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The Mandibular Gland, Probably the Source of the Alarm Substance in *Leptanilla* sp. (Hymenoptera, Formicidae)

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Although ants represent one of the most dominant terrestrial animals [1], some of them are extremely rare, either because of a limited geographical distribution or because of a cryptic life style. The Leptanillinae, a small subfamily of the Old World tropics and subtropics, comprises seven mostly monospecific genera of which four are known from males only [2]. The main genus *Leptanilla* has approx. 20 species, but these are equally very rare and extremely small ants with small colonies [3]. Due to the practical limitations in having live *Leptanilla* available for study, the information available on the biology of these ants is restricted to a behavioural study on the peculiar larval hemolymph feeding by the queen of *L. japonica*, whereas the workers are specialized centipede predators [3], and to a morphological study describing the exocrine system of *Leptanilla* with main emphasis on the abdominal glands [4].

The collection of a colony fragment with approximately 300 live workers and brood of *Leptanilla* sp. in the Bogor Botanical Gardens in West Java, Indonesia, by one of us (F.I.) allowed us to study the internal morphology and chemistry of the head in combination with behavioural observations. This multidisciplinary approach resulted in this first study of the mandibular glands in leptanilline ants. The species studied is new to science, and will be described elsewhere. Voucher specimens are deposited in the Bogor Zoological Museum. The colony fragment could be kept in the laboratory in good condition for 2 months, although the workers refused the centipedes that were collected at the same locality.

To study the internal morphology, heads were fixed in 2% cold glutaraldehyde and further processed along the standard techniques [6] for making semi-thin sections for light microscopy. Chemical analysis of single heads was carried out using the solid sampling technique [7] on a polydimethylsiloxane phase in a 12-m fused silica column in a Hewlett-Packard 5890 gas chromatograph coupled to a 5970B MSD (quadrupole mass spectrometer). Identification of compounds was confirmed by comparison of their mass spectra and retention times with those of standards and using MS databases.

Semi-thin sections through the head revealed that well-developed mandibular glands occur, each gland containing approx. 10 large, rounded secretory cells (diameter approx. 20 μm), that are situated at the ectal side of the reservoir, into which they open through individual duct cells (Fig. 1). Although this arrangement is in line with the general organization of the mandibular gland in ants [6], the relative size of the gland in *Leptanilla* appears larger than in the majority of ants and makes it the largest exocrine gland in the head. Ultrastructural examination of the secretory cells reveals the occurrence of smooth endoplasmic reticulum, which is a common characteristic for pheromone-producing glands [8].

Chemical analysis of individual heads revealed the presence of 4-methyl-4-hepten-3-one (38 ± 8 ng/ant) and skatole (380 ± 44 ng/ant; $n = 5$) as the only two substances. Both substances most probably represent the secretion of the large mandibular gland, as the pro- and postpharyngeal glands (which are the two other cephalic glands) are generally known to produce digestive enzymes and long-chain hydrocarbons, respectively [9, 10]. Skatole has been identified several times in the exocrine secretions of Hymenoptera, although it has not yet been assigned any pheromone function. It has been identified in the heads of the ant *Pheidole fallax* [11], it is the major volatile compound in the abdomen of soldiers of the army ant *Labidus praedator* [12], and it has been identified in the heads of a bethylid wasp *Cephalonomia gallicola* [13]. There are reports of it in two species of Neuroptera and one of Trichoptera [14]. The large amount of skatole stored in the heads of *Leptanilla* sp. certainly suggests a pheromone function. Although volatile ketones have been found in ants previously, 4-methyl-4-hepten-3-one so far has only been characterized as a

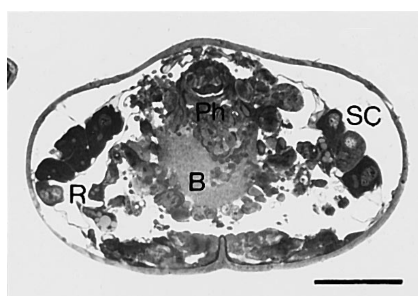

Fig. 1. Semi-thin cross section through the anterior part of the head of a worker of *Leptanilla* sp. showing the well developed mandibular glands. *R*, Reservoir; *SC*, secretory cells; *B*, brain; *Ph*, pharynx. Scale bar, 50 μm

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minor component in the mandibular glands of the ponerine species *Harpegnathos saltator* [15].

As the mandibular gland often serves a function in the production of alarm substances [1, 16, 17], we performed a simple behavioural test by observing the effect of putting a crushed head, thorax or abdomen of a conspecific worker in the foraging area. Whereas the thorax and head did not elicit any noticeable response, the head very clearly caused alarm behaviour: nearby workers excitedly approached the crushed head with open mandibles, with some of them biting it.

From this work we may conclude that the well-developed mandibular glands appear to be the most likely source of the alarm substance in this *Leptanilla* species. Although the mandibular gland is often known to be the source of alarm substances in ants, followed by the pygidial gland in the abdomen [17], our findings represent the first report of this function in the subfamily Leptanillinae. Our behavioural conclusions find support in the morphological observation of the size of the mandibular gland and in the chemical analysis. Unfortunately we were unable to maintain the live ants until the compounds identified could be tested.

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Stable Isotopes of Nitrogen in Fossil Cladoceran Exoskeletons: Implications for Nitrogen Sources in the Central Baltic Sea During the Past Century

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The ratios of stable nitrogen isotopes were analysed in zooplankton exoskeletons extracted from dated sediment cores from the Gotland Basin of the central Baltic Sea. Combined with results on δ^{15}N of bulk sediment, organic carbon concentrations, and abundances of exoskeletons of *Bosmina longispina maritima* in the sediment, the data are used to evaluate significant sources of nitrogen in the food web over the past century. Nitrogen isotopic composition of bulk sediments ranges from 2.5 to 4.5‰, that of exokeletons varies between

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