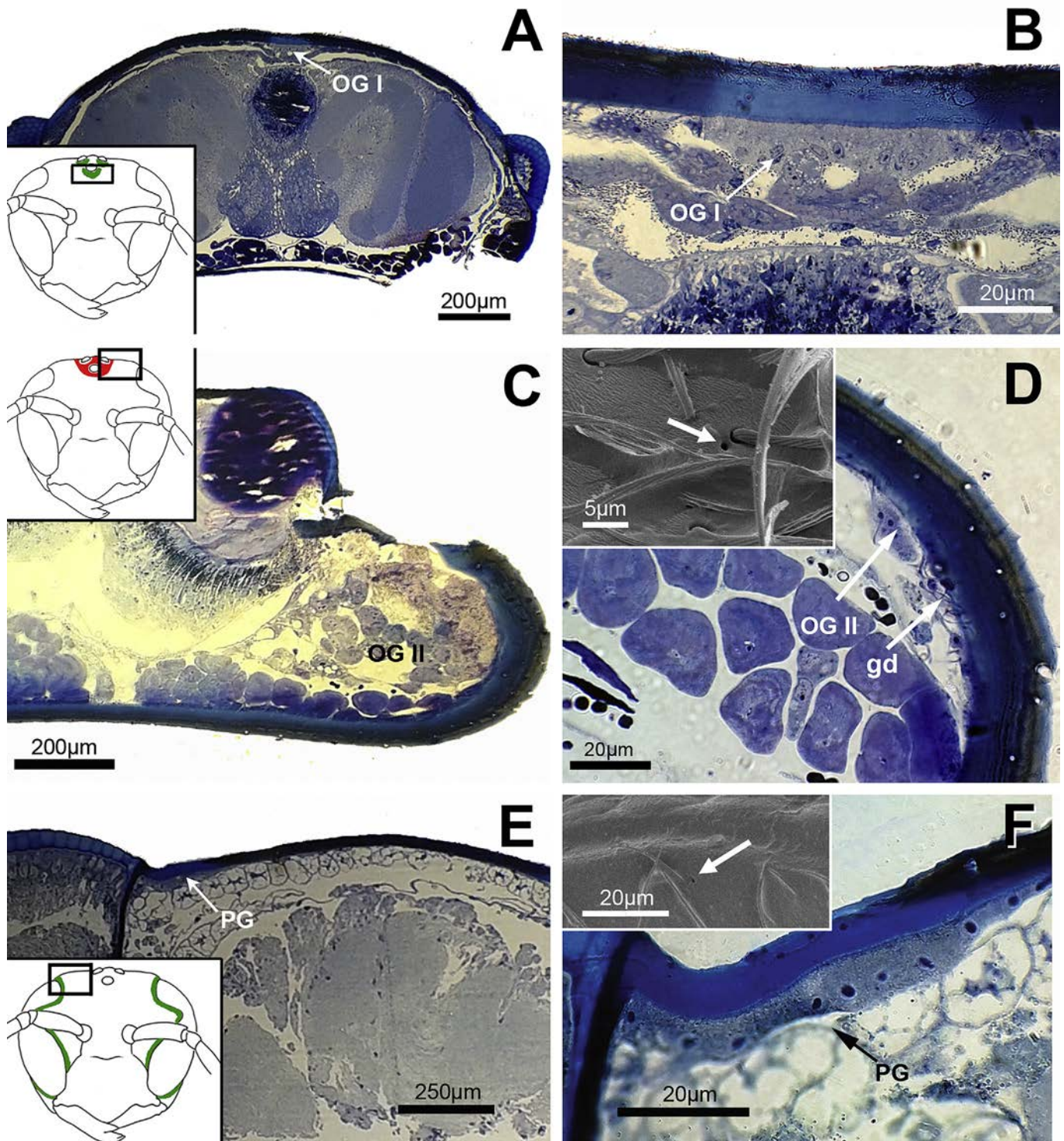


Fig. 1. A–B. Ocellar gland I (OG I) in *Leipomeles spilogastra*. C–D. Ocellar gland II (OG II) in *Leipomeles spilogastra*. Ducts (gd) can be observed. Arrow in SEM inset of D points to a 0.5 μm pore. E–F. Periocular gland (PG) in *Agelaia areata*. Drawing inset indicates the approximate location of the images in the specimen. Arrow in SEM inset of D points to a 0.5 μm pore. Class 1 glands are shown in green, class 3 glands in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

3. Results

A total of 11 glands were observed in the head capsule and mandibles of the 33 Polistinae species studied as follows: ocellar

gland I, ocellar gland II, periocular gland, subantennal gland, hypopharyngeal gland, clypeal gland, posterobasal genal gland, ectal mandibular gland, mesal mandibular gland, intramandibular gland I, and intramandibular gland II.

3.1. Ocellar gland I (class 1)

This is a layer of rectangular-shaped secreting epithelial cells with a large and well-defined nucleus. This gland is found at the anterior side of each ocellus (Fig. 1A–B). The anteroposterior extension of the gland ranges from 460 to 550 μm . Gland width ranges between 190 and 330 μm and cell height on average is 11 μm . This gland was observed in two species only: *Agelaia areata* (Say, 1837) and *Leipomeles spilogastra* (Cameron, 1912) (Suppl. Table 1). No significant modifications of the exoskeleton were observed where the glands were found, however, we observed poor staining in the semithin sections where the gland is located (Fig. 1B), which suggests a difference in the skeleton composition or structure that may facilitate the secretion of the gland product. We did not find previous records of this gland in the literature for vespids or any other hymenopteran.

3.2. Ocellar gland II (class 3)

This is a cluster of spherical glandular cells with ducts that open directly through the exoskeleton around the ocelli (Fig. 1C–D) where a few pores of 0.5 μm of diameter (inset in Fig. 1D), the usual size for this type of glands, were observed; no reservoir was observed. The anteroposterior extension of the gland ranges from 40 to 150 μm . Cell diameter ranges between 18 and 28 μm , the maximum diameter of the nucleus is 11.9 μm . The number of cells varies between 66 and 160 (Suppl. Table 1). This gland was only observed in *Polistes carnifex* (Fabricius, 1775), *Polistes erythrocephalus* Latreille, 1813, *Mischocyttarus angulatus* Richards, 1978 and *L. spilogastra* (Suppl. Table 1). We did not find previous records of this organ in the literature for vespids or any other hymenopteran group.

3.3. Periocular gland (class 1)

This is a layer of rectangular secretory cells attached to the cuticle by their shorter side. Their nuclei are clearly defined. This gland is located on the compound eye perimeter (Fig. 1E–F). The gland width ranges between 50 and 1300 μm , being restricted in most species to the small depression that surrounds the eye, but in other species the gland extends to the antennal fossae. The largest sizes described result from three *Polistes* species which are among the largest species studied (Suppl. Table 1). Cell height ranges between 8 and 62 μm . The diameter of the nucleus varies between 3.5 and 12 μm (Suppl. Table 1). The gland may surround the entire eye in some species, such as *L. spilogastra*, while in others, such as *A. areata*, it only reaches the upper height of the clypeus. This gland was found in 31 of the 33 species, its absence being of interest in *Agelaia brevistigma* Richards, 1978 and *Protopolybia exigua* (De Saussure, 1854) (Suppl. Table 1). A few pores of 0.5 μm of diameter were observed around the depression (inset in Fig. 1F), but no other modification of the exoskeleton was observed in the area where the cells were found. Heselhaus (1922), and later Schuberth and Schönitzer (1993), studied a gland composed of class 1 cells at the frons of the head. This layer is restricted to the frontal fovea of the Apoidea, while the gland we describe surrounds the eye border. We therefore believe this is a different glandular structure that has not been described previously for any other hymenopteran.

3.4. Subantennal gland (class 3)

This is a cluster of glandular cells with ducts that open directly through the exoskeleton inside the antennal socket (Fig. 2A–B); no reservoir was observed. This gland is located ventral to the antennal socket (Fig. 2A–B). The dorsoventral extension of the gland ranges

from 70 to 90 μm , the cell diameter ranges between 20 and 23 μm . Maximum nucleus diameter is 5.9 μm (Suppl. Table 1). This gland was only observed in *Polybia emaciata* Lucas, 1854 and *Protopolybia amarella* Bequaert, 1944. Janet (1894) and Heselhaus (1922) already reported this type of subantennal gland for ants and bees. As far as we know, this is the first formal description of its existence for vespids.

3.5. Hypopharyngeal gland (class 3)

This gland, widely known in the Vespidae and in many other hymenopteran groups, was observed in all the species studied (Fig. 2C–D). This is a large class 3 gland made of two clusters of cells that release their product into the anterior part of the pharynx; dorsoventrally it ranges between 100 and 765 μm . The secretory cell diameter varies between 16 and 76 μm . The nucleus diameter ranges between 2.2 and 30 μm . The number of secretory cells varies in the species studied between 53 and 2078 (Suppl. Table 1). Our observations of the structural organization of this gland follow Landolt and Akre (1979), however, we found that the cell diameter range is larger than previously reported. As these authors indicated, the gland cells measure between 30 and 50 μm .

3.6. Clypeal gland (class 1)

This is a single layer of rectangular secretory cells attached by their shorter side to the internal side of the exoskeleton. This gland may cover the entire clypeus or may be restricted to the ventral third (Fig. 2E–F). Cells of the frontal area of the clypeus are larger than these of the posterior or internal side. Their nuclei are large and well defined. Setae, especially those of the ventral border of the clypeus, may serve as dispersing structures of this gland. The dorsoventral height of the gland ranges between 33 and 1430 μm . Gland width ranges between 70 and 1000 μm . Cell height ranges between 7.4 and 70 μm . Nucleus diameter varies between 3.6 and 12 μm . The gland was observed in 27 of the 33 species studied, however, the limited sampling size in the species where it was not recorded may suggest that the gland is present in all species (Suppl. Table 1). Although the cells of this gland are indistinguishable from those of the periocular gland, we observe no contact between them, suggesting that they are different organs. We did not find mention of this organ in the literature for vespids or any other hymenopteran group.

3.7. Posterobasal genal gland (class 1)

This is a single layer of rectangular cells attached by their shorter side to the cuticle. Cell nuclei are large and well defined. The gland is located at the posterobasal region of the genae, close to the articulation of the mandible, between the epistomal and occipital carinae (Fig. 3A–B). The dorsoventral height of the gland ranges between 115 and 1000 μm . Gland width ranges between 83 and 800 μm . Cell height ranges between 7 and 45 μm . Nucleus diameter varies between 6 and 12 μm (Suppl. Table 1). The gland was present in 17 of the 33 species studied (Suppl. Table 1). The gland was not recorded in species with several individuals sampled, such as *L. spilogastra*, *P. exigua*, *Parachartergus apicalis* (Fabricius, 1804), and *Parachartergus smithii* (De Saussure, 1854) suggesting that its absence may not be due to sampling limitations. We did not find previous mentions of this organ in the literature for vespids or any other hymenopteran group.

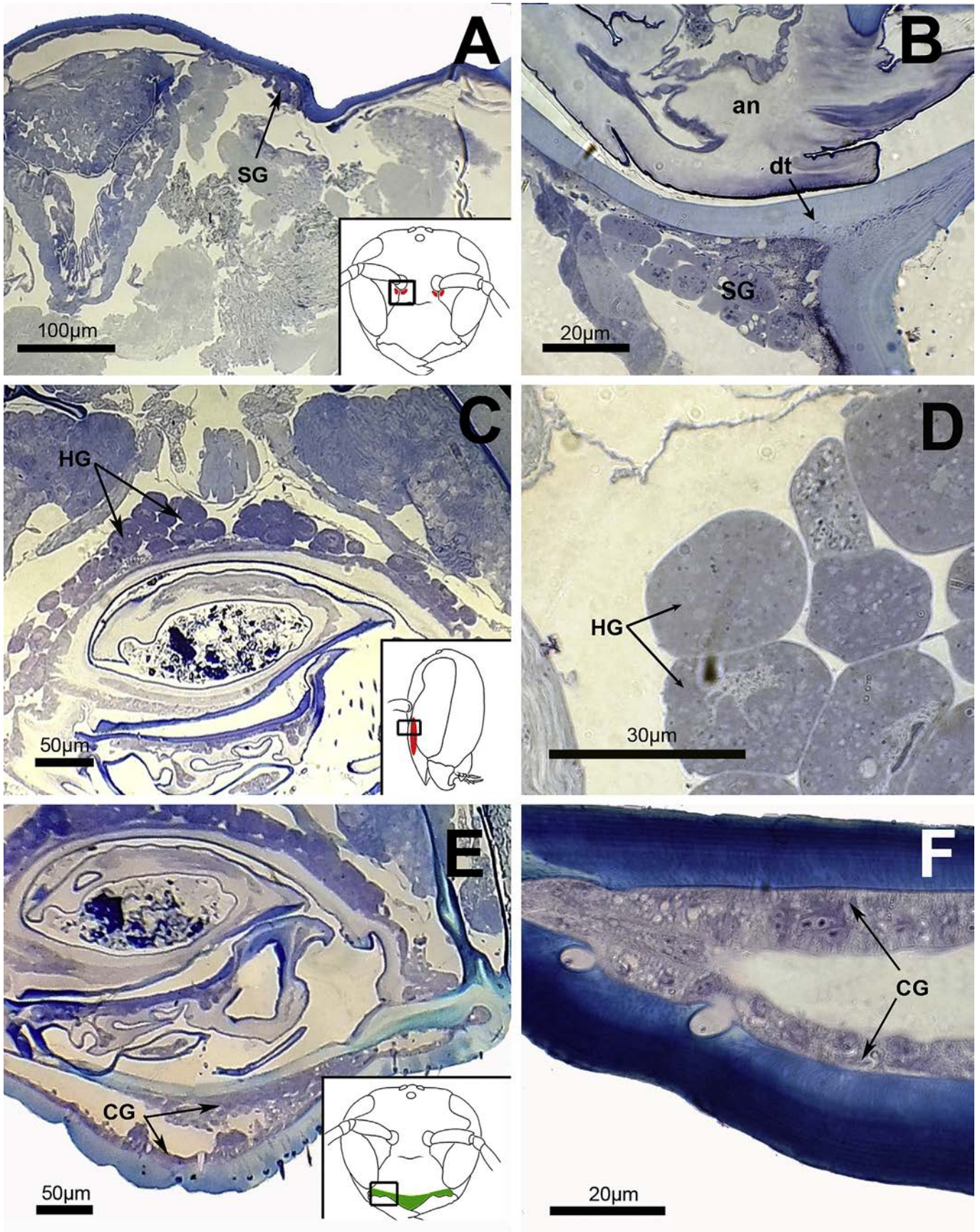


Fig. 2. A–B Subantennal gland (SG) in *Polybia emaciata* (A) and in *Agelaiia areata* (B), ducts (dt) and antenna (an) are indicated. C–D. Hypopharyngeal gland (HG) in *Mischocyttarus angulatus*. E–F. Clypeal gland (CG) in *Mischocyttarus angulatus* and *Brachygastra lecheguana* respectively. Drawing inset indicates the approximate location of the images in the specimen. Class 1 glands are shown in green, class 3 glands in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

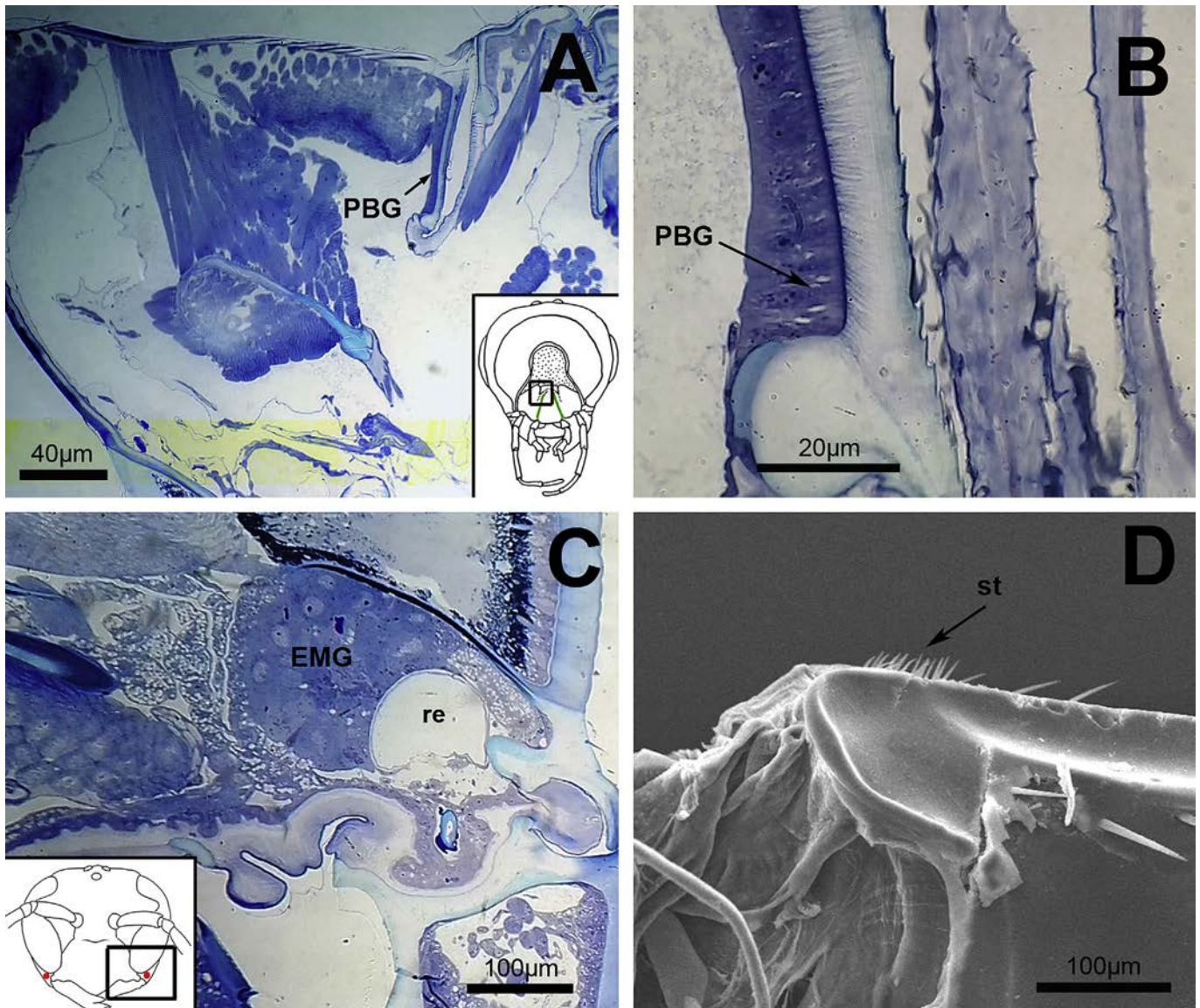


Fig. 3. A–B. Postero-basal genal gland (PBG) in *Epipona niger*. C. Ectal mandibular gland in *Parachartergus smithii*, the reservoir is indicated (re). D. Scanning electron microscopy showing an mesal view of the anterior loop region of the mandible and the brush of setae (st) associated to the ectal mandibular gland in *Parachartergus smithii*. Drawing inset indicates the approximate location of the images in the specimen. Class 1 glands are shown in green, class 3 glands in red.

3.8. Ectal mandibular gland (class 3)

Our observations of the structure of this gland fully concur with previous authors (Landolt and Akre, 1979; Downing and Jeanne, 1982; Downing, 1991), who stated this is a class 3 gland made of a cluster of cells that empty their product through ducts into a reservoir (Fig. 3C–D). This reservoir empties close to a brush of bristles located at the basal anterior area of the external side of the mandible, near the inner side of the anterior condylus. The dorso-ventral length of the gland oscillates between 140 and 580 μm . The cell diameter ranges between 20 and 50 μm . The cell nucleus diameter ranges between 6 and 18 μm . The number of cells ranges between 34 and 476. Landolt and Akre (1979), working with Vespinae queens and the nearctic species *Polistes fuscatus* (Fabricius, 1793) and *Mischocyttarus flavitarsis* (de Saussure, 1854), found these secretory organs could be composed of up to 70 cells. Our records significantly extended the number of cells (Suppl. Table 1).

3.9. Mesal mandibular gland (class 3)

This is a cluster of class 3 cells with ducts that release their product into the microtrichial membrane that connects mandible and maxilla. It ranges dorsoventrally between 80 and 630 μm . The secretory cell diameter varies between 12 and 50 μm . The nucleus diameter ranges between 6 and 26 μm . The number of secretory cells varies between 19 and 395 (Fig. 4A–B, Suppl. Table 1). We observed this gland in 24 of the 33 species studied. This gland was described by Heselhaus (1922), but he named it as the postgenal gland as it is in Apoidea. Later, Landolt and Akre (1979) commented on this gland and described in detail its location indicating that the product could be secreted through the microtrichial membrane. Our results follow this description. The cell sizes we observed are within the range provided in Downing (1991).

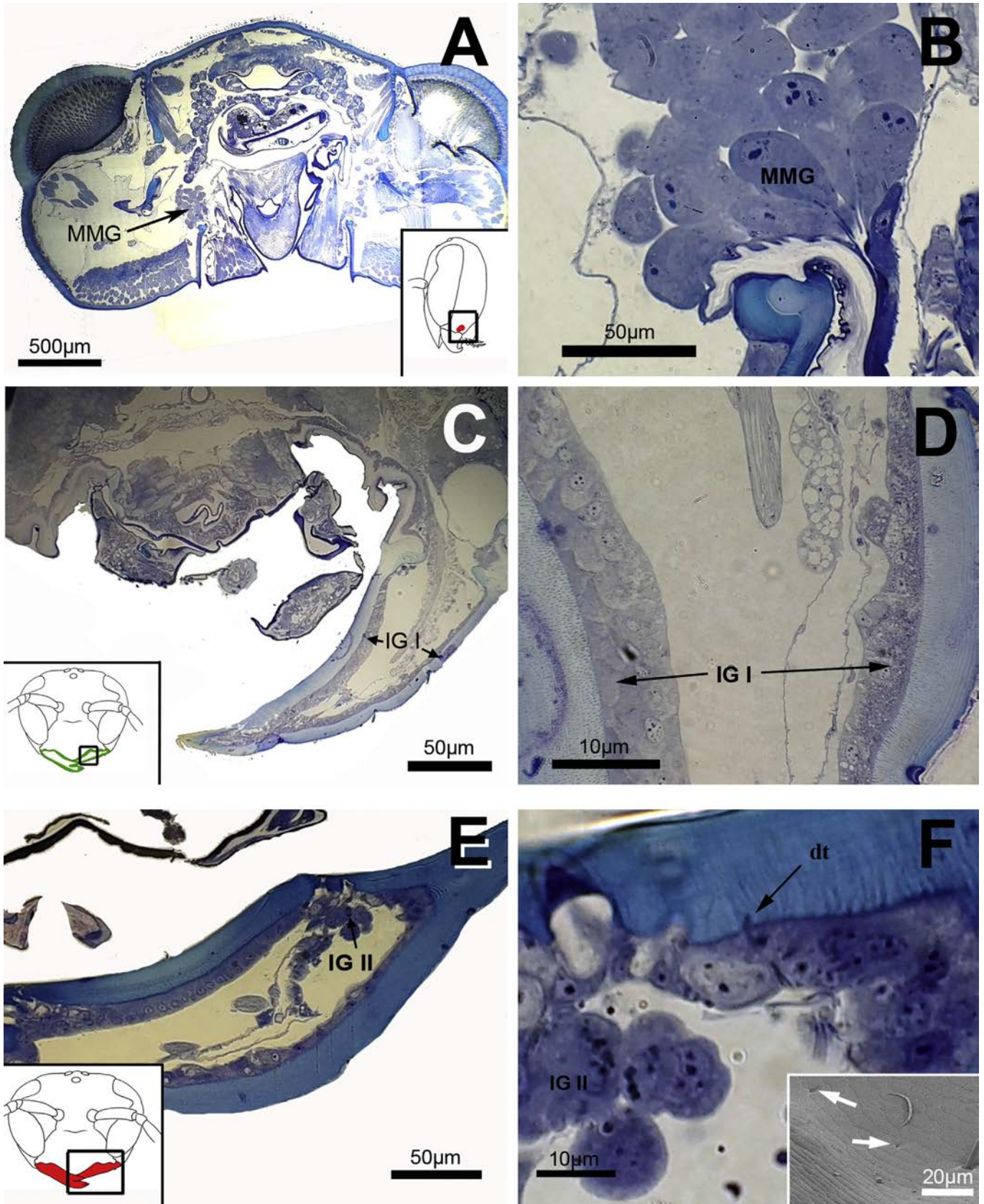


Fig. 4. A–B. Mesal mandibular gland (MMG) in *Parachartergus smithii*, C–D. Intramandibular gland I (IG I) in *Apoica thoracica*, E–F. Intramandibular gland II (IG II) in *Leipomeles spilogastra*. Arrows in SEM inset of F points to a 0.5 µm pore. Drawing inset indicates the approximate location of the images in the specimen. Ducts of this gland (dt) at the base of the mandible are indicated. Class 1 glands are shown in green, class 3 glands in red.

3.10. Intramandibular gland I (class 1)

This is a single layer of secretory rectangular cells attached by their shorter side to the exoskeleton (Fig. 4C–D). This gland covers the entire inner surface of the mandible but the cells are larger at the teeth and where setae are located. In several cases, cells penetrating the cuticle were observed in the sections. Cell height ranges between 10 and 40 μm . Nucleus diameter varies between 3.5 and 30 μm (Suppl. Table 1). The gland was observed in all the species studied. A similar gland was recorded by Cruz-Landim (1962) and Costa Leonardo (1978) for bees, and by Billen and Espadaler (2002) and Amaral and Caetano (2005) for ants, this is the first report for social wasps.

3.11. Intramandibular gland II (class 3)

This is a group of several clusters of rounded cells with ducts that open directly to the mesal and ectal sides of the mandible (Fig. 4E–F). The cluster opens at several places such as the anterior denticle, the median denticle, and the medial basal area. Cell diameter ranges between 10 and 70 μm . Maximum nucleus diameter is 6 μm . The number of cells ranges between 50 and 150 (Suppl. Table 1). This gland was only observed in four species: *M. angulatus*, *Apoica gelida* Van der Vecht, 1973, *L. spilogastra* and *Polybia liliacea* (Fabricius, 1804). A similar organ was described by Nedel (1960) and Costa Leonardo (1978) for bees. However, Nedel (1960) did not find it in the *Vespa* specimens included in his study. Schoeters and Billen (1994) describe this structure for many species of ants.

3.12. Gland size variation

An interesting characteristic of the dataset was the large variation across species in several gland dimensions. In a similar way to studies where a relationship between gland dimensions and body size has been observed (Wilson, 1980; Schoeters and Billen, 1991; Angus et al., 1993), we conducted linear regression analyses to briefly explore this relationship. Species head width, measured with a calibrated scale on a stereoscope, was used as proxy for body size (Suppl. Table 1). We found that several of the gland dimensions were related to body size such as gland height ($R^2 = 0.14$, $F_{1,31} = 6.14$, $p = 0.02$), cell diameter ($R^2 = 0.25$, $F_{1,31} = 11.97$, $p = 0.001$), and the number of cells of the hypopharyngeal gland ($R^2 = 0.34$, $F_{1,31} = 17.26$, $p = 0.0002$, Fig. 5A), while in other cases, such as the number of cells of the mesal mandibular gland

($R^2 = 0.06$, $F_{1,31} = 2.64$, $p = 0.12$, Fig. 5B), there was no statistical relationship between body size and gland dimension.

Additionally in several dispersion plots some species strongly depart of the trend and we have no obvious explanation here. For example, the large four species of *Polistes* plus the medium sized *Agelaia fulvofasciata* (Degeer, 1773) show a large number of cells of the hypopharyngeal gland while other large species such as *Synoeca septentrionalis* Richards, 1978 and *S. surinama* (Linnaeus, 1767) exhibit a lower number of cells for their size (Fig. 5A). A similar situation occurred for the number of cells of the mesal mandibular gland; *Apoica albimaculata* (Fabricius, 1804) and *Polistes canadensis* (Linnaeus, 1758) show an unusually large number of gland cells while comparatively larger species such as these of *Synoeca* and *P. carnifex* (Fabricius, 1775) show values below the regression line.

4. Discussion

4.1. General findings and taxonomic distribution of glands

The present study describes 11 glands in the workers of 33 species distributed along 13 genera of neotropical Polistinae (Suppl. Table 1). Five of those are class 1 glands and six are class 3 glands. A reservoir was observed only in the ectal mandibular gland. Five glands are structures described here for the first time, and three are new records for the subfamily. The hypopharyngeal gland, the ectal mandibular gland, and the mesal mandibular gland were previously reported for queen wasps (Landolt and Akre, 1979). In the present study we recorded these glands also in foragers. The number of species studied allowed us to find several novel glands as their taxonomic distribution was not widespread. Our work almost doubles the number of exocrine glands known for the head of Polistinae and highlights the importance of conducting detailed morphological studies that will reveal the diversity of glands and of chemical communication in social wasps. Future detailed studies in other body regions of social wasps may lead to the discovery of more exocrine glands. This is the case of the new gland found on the antennae of vespids (Romani et al., 2005) and the significant number of glands recently discovered in the legs of social wasps (Nijs and Billen, pers. comm.). A similar trend has been observed in ants (Billen, 2009) and bees (Billen and Vander Plancken, 2014).

As expected, well-known glands such as the hypopharyngeal gland, the ectal mandibular gland and the mesal mandibular gland, are widely distributed in the species studied and suggest an origin prior to all Polistinae. Likewise, the glands described here: perioral gland, clypeal gland, posterobasal genal gland, and

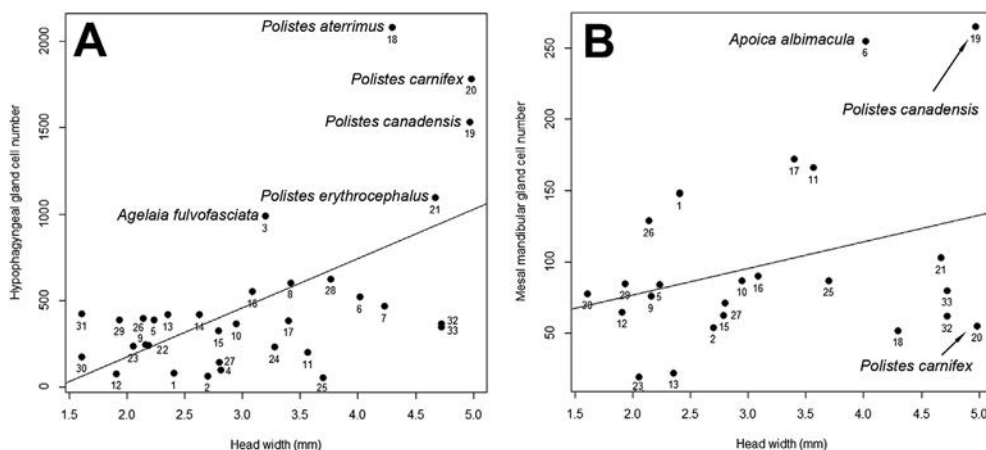


Fig. 5. A. Relationship between head width as indicator of body size and number of gland cells of the hypopharyngeal gland across species of Polistinae, and B. Number of gland cells of the mesal mandibular gland. Numbers 32 and 33 indicate *Synoeca septentrionalis* and *S. surinama* respectively, for other numbers refer to Supplementary Table 1.

intramandibular gland I, were recorded in almost all the species studied indicating that these may have evolved in vespids prior to the origin of the subfamily. Their absence in some species may result from sampling or section deficiencies. Given the number of individuals studied, the absence of some glands seems to be real for the following species: the periocular gland in *P. exigua* and the posterobasal genal gland in *Parachartergus* species and in *L. spilogastra*. On the contrary, some glands were observed in only a few species, opening the discussion about the reason for this restricted taxonomic distribution. These glands are the ocellar gland I, recorded in *A. areata* and *L. spilogastra* only, the ocellar gland II observed in only four species, the subantennal gland found only in *P. emaciata* and *P. amarella*, and the intramandibular gland II observed in only four species (Suppl. Table 1).

The large unexplained variation in the relationship between body size and gland dimensions, as seen by the low R values, indicates that the explanatory power of these lineal models is low. In addition, comparative studies must take into account that species are not independent data and must include the phylogenetic factor into the analysis (Felsenstein, 1985; Harvey and Pagel, 1991). Available comparative methods require a strongly supported phylogeny of the group and despite the largely stable generic phylogeny proposed by Wenzel and Carpenter (1994), currently there is not a single large tree for the Polistinae at the species level where branch lengths are provided. Further studies may analyze in greater detail the variation described.

4.2. Gland functions

In contrast to the extensive and detailed studies about the gland function in bees and ants, this is not the case with social wasps (Landolt and Akre, 1979). The identification of gland function could be a challenging task and several lines of evidence should be considered in this endeavor. At present we have no indications for the role of glands such as the ocellar gland I, ocellar gland II, periocular gland, and the posterobasal genal gland. Where possible, information of comparable glands reported in other social insects will be discussed for glands newly reported here for vespids.

The location of the subantennal gland may suggest that this structure is related to the lubrication of the articulation. Using similar arguments, Billen (2009) has suggested that the class 1 and class 3 glands found at the joints of the legs may help the movement of these structures (Billen and Ito, 2006; Billen, 2009).

The hypopharyngeal gland is exclusive to the order Hymenoptera and in bees produces the royal jelly (Jung-Hoffmann, 1966; Britto and Caetano, 2006). It is homologous to the prothoracic gland of ants, where this is involved in the production of digestive enzymes (Ayre, 1967). A detailed structural and cytochemical study of *Polistes versicolor* (Olivier, 1791) by Britto and Caetano (2008) leads these authors to suggest that the hypopharyngeal gland is related to digestive functions too. In social wasps, Downing and Jeanne (1983) observed no relationship between cell size and dominance in *P. fuscatus* (Fabricius, 1793), and no relationship with nesting season in temperate vespines. Likewise, Britto et al. (2004) found no relationship with age in the neotropical species *P. versicolor*.

There are observations suggesting that social wasps use their clypeus for several functions as in several species the ventral clypeus vestiture fades away with age (Richards, 1978). This may happen through rubbing this head area onto the substrate which at the same time allows clypeal gland secretions to be released. Clypeal rubbing behavior has also been observed in male *Philanthus* wasps (Crabronidae) which in this case has been interpreted as the deposition of mandibular glandular secretions over plant leaves located in their territory (Goettler and Strohm, 2008).

Downing and Jeanne (1982) suggest a social role for the ectal mandibular gland because the duct opening of its reservoir is located at the mandible base and because it is associated to a brush of hairs that can be seen when the mandible is open. They use this fact as part of their argument to suggest a social function of the organ. However, the function of this organ seems to be more extensive. Fortunato et al. (2001), Togni and Giannotti (2007), and Turillazzi (2012) relate the gland to defensive behaviors in *Mischocyttarus cerberus* Ducke, 1918 and *Polistes* species. Fortunato and Coster-Longman (2000) relate the gland to sexual displays in Stenogastrinae males. Landolt and Akre (1979) describe a role of this gland in sexual attraction in *P. fuscatus*. Spradbery (1973) suggests this gland is related to lubrication and feeding, while Downing and Jeanne (1982) provide evidence suggesting a role of this gland in social dominance. Wenzel (1987) found that this gland may be related to male mating behaviors in *Polistes major* Palisot de Beauvois, 1818. García and Noll (2013) studying *Polybia paulista* H. von Ihering, 1896 found a relationship between aging and gland size suggesting that their role may be related to intra-nest functions. A similar situation of multiple functions for the same organ has been discovered in ants where the ectal mandibular gland can have different functions between species and even between castes (Billen and Morgan, 1998; Billen et al., 1998; Morgan et al., 2003; Niculita et al., 2007). Additionally, there are studies indicating that the products or the functions of very different glands may be quite similar (Gobin et al., 2003). Landolt et al. (1999), for example, show evidence indicating that in addition to the alarm pheromone commonly recorded from the venom gland in *Vespula squamosa* (Drury, 1773), additional glands eliciting alarm and defensive behaviors are located in the head.

The mesal mandibular gland has been studied by Downing and Jeanne (1983) and these authors found a relationship between dominance, age, and size and color of the gland which may relate the gland to hierarchical organization; they also observed a relationship between gland color and nest construction activity. The widespread distribution of the gland observed in our study (24 out of the 33 species) concurs with previous findings (Downing, 1991) and may suggest a significant function of this organ in the life of social wasps.

Intramandibular glands have been studied by Cruz-Landim et al. (2011) in the stingless bee *Melipona quadrifasciata* Lepelletier, 1836 and these authors found larger class 1 cells in younger individuals, even before nursing activities are performed, while class 3 cells were larger in the foraging workers. Billen and Espadaler (2002) suggested that the class 1 intramandibular gland in the ant *Pyramica membranifera* (Emery, 1869) may be related to predatory activities. Given its basal location in the mandible, the intramandibular gland may be related to nutrition in the ant *Protanilla wallacei* Hölldobler and Wilson (1990) (Billen et al., 2013). Intramandibular glands had not been reported previously in social wasps. Our findings revealed the existence of two mandibular glands of which intramandibular gland I (class 1) is present in all the species studied while the intramandibular gland II (class 3) was found in four species only. The role of both glands that are novel for social wasps requires further research.

4.3. Cuticular modifications associated to glands

In contrast to the metasomal glands of social wasps, where cuticular modifications are clearly associated (Jeanne and Post, 1982; Jeanne et al., 1983; Samacá et al., 2013), this relationship seems not to be the case for most of the head glands. There are multiple examples of exocrine glands without skeletal

modifications associated, as their product is secreted directly to the exterior (Gobin et al., 2003).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.asd.2015.06.002>.

References

- Amaral, J.B., Caetano, F.H., 2005. The intramandibular gland of leaf-cutting ants (*Atta sexdens rubropilosa* Forel, 1908). *Micron* 37, 154–160.
- Andena, S.R., Noll, F., Carpenter, J.M., 2007. Phylogenetic analysis of the Neotropical social wasps of the genus *Angiopolybia* Araujo, 1946 (Hymenoptera, Vespidae, Epiponini). *Zootaxa* 1427, 57–64.
- Andena, S.R., Carpenter, J.M., Pickett, K.M., 2009a. Phylogenetic analysis of species of the neotropical social wasp *Epipona* Latreille, 1802 (Hymenoptera, Vespidae, Polistinae, Epiponini). *ZooKeys* 20, 325–398.
- Andena, S.R., Carpenter, J.M., Noll, F., 2009b. A phylogenetic analysis of *Synoeca* De Saussure, 1852, a neotropical genus of social wasps (Hymenoptera, Vespidae, Epiponini). *J. N. Y. Entomol. Soc.* 115, 81–89.
- Andena, S.R., Carpenter, J.M., 2012. A phylogenetic analysis of the social wasp genus *Brachygastra* Perty, 1833, and description of a new species (Hymenoptera: Vespidae: Epiponini). *Am. Mus. Novit.* 3753, 1–38.
- Angus, C.J., Jones, M.K., Beattie, A.J., 1993. A possible explanation for size differences in the metapleural glands of ants (Hymenoptera, Formicidae). *Aust. J. Entomol.* 32, 73–77.
- Ayre, G.L., 1967. The relationships between food and digestive enzymes in five species of ants (Hymenoptera; Formicidae). *Can. Entomol.* 99, 408–411.
- Billen, J., Morgan, E.D., 1998. Pheromone communication in social insects: sources and secretions. In: Vander Meer, R.K., Breed, M., Espelie, K., Winston, M. (Eds.), *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*. Westview Press, pp. 3–33.
- Billen, J., Ito, F., Maile, R., Morgan, E.D., 1998. The mandibular gland, probably the source of the alarm substance in *Leptanilla* sp. (Hymenoptera, Formicidae). *Naturwissenschaften* 85, 596–597.
- Billen, J., Espadaler, X., 2002. A novel intramandibular gland in the ant *Pyramica membranifera* (Hymenoptera, Formicidae). *Belg. J. Zool.* 132, 175–176.
- Billen, J., 2006. Morphology and ultrastructure of the Dufour gland in workers of social wasps (Hymenoptera, Vespidae). *Arthropod Struct. Dev.* 35, 77–84.
- Billen, J., 2009. Occurrence and structural organization of the exocrine glands in the legs of ants. *Arthropod Struct. Dev.* 38, 2–15.
- Billen, J., Ito, F., 2006. The basicoxal gland, a new exocrine structure in poneromorph ants (Hymenoptera, Formicidae). *Acta Zool.* 87, 291–296.
- 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., Vander Plancken, L., 2014. Exocrine glands in the legs of the stingless bee *Friesiomeletta varia* (Lepeletier) (Apidae: Meliponini). *Sociobiology* 61, 386–392.
- Billen, J., Wilson, E.O., 2008. Social insect histology from the nineteenth century: the magnificent pioneer sections of Charles Janet. *Arthropod Struct. Dev.* 37, 163–167.
- Bordas, M.L., 1907. Sur les glandes cutanées ou glandes sternales des Vespidae. *Comptes Rendues Soc. Biol. Paris* 62, 978–979.
- Britto, F., Caetano, F.H., Silva De Moraes, R.L.M., 2004. Comparative analysis of morphological, structural and morphometric patterns of *Polistes versicolor* (Olivier) (Hymenoptera: Vespidae) hypopharyngeal glands. *Neotrop. Entomol.* 33, 321–326.
- Britto, F., Caetano, F.H., 2006. Morphological features and occurrence of degenerative characteristics in the hypopharyngeal glands of the paper wasp *Polistes versicolor* (Olivier) (Hymenoptera: Vespidae). *Micron* 37, 742–747.
- Britto, F.B., Caetano, F.H., 2008. Ultrastructural features of the hypopharyngeal glands in the social wasp *Polistes versicolor* (Hymenoptera: Vespidae). *Insect Sci.* 15, 277–284.
- Cely, C., Sarmiento, C.E., 2011. What about intraspecific variation? Reassessment of taxonomic and phylogenetic characters in the genus *Synoeca* de Saussure (Hymenoptera: Vespidae: Polistinae). *Zootaxa* 2899, 43–59.
- Cooper, M., 2000. Five new species of *Agelaia* Lepeletier (Hym., Vespidae, Polistinae) with a key to members of the genus, new synonymy and notes. *Entomol. Mon. Mag.* 136, 177–198.
- Costa Leonardo, A.M., 1978. Glandulas intramandibulares em abelhas sociais. *Cienc. Cult.* 307, 835–838.
- Cruz-Landim, C., 1962. Evaluation of the wax and scent glands in the Apinae (Hymenoptera: Apidae). *J. N. Y. Entomol. Soc.* 21, 2–13.
- Cruz-Landim, C., Gracioli-Vitti, L.F., Abdalla, F.C., 2011. Ultrastructure of the intramandibular gland of workers and queens of the stingless bee, *Melipona quadrifasciata*. *J. Insect Sci.* 11, 1–9.
- Dallwitz, M.L., Paine, T.A., Zurcher, E.J., 1999. User's guide to the DELTA. Available from: <http://www.deltaintkey.com/>. Accessed: nov 2012.
- Delfino, G., Piccioli, M., Calloni, C., 1979. Fine structure of the gland of Van der Vecht organ in *Polistes gallicus* (L) (Hymenoptera Vespidae). *Monit. Zool. Ital.* 13, 221–247.
- Downing, H.A., 1991. The function and evolution of exocrine glands. In: Ross, K.G., Matthews, R.W. (Eds.), *The Social Biology of Wasps*. Cornell University Press, Ithaca, pp. 540–569.
- Downing, H.A., Jeanne, R.L., 1982. A description of the ectal mandibular gland in the paper wasp *Polistes fuscatus* (Hymenoptera: Vespidae). *Psyche* 89, 317–320.
- Downing, H.A., Jeanne, R.L., 1983. Correlation of season and dominance status with activity of exocrine glands in *Polistes fuscatus* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 56, 387–397.
- Felsenstein, J., 1985. Phylogenies and the comparative method. *Am. Nat.* 125, 1–15.
- Fortunato, A., Coster-Longman, C., 2000. Reproductive behavior and relative anatomical structures for the production of pheromones and luminous signal in male *Metischnogaster* (Vespidae: Stenogastrinae). *Insects Soc. Life* 3, 27–28.
- Fortunato, A., Maile, R., Turillazzi, S., Morgan, E.D., Moneti, G., Jones, G., Pieraccini, G., 2001. Defensive role of secretion of ectal mandibular glands of the wasp *Polistes dominulus*. *J. Chem. Ecol.* 27, 569–579.
- García, Z., Noll, F., 2013. Age and morphological changes in the Epiponini wasp *Polybia paulista* Von Ihering (Hymenoptera: Vespidae). *Neotrop. Entomol.* 42, 293–299.
- Gobin, B., Ito, F., Billen, J., 2003. The subepithelial gland in ants: a novel exocrine gland closely associated with the cuticle surface. *Acta Zool.* 84, 285–291.
- Goettler, W., Strohm, E., 2008. Mandibular glands of male European beeswolves, *Philanthus triangulum* (Hymenoptera, Crabronidae). *Arthropod Struct. Dev.* 37, 363–371.
- Harvey, P., Pagel, M., 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Cambridge.
- Herzner, G., Goettler, W., Kroiss, J., Pura, A., Webb, A., Jakob, P.M., Rössler, W., Strohm, E., 2007. Males of a solitary wasp possess a postpharyngeal gland. *Arthropod Struct. Dev.* 36, 123–133.
- Heselhaus, F., 1922. Die Hautdrüsen der Apiden und verwandter Formen. *Zool. Jahrb. Abt. Anat. Ontog. Tiere* 43, 369–464.
- Hölldobler, B., Wilson, E.O., 1990. *The Ants*. Harvard University Press, Cambridge, Massachusetts.
- Huber, J.T., Sharkey, M.J., Fernández, F., 2006. Estructura y glosario. In: Fernández, F., Sharkey, M.J. (Eds.), *Introducción a los Hymenoptera de la Región Neotropical*. Sociedad Colombiana de Entomología y Universidad Nacional de Colombia. Bogotá D. C., pp. 57–92.
- Janet, C., 1894. Sur les nerfs de l'antenne et les organes chordotonaux chez les fourmis. *Comptes Rendus Acad. Sci.* 118, 814–817.
- Jeanne, R.L., Post, D., 1982. Richards' gland and associated cuticular modifications in social wasps of the genus *Polybia* Lepeletier (Hymenoptera, Vespidae, Polistinae, Polybiini). *Insectes Sociaux* 29, 280–294.
- Jeanne, R.L., Downing, A., Post, D., 1983. Morphology and function of sternal glands in polistine wasps (Hymenoptera: Vespidae). *Zoomorphology* 103, 149–164.
- Jung-Hoffmann, I., 1966. Die Determination von Königin und Arbeiterin der Honigbiene (*Apis mellifera* L.). *Z. Bienenforsch.* 8, 296–322.
- Landolt, P., Akre, R., 1979. Occurrence and location of exocrine glands in some social Vespidae (Hymenoptera). *Ann. Entomol. Soc. Am.* 72, 141–148.
- Landolt, P.J., Reed, H.C., Heath, R.R., 1999. An alarm pheromone from the heads of worker southern yellowjackets, *Vespa squamosa* (Drury) (Hymenoptera: Vespidae). *Fla. Entomol.* 82, 356–359.
- Morgan, E.D., Jungnickel, H., Keegans, S.J., Do Nascimento, R., Billen, J., Gobin, B., Ito, F., 2003. Comparative survey of abdominal gland secretions of the ant subfamily Ponerinae. *J. Chem. Ecol.* 29, 95–114.
- Nedel, J.O., 1960. Morphologie und Physiologie der Mandibeldrüse einiger Bienen-Arten (Apidae). *Z. Morphol. Ökol. Tiere* 49, 139–183.
- 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., Quenchedy, A., 1974. Fine structure of insect epidermal glands. *Annu. Rev. Entomol.* 19, 61–80.
- Noirot, C., Quenchedy, A., 1991. Glands, gland cells, glandular units: some comments on terminology and classification. *Ann. Soc. Entomol. Fr.* 27, 123–128.

- Pickett, K.M., Wenzel, J., 2007. Revision and cladistic analysis of the nocturnal social wasp genus, *Apoica* Lepeletier (Hymenoptera: Vespidae: Polistinae, Epiponini). *Am. Mus. Novit.* 3562, 1–30.
- Richards, O.W., 1978. *The Social Wasps of the Americas Excluding the Vespinae*. British Museum (Natural History), London.
- Rocha, T., Caetano, F., 2003. Ultramorphology and histology of the *Polistes versicolor* (Olivier) (Vespidae) thorax salivary gland compared with other Hymenoptera. *Neotrop. Entomol.* 32, 585–590.
- Romani, R., Isidoro, N., Riolo, P., Bin, F., Fortunato, A., Turillazzi, S., Beani, L., 2005. A new role for antennation in paper wasps (Hymenoptera, Vespidae): antennal courtship and sex dimorphic glands in antennomeres. *Insectes Sociaux* 52, 96–102.
- Samacá, E., Billen, J., Sarmiento, C.E., 2013. Morphology of the fifth sternal glands of Neotropical social wasps (Hymenoptera, Vespidae, Polistinae). *Invertebr. Biol.* 132, 163–172.
- Schoeters, E., Billen, J., 1991. Morphologie des glandes pro- et postpharyngiennes chez *Atta sexdens*. *Actes des Colloq. Insectes Sociaux* 7, 153–160.
- Schoeters, E., Billen, J., 1994. The intramandibular gland, a novel exocrine structure in ants (Insecta, Hymenoptera). *Zoomorphology* 114, 125–131.
- Schuberth, J., Schönitzer, K., 1993. Vergleichende Morphologie der Fovea facialis und der Stirnseitendrüse bei Apoidea und Sphecidae (Hymenoptera, Aculeata). *Linz. Biol. Beiträge* 25, 205–277.
- Sereno, P.C., 2007. Logical basis for morphological characters in phylogenetics. *Cladistics* 23, 565–587.
- Silveira, O.T., Araújo dos Santos Jr., J.N., 2011. Comparative morphology of the mandibles of female polistine social wasps (Hymenoptera, Vespidae, Polistinae). *Rev. Bras. Entomol.* 55, 479–500.
- Smith, A., O'Donnell, S., Jeanne, R.L., 2001. Correlated evolution of colony defense and social structure: a comparative analysis in eusocial wasps (Hymenoptera: Vespidae). *Evol. Ecol. Res.* 3, 331–344.
- Spradbery, P., 1973. *Wasps*. University of Washington Press, Seattle.
- Togni, O.C., Giannotti, E., 2007. Nest defense behavior against the attack of ants in colonies of pre-emergent *Mischocyttarus cerberus* (Hymenoptera, Vespidae). *Sociobiology* 50, 675–694.
- Turillazzi, S., 2012. *The Biology of Hover Wasps*. Springer-Verlag, Berlin.
- Wenzel, J.W., 1987. Male reproductive behavior and mandibular glands in *Polistes major* (Hymenoptera Vespidae). *Insectes Sociaux* 34, 44–57.
- Wenzel, J.W., Carpenter, J.M., 1994. Comparing methods: adaptive traits and tests of adaptation. In: Eggleton, P., Vane-Wright, R. (Eds.), *Phylogenetics and Ecology*. Academic Press, London, pp. 79–101.
- Wilson, E.O., 1980. Caste and division of labor in leaf-cutter ants: I. The overall pattern in *Atta sexdens*. *Behav. Ecol. Sociobiol.* 7, 143–156.