Arthropod Structure & Development 45 (2016) 333–340

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

Morphology and ultrastructure of the allomone and sex-pheromone producing mandibular gland of the parasitoid wasp *Leptopilina heterotoma* (Hymenoptera: Figitidae)

Johannes Stökl*, Gudrun Herzner

Institute of Zoology, University of Regensburg, Germany

ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form
22 June 2016
Accepted 22 June 2016
Available online 4 July 2016

Keywords:
Mandibular gland
Iridomyrmecin
Hymenoptera
Figitidae
Drosophila

ABSTRACT

Chemical communication by the parasitoid wasp *Leptopilina heterotoma* is based largely on (−)-iridomyrmecin. The female wasps use (−)-iridomyrmecin as a defensive allomone, a chemical cue to avoid competition with con- and heterospecific females, and as a major component of their sex pheromone to attract males. Males of *L. heterotoma* produce (+)-isoiridomyrmecin, which is also used for chemical defense. In this study we show that females and males of *L. heterotoma* produce the iridomyrmecins in a pair of mandibular glands. Each gland consists of a secretory part composed of class 3 gland cells and their accompanying duct cells, as well as a reservoir bordered by a thin intima. The gland discharges between the mandible base and the clypeus. Males have considerably smaller glands than females, which corresponds to the lower amount of iridomyrmecins produced by males. Chemical analyses of the mandibular gland contents showed that the gland of females contained mainly (−)-iridomyrmecin, as well as low amounts of the other previously described iridoid pheromone compounds, while the glands of males contained only (+)-isoiridomyrmecin. The morphology and sizes of the mandibular glands of males and females of *L. heterotoma* have evolved to the multi-functional use of iridomyrmecin.

1. Introduction

Most insects rely on semiochemicals for their communication with conspecifics and interaction with the environment (Wyatt, 2014). Most of those semiochemicals are produced (synthesized) in exocrine glands and either stored in a reservoir attached to the gland or directly released into the environment. Exocrine glands can be found in all parts of an insect's body, with the most prominent glands being located in the head, the thorax and the abdomen (Cruz-Landim and Abdalla, 2002; Billen et al., 2013). However, the legs (e.g. Billen, 2009; Jarau et al., 2012) or the antennae (e.g. Isidoro et al., 1999) of insects can also bear exocrine glands. In addition to intra- and interspecific communication (Blomquist and Vogt, 2003; Schulz, 2005; Wyatt, 2014), the secretions of these glands serve a range of other functions, such as defense against predators, parasites and pathogens (Hemp and Dettner, 1997; Herzner and Strohm, 2007).

The chemical communication by the wasp *Leptopilina heterotoma*, a parasitoid of *Drosophila* larvae, is based largely on (−)-iridomyrmecin and serves as a prime example for semiochemical parsimony. Females of *L. heterotoma* produce (−)-iridomyrmecin and minor amounts of four other iridoid compounds and use this secretion as an allomone to repel predators such as ants (Stökl et al., 2012). Synthetic (−)-iridomyrmecin proved to be highly repellent to ants in bioassays, and headspace sampling of *L. heterotoma* females and ants showed that the females release higher amounts of (−)-iridomyrmecin on encounter with ants. Females use (−)-iridomyrmecin not only as a defensive allomone, but also as a chemical cue to recognize and avoid host patches that have already been occupied and exploited by conspecific females. Since other species, like e.g. *Leptopilina boulardi*, use (−)-iridomyrmecin as well, *L. heterotoma* females can additionally avoid hosts already parasitized by these heterospecific wasps (Weiss et al., 2013). Finally, (−)-iridomyrmecin is the major component of the female sex pheromone of *L. heterotoma*. However, (−)-iridomyrmecin alone is not attractive to males. Only a mix of (−)-iridomyrmecin with minor amounts of four other iridoid compounds [including (+)-isoiridomyrmecin and iridodial] produced by the females attracts conspecific males (Weiss et al., 2013). In total, 16

* Corresponding author. Tel.: +49 941 943 2156; fax: +49 941 943 5583.
E-mail address: johannes.stoekl@ur.de (J. Stökl).

http://dx.doi.org/10.1016/j.asd.2016.06.003
1467-8039/

stereoisomers of iridomyrmecin exist, with (−)-iridomyrmecin and (+)-isoiridomyrmecin being two noteworthy examples. We will use the term "iridomyrmecin" without further specification to refer to all stereoisomers produced by the wasps. Males of *L. heterotoma* also defend themselves chemically against ants. They produce lower amounts of the secretion, however, and only one stereoisomer, (+)-isoiridomyrmecin (Stökl et al., 2012).

Despite their fascinating and manifold functions, the source of the iridomyrmecins in *L. heterotoma* has not as yet been identified. Mandibular glands have been reported to produce and secrete the allomones and sex pheromones in several wasp species (e.g. Fales et al., 1980; Goettler and Strohm, 2008), including the hyperparasitoid wasp *Alloxysta brevis*, which like *L. heterotoma* is a member of the family Figitidae, and which uses a mixture of transfused iridomyrmecins for defense against ants (Völkl et al., 1994; Hilgraf et al., 2012). In *L. heterotoma*, the iridomyrmecins are found only in the head, but not in the thorax and abdomen (Stökl et al., 2012). We therefore hypothesized that in *L. heterotoma* the iridomyrmecins are likewise produced in mandibular glands.

In this study we investigated the morphology and ultrastructure of the prominent head glands of *L. heterotoma* females and males by doing histological as well as electron microscopic analyses and producing 3D-reconstructions based on a continuous series of semi-thin sections. We analyzed the contents of single excised glands by gas chromatography and mass spectrometry in both sexes to clarify the source(s) of the iridoid compounds.

2. Material and methods

2.1. Rearing of insects

We used *Drosophila melanogaster* as host to rear *L. heterotoma*. To rear a cohort of *L. heterotoma,* 20–30 flies of mixed age and sex were placed in a jar containing an approximately 2 cm thick layer of standard corn based rearing medium. After 48 h the flies were removed and five to 10 mated females of *L. heterotoma* were put in the jar. Wasps were kept at 25 °C, 60% relative humidity and a 16:8 h light:dark cycle.

2.2. Specimen preparation for histological study

Histological studies of *L. heterotoma* females' (N = 11) and males' (N = 16) heads were conducted using light microscopy following standard histological methods. Males and females of *L. heterotoma* (1–7 days old) were anaesthetized with carbon dioxide and fixed immediately in formalin-ethanol-acetic acid fixative (Scheuring). Subsequently, they were rinsed in 80% ethanol and dehydrated in a graded ethanol series and propylene oxide, and finally embedded in Epon 812 (Polysciences Europe GmbH, Eppelheim, Germany). Continuous series of semi-thin sagittal or frontal sections (3 μm) were cut on a Reichert Ultracut microtome (Leica Microsystems AG, Wetzlar, Germany) equipped with a diamond knife, and subsequently stained with toluidine blue. Sections were examined under a Leica DMLS compound microscope and photographed with a Nikon Digital Sight DS-2Mv digital camera using the Nikon NIS F 2.20 software (Nikon Corp., Tokyo, Japan).

2.3. Image analysis and 3D-reconstruction

3D-reconstructions of one *L. heterotoma* female head and one male head based on the series of semi-thin sections were conducted using the 3D visualization software Reconstruct (version 1.1.0.0, synapses.clm.utexas.edu/software-0, Fiala, 2005). The photographs of the series of head sections were loaded into the program and manually aligned to each other. The structures of interest (head glands, pharynx, head capsule, compound eyes, and mandibles) were manually marked in each picture and the 3D surfaces computed with the Boissonnet surface algorithm. Furthermore, gland volumes and head capsule volumes were calculated by the Reconstruct software. The number of secretory units was determined by the compilation of all serial sections. The datasets were subsequently transferred to the software Blender (version 2.72b, www.blender.org) to slightly smooth surfaces.

2.4. Transmission electron microscopy

Heads of three freshly killed *L. heterotoma* males and females, respectively, were fixed for transmission electron microscopy (TEM) in a solution of 5% glutardialdehyde and 2% paraformaldehyde in 0.1M Sörensen phosphate buffer (pH 7.4) and kept overnight in a refrigerator at 4 °C. After postfixation in 2% osmium tetroxide (OsO_4) in 0.1M Sörensen phosphate buffer (pH 7.4) on ice for 2 h, the glands were dehydrated in a graded ethanol series. They were then embedded in Epon 812 (with acetone as the intermediary solution). Ultrathin sagittal sections were made with a 45° diamond knife on a Reichert Ultracut E microtome (Leica Microsystems AG, Wetzlar, Germany). Sections were stained with 2% uranyl acetate and Reynolds' lead citrate and examined with a Zeiss EM 10 at 80 kV. Micrographs were taken with a TRS slow scan CCD camera (type s-7899-v) and the software TRS Image Sys Prog (Version 1.1.1.65).

2.5. Gland preparation

For the preparation of the glands, freshly killed wasps were immobilized with dental wax and the gland excised in water under a stereo microscope. We excised the gland together with a small piece of cuticle above the mandible and the mandible itself, because the gland reservoir is very fragile and ruptures if touched directly. Excised glands were photographed in several focal planes with a Keyence VHX 600 digital microscope (Keyence Deutschland GmbH, Neu-Isenburg, Germany) and afterwards extracted in 10 μl dichloromethane (DCM). Glands were transferred to DCM with as little water as possible. Photographs from different focal planes were stacked in Photoshop CS4 (Adobe Systems, Munich, Germany) and brightness and contrast were slightly adjusted.

2.6. Chemical analysis

Extracts of the glands were analyzed by gas chromatography (GC) and mass spectrometry (MS). For this, 1 μl of the extract was injected splitless into a GC2010 GC connected to a 2010plus MS (both Shimadzu, Duisburg, Germany). The GC was equipped with a non-polar capillary column (BPX-5, 30 m long, 0.25 mm inner diameter, 0.25 μm film thickness; SGE Analytical Science, Milton Keynes, UK). Helium was used as carrier gas with a constant linear velocity of 50 cm s^{-1}. The temperature program of the GC-oven started at 80 °C and was raised by 5 °C min^{-1} to 280 °C, and the injector was set to a temperature of 280 °C. The MS was run in electron impact (EI) mode at 70 eV and set to a scan range from 35 to 600 mz^{-1}. In addition, we also extracted the heads of male and female *L. heterotoma* in 10 μl DCM per head and analyzed those extracts as described above. The iridoid compounds produced by females and males of *L. heterotoma* were identified using the mass spectra and retention indices of the compounds available from previous studies (Stökl et al., 2012; Weiss et al., 2013). We successfully excised and extracted the glands of six females and four males of *L. heterotoma*.

3. Results

3.1. Morphology

Histological examinations revealed that females and males of *L. heterotoma* possess conspicuous mandibular glands. The 3D-reconstructions illustrate that these paired glands are located laterally in the anterior part of the head and extend from the base of the mandible dorsally, parallel to the pharynx, at each side of the head (Fig. 1).

The glands of both sexes comprise a spherical, sac-like gland reservoir that is bordered by a thin intima (Figs. 1 and 2). A cluster of secretory cells, with their corresponding duct cells, is associated with the apical region of each reservoir (Figs. 1 and 3). End apparatus at the junction of the secretory cells and duct cells, as well as the conducting canals that drain the secretion in the reservoir, are clearly visible (Fig. 3). This bicellular system is characteristic of class 3 gland cells, according to the standard classification by Noirot and Quennedey (1974). Each secretory part of the mandibular gland consists of a cluster of 26 secretory units (gland cells + duct cells) in the reconstructed female and a cluster of 16 secretory units in the male. The secretory cells possess rather large nuclei.

In the reconstructed *L. heterotoma* female the calculated total gland volume (reservoirs plus gland cells) is 1.14 nl, in the

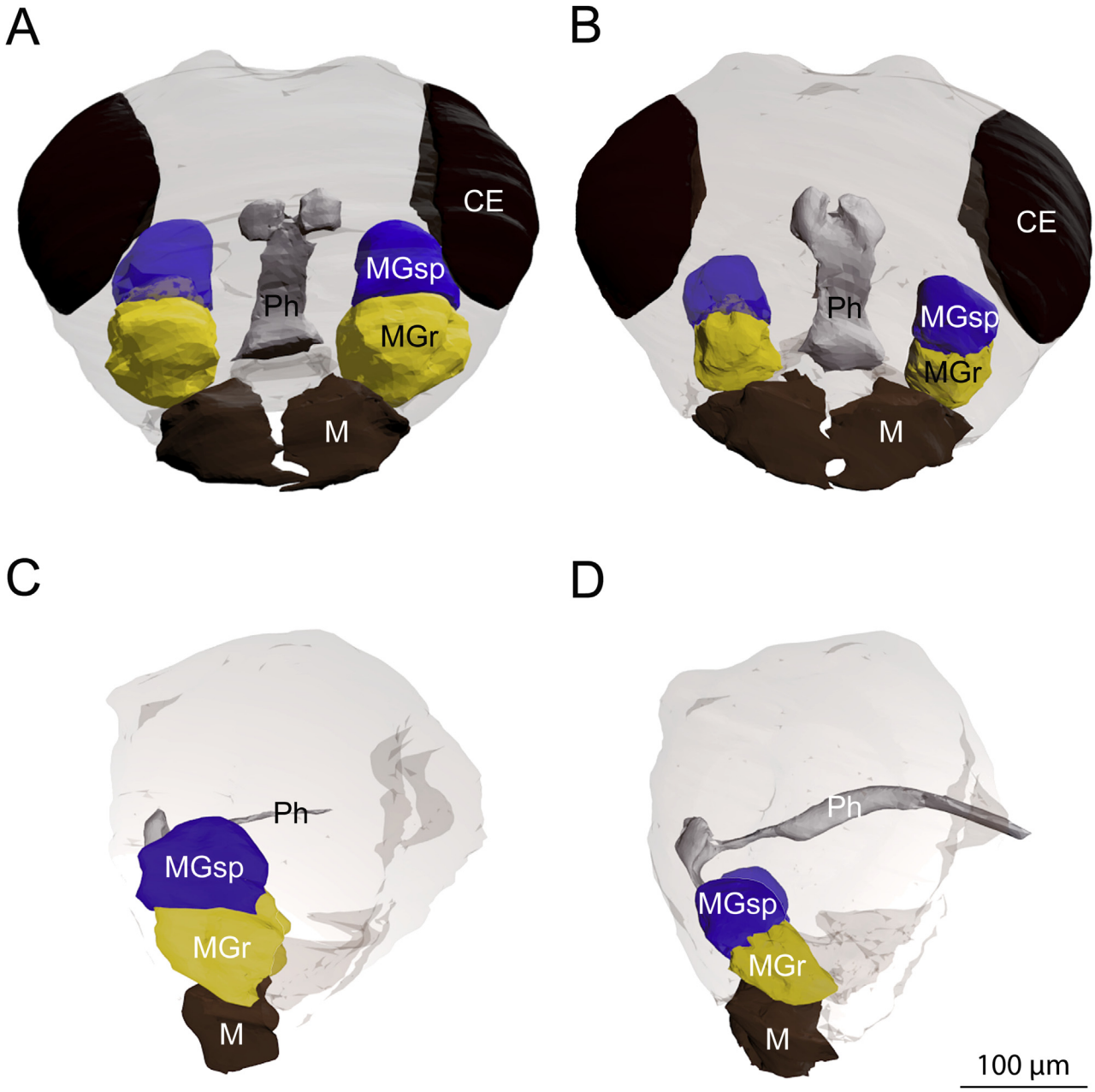

Fig. 1. 3D-reconstruction of the mandibular glands of *Leptopilina heterotoma*. A) Female, frontal view, B) Male, frontal view, C) Female, lateral view, D) Male, lateral view. Please note that in the frontal views the mandibular gland secretory part on the right side is depicted in transparent, in the lateral views the compound eyes are omitted. Abbreviations: CE, compound eye; M, mandible; MGr, mandibular gland reservoir; MGsp, secretory part of mandibular gland comprising secretory cells and their accompanying duct cells; Ph, Pharynx.

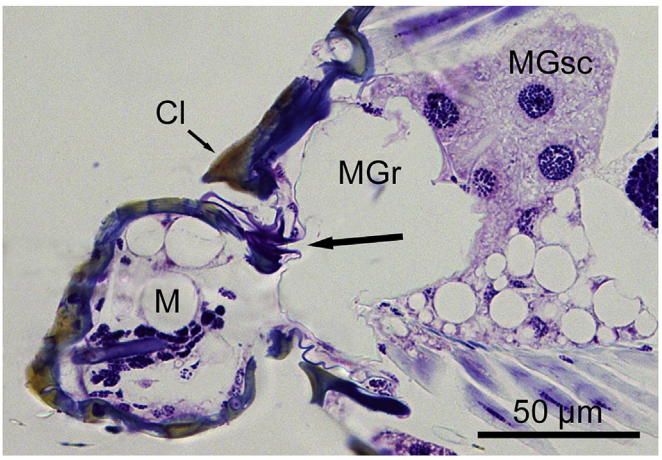

Fig. 2. Sagittal semithin section through anterior part of the head of a female *Leptopilina heterotoma*, showing the mandibular gland reservoir (MGr) and secretory cells (MGsc), as well as the opening of the gland through the articular membrane at the anterior hinge of the left mandible (Arrow). All features shown here for females were also observed in males. Cl, clypeus; M, mandible.

reconstructed male 0.41 nl. The relative gland volume (mandibular gland volume to head capsule volume ratio) is 0.07 for the female and 0.03 for the male.

The mandibular gland reservoir opens to the outside through the articular membrane, where the mandible is hinged to the cranial margin by the anterior secondary joint on the outer side of its base (Fig. 2). This means that the gland secretion is emitted dorsally to the mandible between the mandible base and the clypeus. The gland reservoir does not decrease in diameter towards its proximal end to form an excretory duct. Rather, the articulate membrane forms a funnel-shaped structure through which the secretion is guided to the outside (Fig. 2).

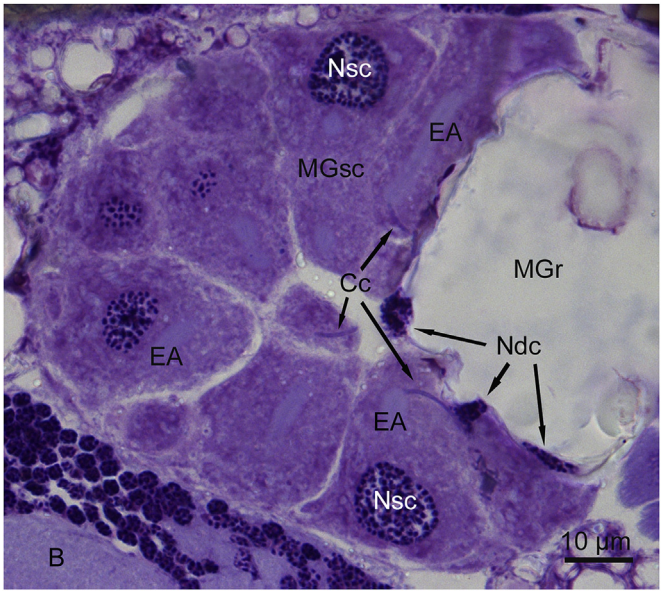

Fig. 3. Detail of the mandibular gland of a *Leptopilina heterotoma* female, showing conducting canals (Cc) of mandibular gland duct cells, the end apparatus (EA) at the junction of the secretory cells (MGsc) and their accompanying duct cells, and the mandibular gland reservoir (MGr). Nsc, nuclei of secretory cells; Ndc, nuclei of duct cells; B, brain. All features shown here for females could also be observed in males.

3.2. Ultrastructure

There were no noticeable differences in the ultrastructure of the mandibular gland between male and female *L. heterotoma*. The transmission electron microscopic investigations of the ultrathin sections of *L. heterotoma* heads confirmed that the mandibular gland is a class 3 gland (after the standard classification of Noirot and Quennedey, 1974), consisting of several two-cell units, in which each secretory cell is associated with a duct cell. The most prominent ultrastructural feature of these two-cell units is the ramified end apparatus, which is formed by a central receiving canal lined by porous cuticle that is surrounded by tightly packed microvilli (Fig. 4A), whose appearance may be regular or somewhat distorted depending on the accumulation of secretion. The nuclei of the secretory cells are rather large, spherical or ovoid and show numerous small condensations of chromatin (Fig. 4B). The cytoplasm comprises a high number of polymorphous mitochondria (Fig. 4B) and some dark-staining secretory granules, but no clear endoplasmatic reticulum nor Golgi apparatus. On their basal side the secretory cells show conspicuous infoldings of the plasma membrane forming a basal labyrinth (Fig. 4C). The secretory cells are individually linked to the reservoir by means of conducting canals, which are formed by the duct cells. The conducting canals are lined by massive cuticle and filled with secretion (Fig. 4D). The nuclei of the duct cells are of irregular shape. The reservoir is lined by a thin luminal cuticle.

3.3. Preparation

The gland excised from females of *L. heterotoma* was attached to the mandible. The gland reservoir was filled with a white fluid and thus clearly distinguishable from the secretory cells (Fig. 5A). Glands excised from males were smaller than the glands of females, but otherwise very similar (Fig. 5B).

3.4. Chemical analysis

The mandibular glands excised from *L. heterotoma* females contained mainly (−)-iridomyrmecin and low amounts of four other iridoids, including (+)-isoiridomyrmecin (Fig. 5C). The mandibular glands excised from males of *L. heterotoma* contained only (+)-isoiridomyrmecin (Fig. 5D). The gland extracts contained the same compounds as the head extracts in *L. heterotoma* females and males, respectively (Fig. 5E,F).

4. Discussion

We demonstrated that *L. heterotoma* produce iridomyrmecin and the other iridoid compounds in a pair of mandibular glands. Both sexes of *L. heterotoma* possess the mandibular glands and each gland consists of a reservoir and a secretory part. The secretory part comprises a cluster of secretory units with one secretory cell and one duct cell each. The glands discharge between the clypeus and the mandible base through the articular membrane. The mandibular glands accounts for 3% and 7% of the head capsule volume in the reconstructed male and female, respectively. The absolute and relative size of the mandibular gland is almost three times higher in females than in males, which is in agreement with the data on the defensive chemistry of *L. heterotoma*: Males carry much less iridomyrmecin in their gland reservoir than females and also release smaller amounts of iridomyrmecin on encounter with ants (Stökl et al., 2012). The large reservoirs of the mandibular gland provide the opportunity to store a considerable quantity of gland secretion and enable the wasps to discharge secretion several times in succession. Wasps defending themselves released much lower

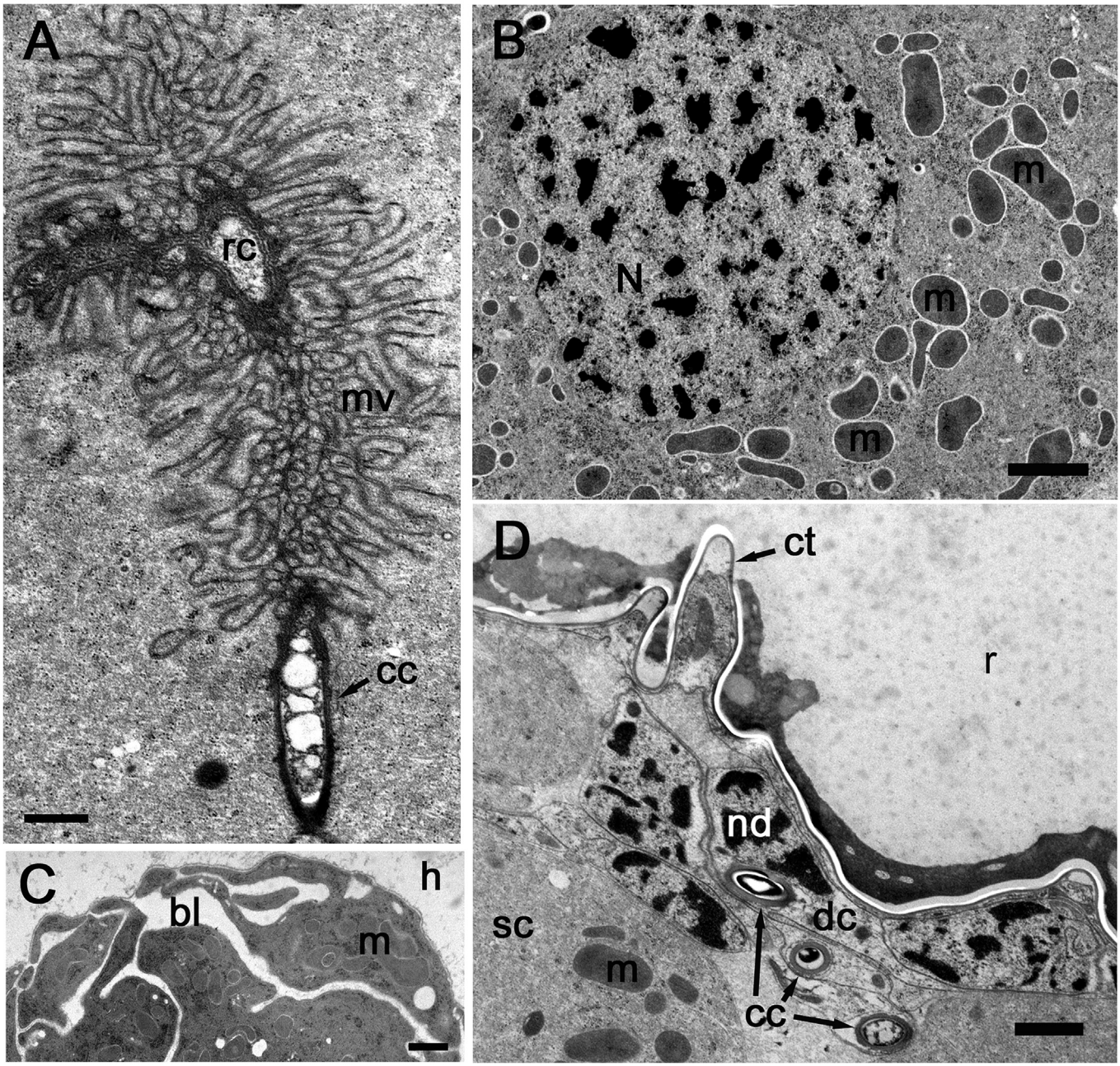

Fig. 4. Electron micrographs of the mandibular gland of *Leptopilina heterotoma*. A) Detail of an end apparatus showing the receiving canal with its porous cuticle and the surrounding microvilli as well as the conducting canal that is lined with massive cuticle and filled with secretion. Scalebar = 0.5 μm. B) Numerous polymorphous mitochondria surrounding the nucleus of a secretory cell. Scalebar = 2 μm. C) Basal side of a secretory cell with deep infoldings of the plasma membrane forming a basal labyrinth. Scalebar = 1 μm. D) Contact zone between secretory cell, duct cell and gland reservoir. The cuticle lined conducting canals are embedded by the duct cells and filled with secretion. Scalebar = 0.5 μm bl, basal labyrinth; cc, conducting canal; ct, cuticle; dc, duct cell; h, hemolymphatic space; m, mitochondria; mv, microvilli; N, nucleus of secretory cell; nd, nucleus of duct cell; r, reservoir; rc, receiving canal; sc, secretory cell.

amounts of iridomyrmecin than found in the extracts of the wasps (Stökl et al., 2012), indicating that the wasps can use their defensive secretion economically.

The ultrastructural investigations clearly corroborate a secretory function of the gland cells and the classification of the mandibular gland as a class 3 gland (Noirot and Quennedey, 1974). The surface increasing basal labyrinth indicates an uptake of precursor molecules or metabolites from the hemolymph into the secretory cells. The active nuclei and the occurrence of numerous mitochondria are in agreement with a high metabolic rate of the secretory cells. In the end apparatus at the junction of the secretory cells and the duct cells the considerable surface enlargement due to the tightly packed microvilli allows for a high secretory activity. The cuticle of the receiving canal in the secretory cell is porous and, thus, permits the passage of secretion. Upon leaving the secretory cell, the cuticle of the canal becomes massive and shows no pores to form the conducting canal in the duct cell that drains the secretion off to the reservoir.

Mandibular glands similar to the gland of *L. heterotoma* have been described from several other Hymenoptera. For example, queens of the pharaoh ant *Monomorium pharaonis* also possess mandibular glands composed of a secretory part with bicellular

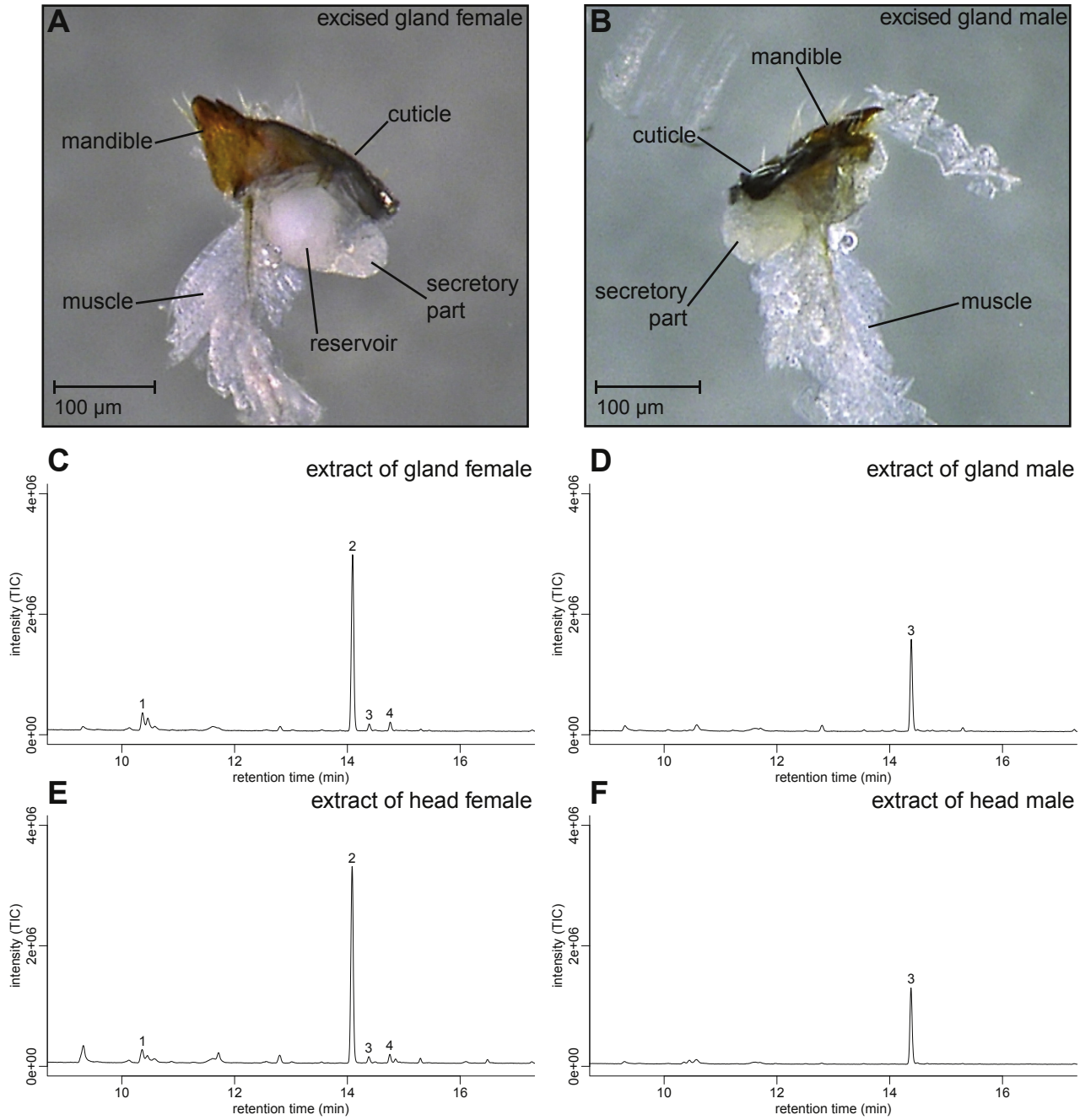

Fig. 5. Excised mandibular glands of A) a female and B) a male of *L. heterotoma* with the mandible, some cuticle, and muscle attached to it. C) and D) show total ion chromatograms (TIC) of the extracts of the excised glands shown in A) and B), respectively. Total ion chromatograms (TIC) of extracts of heads of E) female and F) male *L. heterotoma*. C) to F) Peak numbers: 1 iridodial (two stereoisomers), 2 (−)-iridomyrmecin, 3 (+)-isoiridomyrmecin, 4 stereoisomer of iridomyrmecin with unknown configuration. Peaks without numbers are non-iridoid compounds.

gland units connected to a reservoir (Boonen et al., 2013). Females of the bethylid wasp *Goniozus nephantidis* release spiroacetal during aggressive interactions from a mandibular gland. This gland also consists of class 3 secretory cells connected to a cuticular reservoir (Goubault et al., 2008). Furthermore, the mandibular glands of honeybees and several other bee species show a similar morphology (Cruz-Landim, 2009).

Defensive secretions are known to be produced in mandibular glands in several hymenopteran species, mostly ants (Morgan et al., 1999), but also braconid (Byers and Levi-Zada, 2011) and mutillid wasps (Fales et al., 1980). Most interestingly, the closely related parasitoid wasp to *L. heterotoma, A. brevis* uses a secretion containing two trans-fused stereoisomers of iridomyrmecin, 6-methyl-5-hepten-2-one, and actinidine to defend itself against ants while searching for hosts in ant-attended aphid colonies (Völkl et al., 1994; Hilgraf et al., 2012). The defensive secretion was found in head extracts of *A. brevis*, and since the only glands detected in the heads of these wasps were paired mandibular glands, these glands are likely the source of the secretion (Völkl et al., 1994). The mandibular glands of *A. brevis* seem to have a similar relative size as the glands of *L. heterotoma*, and the opening of the gland reservoir has been reported also to be located at the base of the mandible

(Völkl et al., 1994). However, because no histological data on the mandibular gland of *A. brevis* are available a detailed comparison of the structure and morphology of the glands of *A. brevis* and *L. heterotoma* is unfortunately not possible.

Both *Alloxysta* and *Leptopilina* belong to the family Figitidae (subfamily Charipinae and Eucoilinae, respectively), but their ecology and lifecycle are quite different (Sullivan, 1987; Fleury et al., 2009). Still, all species of these two genera that have been investigated to date, 24 species of *Alloxysta* (Hübner et al., 2002) and five species of *Leptopilina* (Stökl et al., 2012; Weiss et al., 2013, 2015 and unpublished data) produce some stereoisomer of iridomyrmecin and other iridoid compounds as defensive secretions. The family Figitidae contains almost 3000 species in 132 genera (Ronquist, 1999), but unfortunately not a single species from a genus other than *Alloxysta* and *Leptopilina* has been chemically investigated so far (El-Sayed, 2013). Therefore, more studies are needed to ascertain whether the presence of iridoid producing glands in *Alloxysta* and *Leptopilina* evolved independently or whether this gland and the use of iridoids is widespread within the Figitidae. An iridoid producing gland is definitely not a common feature of all parasitoids of *Drosophila*. For example, the wasp *Asobara tabida* (Braconidae) also parasitizes larvae of *Drosophila* and occurs sympatrically with *L. heterotoma,* but does not produce any iridoid compound (Stökl et al., 2014).

A function of the trans-fused iridomyrmecins as sex pheromone has also been suggested for *Alloxysta* (Petersen, 2000), but was never demonstrated. In general, sex pheromones produced by mandibular glands seem to be rare within parasitoid wasps. To date the sex pheromones of about 20 species of parasitoid wasps have been identified (Ruther, 2013), but only in one species (*Macrocentrus grandii*) is the sex pheromone known to be produced in a mandibular gland (Swedenborg and Jones, 1992; Ruther, 2013). Another example, although a predatory rather than parasitoid wasp (Godfray, 1994), is the European beewolf *Philanthus triangulum* (Hymenoptera, Crabronidae). Males of *P. triangulum* possess a pair of large mandibular glands, which have been reported to produce a scent marking pheromone that is used to mark a small territory in which mating with the females takes place (Goettler and Strohm, 2008). However, in contrast to *L. heterotoma*, the class 3 gland cells of male *P. triangulum* mandibular glands are organized in acini (Goettler and Strohm, 2008), and the secretion is stored in an additional gland, the postpharyngeal gland, which thus functions as pheromone reservoir (Kroiss et al., 2006; Herzner et al., 2007).

Iridomyrmecin was also described in species other than *Leptopilina* and *Alloxysta*. It was first found in and named after the Argentine ant *Linepithema humilis* (formely *Iridomyrmex*), which produces (+)-iridomyrmecin (Cavill and Houghton, 1974; Cavill et al., 1976), and, like in *L. heterotoma*, uses it for several functions. The ants use (+)-iridomyrmecin, in combination with dolichodial and (*Z*)-9-hexadecenal, as trail pheromone (Choe et al., 2012), as a chemical cue inhibiting necrophoresis (Choe et al., 2009), and probably also as defensive allomone. However, the argentine ant does not produce iridomyrmecin in a mandibular gland, but rather in the pygidial gland in the gaster. The location of the gland might in this case be influenced by the trail laying or defensive (stinging) behavior of the ant. Outside the Hymenoptera, two stereoisomers of iridomyrmecin have been found in the mesothoracic glands of the anthicid beetles *Formicomus gestroi* and *Formicomus pedestris* (Hemp and Dettner, 1997). The beetles use the secretions from those glands to deter predators, i.e. ants.

In conclusion, our data on the morphology and ultrastructure of the mandibular gland of *L. heterotoma* fit very well with the available data on the ecological and behavioral uses of the gland secretion. This makes *L. heterotoma* a rare example of a species in which we can combine gland morphology with ecological, behavioral, and chemical data to understand the complexity of pheromone evolution.

Acknowledgment

We want to thank Agnes Paech, Margot Schilling, Margit Schimmel and Angelika Pach for technical assistance, Michael Brummer for rearing the insects, and Tomer Czaczkes for comments on an earlier version of the manuscript. Part of this study was funded by the German Research Council (Deutsche Forschungsgemeinschaft, DFG; grants STO 966/1-1, STO 966/1-2, and STO 966/2-1 to J.S.).

References

Billen, J., 2009. Occurrence and structural organization of the exocrine glands in the legs of ants. Arthropod Struct. Dev. 38, 2–15.
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.
Blomquist, G.J., Vogt, R.G., 2003. Insect Pheromone Biochemistry and Molecular Biology. Elsevier Academic Press, London, UK.
Boonen, S., Eelen, D., Børgesen, L., Billen, J., 2013. Functional morphology of the mandibular gland of queens of the ant *Monomorium pharaonis* (L.). Acta Zool. 94, 373–381.
Byers, J., Levi-Zada, A., 2011. Individual variation of (*S*)-4-methyl-3-heptanone in heads of braconid wasp, *Leiophron uniformis*, and *Pogonomyrmex* ants indicates costs of semiochemical production. Chemoecology 21, 35–44.
Cavill, G.W.K., Houghton, E., 1974. Volatile constituents of the Argentine ant, *Iridomyrmex humilis*. J. Insect Physiol. 20, 2049–2059.
Cavill, G.W.K., Houghton, E., McDonald, F.J., Williams, P.J., 1976. Isolation and characterization of dolichodial and related compounds from the argentine ant, *Iridomyrmex humilis*. Insect Biochem. 6, 483–490.
Choe, D.-H., Millar, J.G., Rust, M.K., 2009. Chemical signals associated with life inhibit necrophoresis in Argentine ants. Proc. Natl. Acad. Sci. 106, 8251–8255.
Choe, D.-H., Villafuerte, D.B., Tsutsui, N.D., 2012. Trail pheromone of the argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). PLoS One 7, e45016.
Cruz-Landim, C. da, 2009. Abelhas: Morfologia e função de sistemas. Editora UNESP, Sao Paulo, Brasil.
Cruz-Landim, C. da, Abdalla, F.C. (Eds.), 2002. Glândulas exócrinas das abelhas. FUNPEC-Editora, Ribeirão Preto, SP, Brazil.
El-Sayed, A.M., 2013. The Pherobase: Database of Pheromones and Semiochemicals [WWW document]. www.pherobase.com.
Fales, H.M., Jaouni, T.M., Schmidt, J.O., Blum, M.S., 1980. Mandibular gland allomones of *Dasymutilla occidentalis* and other mutillid wasps. J. Chem. Ecol. 6, 895–903.
Fiala, J.C., 2005. Reconstruct: a free editor for serial section microscopy. J. Microsc. 218, 52–61.
Fleury, F., Gibert, P., Ris, N., Allemand, R., 2009. Ecology and Life History Evolution of Frugivorous *Drosophila* Parasitoids. Parasitoids of Drosophila. Academic Press, London, pp. 3–44.
Godfray, H.C.J., 1994. Parasitoids. Behavioral and Evolutionary Ecology. Princeton University Press, Chichester.
Goettler, W., Strohm, E., 2008. Mandibular glands of male European beewolves, *Philanthus triangulum* (Hymenoptera, Crabronidae). Arthropod Struct. Dev. 37, 363–371.
Goubault, M., Batchelor, T.P., Romani, R., Linforth, R.S.T., Fritzsche, M., Francke, W., Hardy, I.C.W., 2008. Volatile chemical release by bethylid wasps: identity, phylogeny, anatomy and behaviour. Biol. J. Linn. Soc. 94, 837–852.
Hemp, C., Dettner, K., 1997. Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). Entomol. Gener. 22, 97–108.
Herzner, G., Goettler, W., Kroiss, J., Purea, A., Webb, A.G., Jakob, P.M., Rössler, W., Strohm, E., 2007. Males of a solitary wasp possess a postpharyngeal gland. Arthropod Struct. Dev. 36, 123–133.
Herzner, G., Strohm, E., 2007. Fighting fungi with physics: food wrapping by a solitary wasp prevents water condensation. Curr. Biol. 17, 7.
Hilgraf, R., Zimmermann, N., Lehmann, L., Tröger, A., Francke, W., 2012. Stereoselective synthesis of trans-fused iridoid lactones and their identification in the parasitoid wasp *Alloxysta victrix*, Part II: Iridomyrmecins. Beilstein J. Org. Chem. 8, 1256–1264.
Hübner, G., Völkl, W., Francke, W., Dettner, K., 2002. Mandibular gland secretions in alloxystine wasps (Hymenoptera, Cynipoidea, Charipidae): do ecological or phylogenetical constraints influence occurrence or composition? Biochem. Syst. Ecol. 30, 505–523.
Isidoro, N., Bin, F., Romani, R., Pujade-Villar, J., Ros-Farre, P., 1999. Diversity and function of male antennal glands in Cynipoidea (Hymenoptera). Zool. Scr. 28, 165–174.
Jarau, S., Žáček, P., Šobotník, J., Vrkoslav, V., Hadravová, R., Coppée, A., Vašíčková, S., Jiroš, P., Valterová, I., 2012. Leg tendon glands in male bumblebees (*Bombus*

terrestris): structure, secretion chemistry, and possible functions. Naturwissenschaften 99, 1039–1049.
Kroiss, J., Schmitt, T., Schreier, P., Strohm, E., Herzner, G., 2006. A selfish function of a "social" gland? A postpharyngeal gland functions as a sex pheromone reservoir in males of the solitary wasp *Philanthus triangulum*. J. Chem. Ecol. 32, 2763–2776.
Morgan, E.D., do Nascimento, R., Keegans, S., Billen, J., 1999. Comparative study of mandibular gland secretions of workers of ponerine ants. J. Chem. Ecol. 25, 1395–1409.
Noirot, C., Quennedey, A., 1974. Fine structure of insect epidermal glands. Annu. Rev. Entomol. 19, 61–80.
Petersen, G., 2000. Signalstoffe in der innerartlichen Kommunikation des Hyperparasitoiden *Alloxysta victrix* (Hymenoptera: Cynipidae) und ihre Wirkung auf den Primärparasitoiden *Aphidius uzbekistanicus* und die Große Getreideblattlaus *Sitobion avenae* (PhD Thesis, Kiel).
Ronquist, F., 1999. Phylogeny, classification and evolution of the Cynipoidea. Zool. Scr. 28, 139–164.
Ruther, J., 2013. Novel Insights into Pheromone-mediated Communication in Parasitic Hymenopterans. Chemical Ecology of Insect Parasitoids. Wiley-Blackwell, Hoboken, NJ, USA, pp. 112–144.
Schulz, S. (Ed.), 2005. The Chemistry of Pheromones and Other Semiochemicals II. Springer, New York.
Stökl, J., Dandekar, A.-T., Ruther, J., 2014. High chemical diversity in a wasp pheromone: a blend of methyl 6-methylsalicylate, fatty alcohol acetates and cuticular hydrocarbons releases courtship behavior in the *Drosophila* parasitoid *Asobara tabida*. J. Chem. Ecol. 40, 159–168.
Stökl, J., Hofferberth, J., Pritschet, M., Brummer, M., Ruther, J., 2012. Stereoselective chemical defense in the *Drosophila* parasitoid *Leptopilina heterotoma* is mediated by (−)-iridomyrmecin and (+)-isoiridomyrmecin. J. Chem. Ecol. 38, 331–339.
Sullivan, D.J., 1987. Insect hyperparasitism. Annu. Rev. Entomol. 32, 49–70.
Swedenborg, P.D., Jones, R.L., 1992. (*Z*)-4-Tridecenal, a pheromonally active air oxidation product from a series of (*Z,Z*)-9,13 dienes in *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae). J. Chem. Ecol. 18, 1913–1931.
Völkl, W., Hübner, G., Dettner, K., 1994. Interactions between *Alloxysta brevis* (Hymenoptera, Cynipoidea, Alloxystidae) and honeydew-collecting ants: how an aphid hyperparasitoid overcomes ant aggression by chemical defense. J. Chem. Ecol. 20, 2901–2915.
Weiss, I., Hofferberth, J., Ruther, J., Stökl, J., 2015. Varying importance of cuticular hydrocarbons and iridoids in the species-specific mate recognition pheromones of three closely related *Leptopilina* species. Front. Ecol. Evol. 3, 19.
Weiss, I., Rössler, T., Hofferberth, J., Brummer, M., Ruther, J., Stökl, J., 2013. A nonspecific defensive compound evolves into a competition-avoidance cue and a female sex-pheromone. Nat. Commun. 4, 2767.
Wyatt, T.D., 2014. Pheromones and Animal Behavior, second ed. Cambridge University Press, Cambridge.