

Antennal glands of the slave-making ant *Polyergus rufescens* and its slave species *Formica cunicularia* (Hymenoptera, Formicidae)

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Abstract: The fine morphology of glandular structures associated with the antennae is reported for the first time in a social parasite ant, the obligate slave-maker *Polyergus rufescens* (Latreille, 1798). In this species, external pores have been detected through scanning electron microscopy only on the scape of the female castes (queen and worker). Each pore is associated internally with a bicellular secretory unit by means of a cuticular duct. The number of secretory cells appears to be higher in queens than in workers. Similar exocrine structures have been found also in workers of *Formica cunicularia* Latreille, 1798, a common host species of *P. rufescens*. The possible functional role of this gland is discussed.

Résumé : Nous présentons pour la première fois la morphologie fine des structures glandulaires associées à l'antenne chez une fourmi parasite sociale, l'esclavagiste obligatoire *Polyergus rufescens* (Latreille, 1798). Chez cette espèce, on trouve au microscope électronique à balayage des pores externes seulement sur le scape des castes femelles (reine et ouvrière). Chaque pore est relié à l'intérieur à une unité sécrétrice bicellulaire par un conduit cuticulaire. Le nombre de cellules sécrétrices semble être plus élevé chez les reines que chez les ouvrières. Des structures exocrines semblables se retrouvent aussi chez les ouvrières de *Formica cunicularia* Latreille, 1798, une espèce-hôte commune de *P. rufescens*. Le rôle fonctionnel possible de cette glande fait l'objet d'une discussion.

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Introduction

Polyergus rufescens (Latreille, 1798) is an extremely specialized social parasite (obligate slave-maker) that periodically conducts group raids against colonies of the related genus *Formica* (*Serviformica*) to sack the resident brood that is reared to eclosion and integrated into the work force of the parasite colony (Hölldobler and Wilson 1990; Mori et al.

1991; Le Moli et al. 1994). The parasitic habit of this species is also evident in the context of colony founding: newly mated females are not able to start a new colony alone, so they invade a *Formica* colony, kill the resident queen, and become accepted by the resident workers. This is a crucial phase of the reproductive biology of the genus *Polyergus*, since during nest invasion the parasite queen must cope with fierce hostility from the resident workers (Mori et al. 2001). In both contexts, *P. rufescens* uses efficient behavioural and morphofunctional adaptations, among which the exocrine glands play a major role. For example, chemical secretions from the mandibular gland are used by workers as "propaganda" allomones during host-nest invasion (Visicchio et al. 2001), while secretions from Dufour's gland are used by newly mated queens to appease residents during host-nest usurpation (Mori et al. 2000a, 2000b, 2001).

Because of the amazing development of the exocrine system in ants and the importance of exocrine glands for virtually all aspects of their biology (see Hölldobler and Wilson 1990; Billen and Morgan 1998), in recent years we have conducted a series of morphofunctional investigations on the glandular equipment of *P. rufescens* (Billen et al. 2001; Grasso et al. 2004, 2005). Here we continue the survey, since investigations on the morphofunctional features of glands, their development, roles, and diversity in these spe-

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cialized ants may further clarify the astonishing puzzle of their biology and parasitic habit.

We focused our attention on the antennae, since to date the presence of antennal glands in ants has been investigated in only a few species (Bolton 1999; Billen 2000; Isidoro et al. 2000), not including either *P. rufescens* or its slave species *Formica cunicularia* Latreille, 1798.

Materials and methods

Individuals of *P. rufescens* and *F. cunicularia* used for morphological studies were collected from field colonies located in Parma (northern Italy).

For scanning electron microscopy (SEM) observations, 10 winged queens, 10 workers, and 10 males of *P. rufescens*, as well as 10 workers of *F. cunicularia* were used. Individuals were beheaded and the heads were immersed in a 50% ethanol–water solution. After dehydration in a graded ethanol series, the heads with the antennae were examined through a Philips® XL 30 scanning electron microscope after having been processed through a critical-point drier and a gold-coating unit.

For transmission electron microscopy (TEM) observations, the same number of individuals of both species (see above) were anaesthetized in CO₂ and immediately immersed in 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer + 5% glucose, pH 7.2–7.3. Then the first antennomere (the scape, or A1) was detached from the head capsule to help fixative penetration and left at 4 °C for about 2 h. After overnight rinsing in cacodylate buffer the specimens were postfixed in 1% osmium tetroxide at 4 °C for about 1 h and rinsed in the same buffer. Dehydration in a graded ethanol series was followed by embedding in Epon–Araldite mixture with propylene oxide as the bridging solvent. Thin sections were cut with a diamond knife on a LKB® Nova ultramicrotome and mounted on collodion-coated 50-mesh (50 lines/inch, where 1 inch = 25.4 mm) grids. Finally, the sections were observed with a Philips EM 400T transmission electron microscope after staining with uranyl acetate (20 min at room temperature) and lead citrate (5 min at room temperature).

Results

External morphology and localization

In *P. rufescens* the antennae are sexually dimorphic and composed of 12 antennomeres in females (both queens and workers) and 13 in males. In both cases the scape makes up about 30% of the entire antennal length, although scapes vary in shape. In queens and workers the scape (about 1 mm long) resembles a juggler's club, being slender at the proximal end and wider distally (Figs. 1A–1C). In *P. rufescens* the latter feature is more pronounced in the queens, while in males the diameter of the scape (which is about 650 µm long) is about the same from the base to the apical part (Fig. 1D).

In *F. cunicularia* workers the antennae are composed of 12 antennomeres as in *P. rufescens*, but the scape is longer (about 1.4 mm) and its distal part narrower (Fig. 1E).

In both species the antennal glands consist of bicellular secretory units composed of a secretory cell that produces a secretion which is released through external cuticular pores

connected internally via a cuticular conducting canal produced by the associated duct cell. Internal examination after tissue removal revealed the presence of the ducts belonging to the glandular units. Such ducts (length about 50 µm, diameter about 0.5 µm) are located in the distal (clublike) region of the scape, and open on the ventrolateral wall of the antennomere.

Ultrastructure of the antennal glands

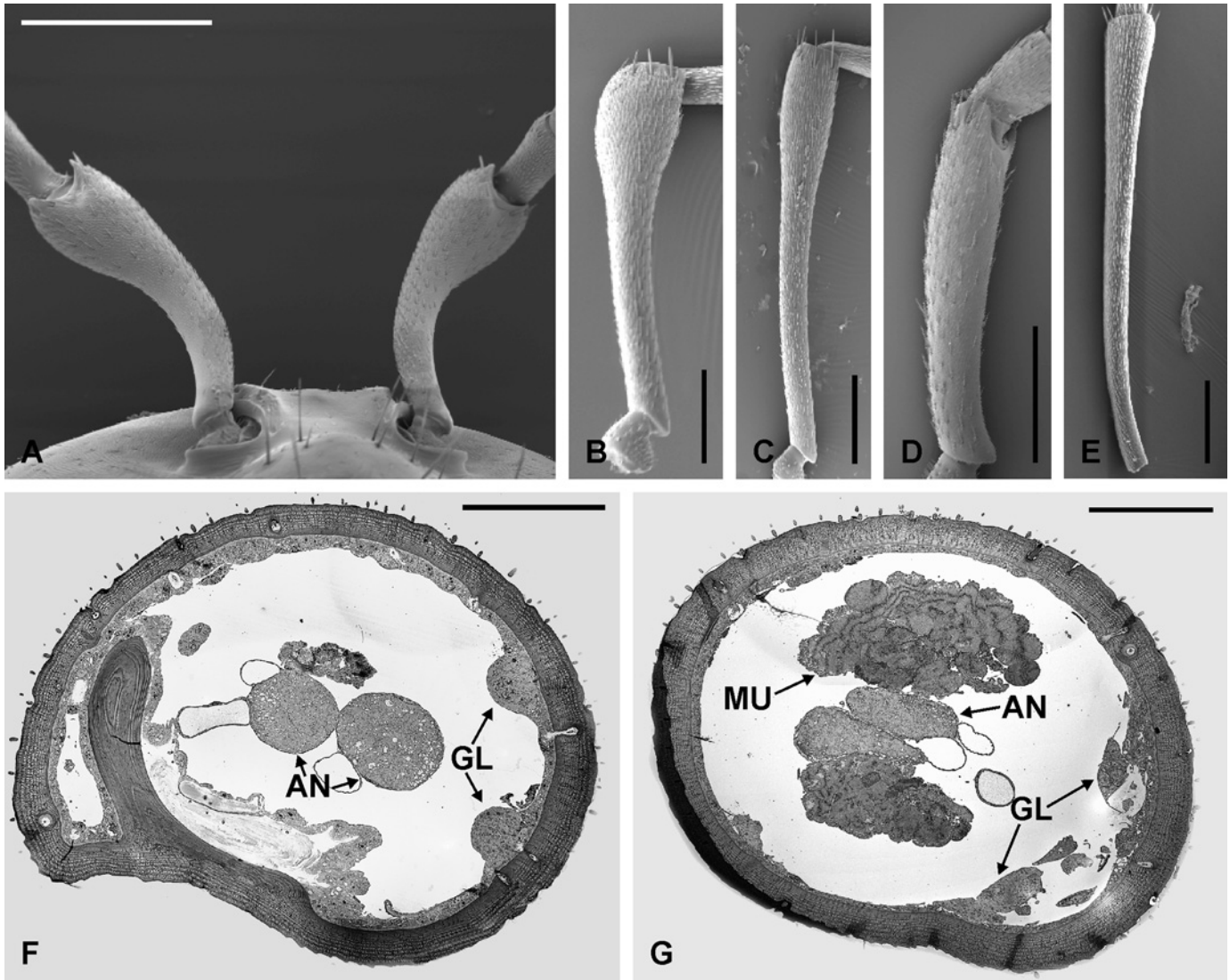
In all the individuals of both species, except male *P. rufescens*, that were examined, serial cross and oblique thin sections revealed the presence of isolated cell masses (numbering 15–20; $n = 10$ (no. of examined individuals), which are located immediately underneath the cuticle in both workers and queens of *P. rufescens* (Figs. 1F, 1G). Each mass is made up of a secretory unit and a duct cell. In cross section, the glandular units of queens appear quite different from those of workers: in the former they are more irregular in shape and flattened, while in the latter they are more regular in shape (almost spherical) (Figs. 1F, 1G). Moreover, in queens the glands appear to be positioned more towards the antennal lumen, while in workers they are attached to the inner cuticular wall. In *F. cunicularia* workers the antennal glands occupy the same position as in *P. rufescens* workers, and have been found to number about 20 ($n = 10$), each connected to a single conducting canal.

In both species the secretory cells show similar cytological features. The main ultrastructural attribute of these cells is the receiving canal, which occupies most of the cell volume (Figs. 2A, 2B). The receiving canal, located in the apical region of the secretory cell, is short and surrounded by electron-dense secretory vesicles. TEM sections show the presence of large rounded nuclei located laterally in the cytoplasm and characterized by abundant chromatin condensations and evident nucleoli. The secretory cells do not exhibit infoldings of the basal plasma membrane, possibly because of discontinuous activity of the secretory cells themselves. The cytoplasm contains mitochondria and Golgi apparatus as well as numerous ribosomes either freely organised in the cytoplasm or arranged in infrequent rough endoplasmic reticulum cisternae (Figs. 2C, 2D). The duct cells are located in close proximity to the cuticle. Their main cytological feature is the presence of a smaller (compared with that of the secretory cells) and more electron-dense nucleus surrounded by sparse cytoplasm that lacks most of the organelles (Fig. 2E). The nucleus is flattened and elongated, and the cuticular duct is either very close to or almost embedded in the nucleus.

Discussion

This is the first description of antennal glands in the obligate slave-maker *P. rufescens* and its slave species *F. cunicularia*. This report is particularly important because detailed descriptions of antennal glands were known for only a few ant species (Billen 2000; Isidoro et al. 2000) and no data were available on social parasitic ants. The glands consist of class 3 secretory cells (Noirot and Quennedey 1974, 1991) opening through cuticular pores on the antennal scape. This is consistent with data on other ants, but the position within the antennae is quite different, suggesting, at least in

Fig. 1. (A) Scanning electron micrograph showing the antennal scapes attached to the head capsule of a *Polyergus rufescens* queen in ventral view. (B–D) Scanning electron micrographs of the antennal scape of a *P. rufescens* queen (B), worker (C), and male (D). (E) Scanning electron micrograph of the antennal scape of a *Formica cunicularia* worker. (F and G) Transmission electron micrograph of a cross section of the distal part of the antennal scape of a *P. rufescens* worker (F) and queen (G), showing the locations of the glandular units (GL). AN, antennal nerves; MU, muscles. Scale bars: 500 μm in A; 250 μm in B–E; 50 μm in F and G.



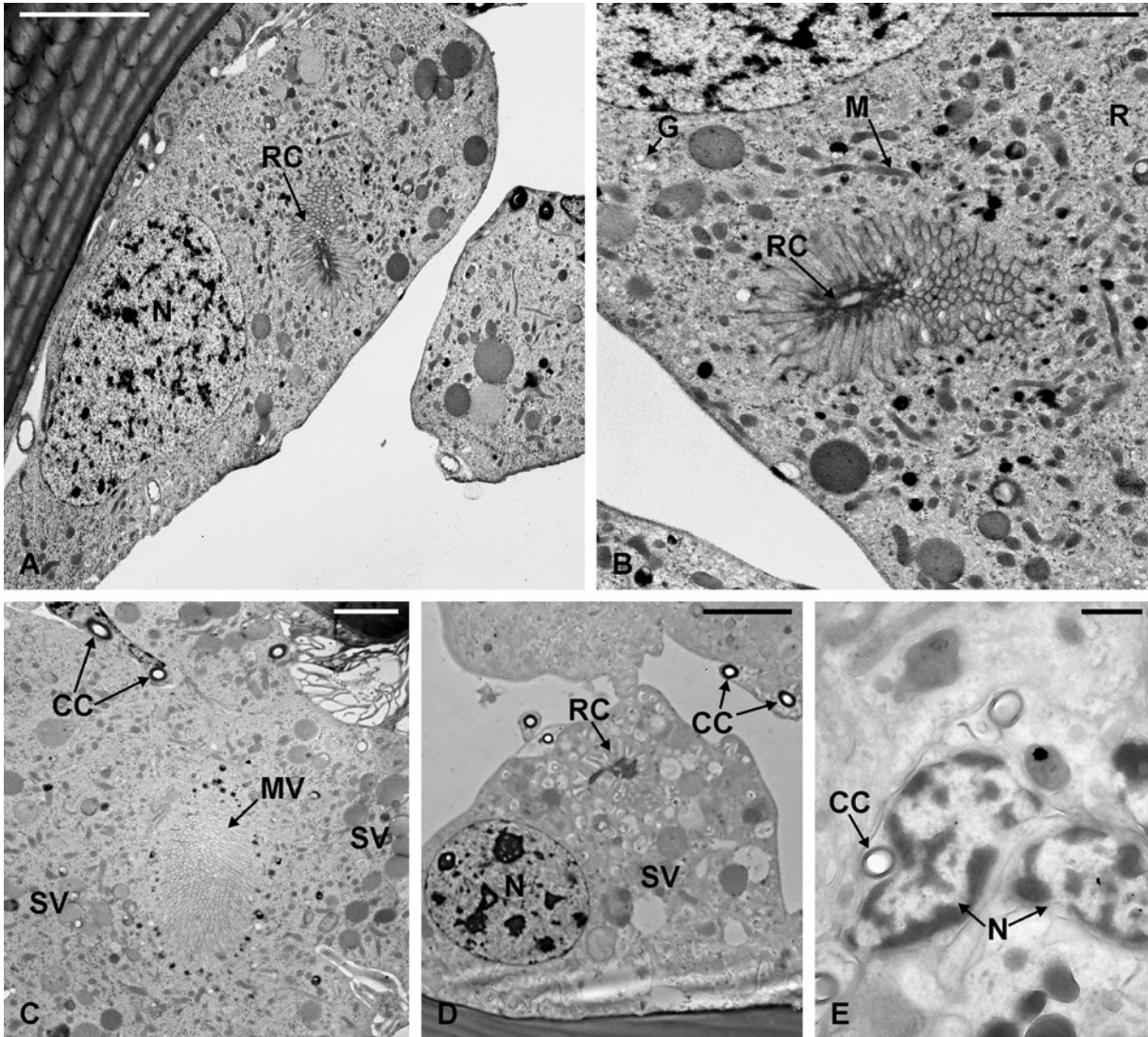
some cases, a nonhomologous origin of the structures. In particular, in queens and workers of *Solenopsis wagneri* Santschi, 1916 (= *Solenopsis invicta* Buren, 1972) the gland is associated with the antennomeres of the funiculus (Isidoro et al. 2000) and not with the antennal scape. In *Eciton burchelli* (Westwood, 1842) the glandular units are located in the distal portion of the scape but in a dorsal position, while in our case they open on the ventrolateral wall of the scape. Furthermore, this type of bicellular scape gland differs strongly from the structures reported for dacetone ants by Bolton (1999), which, as suggested by Billen (2000), are likely formed by glandular epithelium.

The function of these exocrine structures is actually unknown. However, based on previous and present data, hypotheses concerning the functional roles of the antennal glands can be summarized as follows.

In several species of Hymenoptera belonging to either the Terebrantia or the Aculeata, glands located in the antennae of males play a role in sex recognition (Bin and Vinson 1986; Isidoro et al. 1996, 1999; Bin et al. 1999; Romani et al. 2003, 2005). Although males of *P. rufescens* do not exhibit antennal glands, involvement of the gland in the reproductive biology of this species is still possible. In fact, females of *P. rufescens* (not the males) are responsible for sexual calling after nuptial flights (female calling syndrome) (Mori et al. 2001).

Another hypothesis concerning the function of the gland may involve the parasitic nature of this species. In the genus *Polyergus* both newly mated queens and workers must invade alien nests, the former to usurp the resident queen(s) and the latter to sack the resident brood. We have already demonstrated that various exocrine structures play a crucial

Fig. 2. (A–C) Transmission electron micrographs showing ultrastructural details of an antennal gland of a *P. rufescens* queen, including the conducting canals (CC), Golgi complex (G), mitochondria (M), large nuclei (N), free ribosomes (R), receiving canal (RC) bordered by microvilli (MV), and secretory vesicles (SV). (D) Transmission electron micrograph of an antennal gland of a *F. cunicularia* worker. CC, conducting canals; N, large nuclei; RC, receiving canal; SV, secretory vesicles. (E) Transmission electron micrograph of an antennal gland of a *P. rufescens* queen, showing details of the duct cells. CC, conducting canals; N, nuclei. Scale bars: 5 μm in A and D; 2.5 μm in B, C, and E.



role as chemical weapons to facilitate both host-nest usurpation and brood raiding (see Mori et al. 2000a, 2000b; Billen et al. 2001; Visicchio et al. 2001). Hence, in these contexts, the possible involvement of the antennal glands as the source of allomones should not be excluded. The presence of antennal-scape glands also in the slave species (*F. cunicularia*) is consistent with manipulation of host behaviour.

Apart from the communication aspects, potential functions of the antennal glands also include maintenance and homeostasis of the individual. For example, the antennal glands of ants, in view of their peculiar location, may be involved in the cleaning and protection of antennal receptors. The secretion produced by these glands (in species where it has antifungal or antibacterial properties), when smeared or rubbed on the antennae (or other body parts), may play a role in defence against pathogens, as is well documented for

other exocrine structures in ants and other Hymenoptera (see Hölldobler and Wilson 1990; Bot et al. 2002 and references therein; Kaltenpoth et al. 2005). The absence of the glands in males (as in *P. rufescens*) should not present a problem for this hypothesis, since male ants are intensely cared for and groomed by workers during their lifetime (Hölldobler and Wilson 1990). Present and future investigations using a multidisciplinary (morphological, chemical, and behavioural) approach, together with comparative analyses within the Formicidae, will hopefully clarify this topic.

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References

- Billen, J. 2000. A novel exocrine gland in the antennal scape of the army ant *Eciton burchelli*. Belg. J. Zool. **130**: 159–160.
- Billen, J., and Morgan, E.D. 1998. Pheromone communication in social insects — sources and secretions. In *Pheromone communication in social insects: ants, wasps, bees, and termites*. Edited by R.K. Vander Meer, M.D. Breed, M.L. Winston, and K.E. Espelie. Westview Press, Boulder, Colo., and Oxford, U.K. pp. 3–33.
- Billen, J., Grasso, D.A., Mori, A., and Le Moli, F. 2001. Structural and functional changes of the Dufour gland in gynes of the Amazon ant *Polyergus rufescens* (Hymenoptera, Formicidae). *Zoomorphology*, **121**: 55–61.
- Bin, F., and Vinson, S.B. 1986. Morphology of the antennal sex-gland in male *Trissolcus basalis* (Woll.), an egg parasitoid of the green stink bug, *Nezara viridula*. *Int. J. Insect Morphol. Embryol.* **15**: 129–138.
- Bin, F., Waeckers, F.L., Romani, R., and Isidoro, N. 1999. Tyloids in *Pimpla turionellae* (L.) are release structures of male antennal glands involved in courtship behaviour (Hymenoptera: Ichneumonidae). *Int. J. Insect Morphol. Embryol.* **28**: 61–68.
- Bolton, B. 1999. Ant genera of the tribe Dacetoniini (Hymenoptera: Formicidae). *J. Nat. Hist.* **33**: 1639–1689.
- Bot, A.N.M., Ortius-Lechner, D., Finster, K., Maile, R., and Boomsma, J.J. 2002. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. *Insectes Soc.* **49**: 363–370.
- Grasso, D.A., Romani, R., Castracani, C., Visicchio, R., Mori, A., Isidoro, N., and Le Moli, F. 2004. Mandible associated glands in queens of the slave-making ant *Polyergus rufescens* (Hymenoptera, Formicidae). *Insectes Soc.* **51**: 74–80.
- Grasso, D.A., Mori, A., Le Moli, F., and Billen, J. 2005. Morpho-functional comparison of the Dufour gland in the female castes of the Amazon ant *Polyergus rufescens* (Hymenoptera, Formicidae). *Zoomorphology*, **124**: 149–153.
- Hölldobler, B., and Wilson, E.O. 1990. *The ants*. Springer-Verlag, Berlin, Heidelberg, and New York.
- Isidoro, N., Bin, F., Colazza, S., and Vinson, S.B. 1996. Morphology of antennal gustatory sensilla and glands in some parasitoid Hymenoptera with hypothesis on their role in sex and host recognition. *J. Hymenopt. Res.* **5**: 206–239.
- Isidoro, N., Bin, F., Romani, R., Pujade-Villar, J., and Ros-Farré, P. 1999. Diversity and function of male antennal glands in Cynipoidea. *Zool. Scr.* **28**(1–2): 165–174.
- Isidoro, N., Romani, R., Velasquez, D., Renthall, R., Bin, F., and Vinson, S.B. 2000. Antennal glands in queen and worker of the fire ant, *Solenopsis invicta* Buren: first report in female social Aculeata (Hymenoptera, Formicidae). *Insectes Soc.* **47**: 236–240.
- Kaltenpoth, M., Göttler, W., Herzner, G., and Strohm, E. 2005. Symbiotic bacteria protect wasp larvae from fungal infestation. *Curr. Biol.* **15**: 475–479.
- Le Moli, F., Grasso, D.A., Mori, A., and Ugolini, A. 1994. Eco-ethological factors affecting the scouting and the raiding behaviour of the slave-making ant, *Polyergus rufescens* Latr. (Hymenoptera, Formicidae). *Ethology*, **96**: 289–302.
- Mori, A., Grasso, D.A., and Le Moli, F. 1991. Eco-ethological study on raiding behaviour of the European Amazon ant, *Polyergus rufescens* Latr. (Hymenoptera: Formicidae). *Ethology*, **88**: 46–62.
- Mori, A., Grasso, D.A., Visicchio, R., and Le Moli, F. 2000a. Colony founding in *Polyergus rufescens*: the role of the Dufour's gland. *Insectes Soc.* **47**: 7–10.
- Mori, A., Visicchio, R., Sledge, M.F., Grasso, D.A., Le Moli, F., Turillazzi, S., Spencer, S., and Jones, G.R. 2000b. Behavioural assays testing the appeasement allomone of *Polyergus rufescens* queens during host-colony usurpation. *Ethol. Ecol. Evol.* **12**: 315–322.
- Mori, A., Grasso, D.A., Visicchio, R., and Le Moli, F. 2001. Comparison of reproductive strategies and raiding behaviour in facultative and obligatory slave-making ants: the case of *Formica sanguinea* and *Polyergus rufescens*. *Insectes Soc.* **48**: 302–314.
- Noirot, C., and Quennedey, A. 1974. Fine structure of insect epidermal glands. *Annu. Rev. Entomol.* **19**: 61–89.
- Noirot, C., and Quennedey, A. 1991. Glands, gland cells, glandular units: some comments on terminology and classification. *Ann. Soc. Entomol. Fr.* **27**: 123–128.
- Romani, R., Isidoro, N., Riolo, P., and Bin, F. 2003. Antennal glands in male bees: structures for sexual communication by pheromones? *Apidologie*, **34**: 603–610.
- Romani, R., Isidoro, N., Riolo, P., Bin, F., Fortunato, A., Turillazzi, S., and Beani, L. 2005. A new role for antennation in paper wasps (Hymenoptera: Vespidae): antennal courtship and sex dimorphic glands in antennomeres. *Insectes Soc.* **52**: 96–102.
- Visicchio, R., Mori, A., Grasso, D.A., Castracani, C., and Le Moli, F. 2001. Glandular sources of recruitment, trail, and propaganda semiochemicals in the slave-making ant *Polyergus rufescens*. *Ethol. Ecol. Evol.* **13**: 361–372.