

# Reproductive conflict between laying workers in the ant *Aphaenogaster senilis*

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**Abstract** Since workers of the ant *Aphaenogaster senilis* can lay male eggs, reproductive conflict may occur between these workers. We examined the occurrence of worker conflicts in groups of workers either with or without the queen. Intranidal aggression was observed in each nest for 10 min each day, and the immatures produced were counted once a week for two months. Pairs of workers involved in aggression were taken regularly from each nest and used for chemical, morphological and anatomical analyses. The attacker and the attacked workers differed in their cuticular hydrocarbon profiles. The attacker and the attacked ants were at the same middle-aged fertile stage. The attacker ant was significantly larger and more fertile than the attacked ant, and more mature physiologically (poison gland was darker). There was apparently no stable hierarchy between laying workers. In the first weeks under queenless conditions, most eggs and larvae were destroyed, but they were later reared to obtain males. The intranidal worker aggression in this highly evolved ant is discussed in relation to dominance and worker policing.

**Keywords** Aggression · Ant · Cuticular hydrocarbons · Dominance · Worker reproduction

## Introduction

Members of colonies of social insects benefit from cooperation (Hölldobler and Wilson 1990). However, apparently peaceful colonies may hide reproductive conflicts of interest (Boomsma and Franks 2006; Ratnieks et al. 2006). Such hidden conflicts may occur, for example, when the workers are able to lay haploid eggs by arrhenotokous parthenogenesis, which may reduce colony fitness. In this situation, workers can try to monopolise reproduction by dominance behaviour or they can destroy the eggs of nestmates to favour their own eggs, a behaviour called policing. Another reproductive conflict can appear in species where the queen inhibits the reproduction of workers. In such species, workers can try to escape this inhibition in order to reproduce. These conflicts are more likely in species which have either one multiple mated queen or multiple related queens, inducing differences in relatedness (Bourke and Franks 1995; Crozier and Pamilo 1996). In monogynous and monoandrous species like *Camponotus floridanus*, policing is efficient for worker-laid eggs, but not between workers (Smeeton 1982; Endler 2004; Endler et al. 2007). Several studies also reported that large or major workers have a higher reproductive potential than small or minor workers, indicating that individual selection could also take place (see references in Clémencet et al. 2007). In the ant *Cataglyphis cursor*, workers can lay parthenogenetic eggs after the death of the queen to produce gynes (virgin queens) and workers. These workers engage in dominance aggression and a potential for nepotism may occur for gyne producing, but not for

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male rearing (Clémencet et al. 2007). In the genus *Aphaenogaster*, queenless workers lay parthenogenetic male eggs. In *A. cockerelli*, workers who have matured eggs in their ovaries are attacked by their nestmates when they are returned to their colony (Hölldobler and Carlin 1989). Iwanishi et al. (2003) reported intranidal worker aggression in a congeneric species, *A. japonica* (a subspecies of *A. smythiesi*), in queenless groups, and when the ants were reunited. Aggression against workers that have been kept separated in the above reports would be due to both a divergence of their chemical profiles after separation (see Provost et al. 1993; Boulay and Lenoir 2001) and a change in the odour of laying workers versus sterile ones. It is now well known that queens change their odour when they begin to lay eggs (Monnin 2006). These effects on aggression may have happened in the above experiments on *Aphaenogaster*. Thus, the diversification of the cuticular hydrocarbon composition should be taken into account.

This study was undertaken to observe intranidal worker aggression and identify the proximate mechanisms involved in these aggressions in *Aphaenogaster senilis*. Colonies of this species are headed by one single queen who inhibits the sexual production (Ledoux 1976; Boulay et al. 2007), and recent studies indicated that the queen is monoandrous (Chéron et al., unpublished), like *A. japonica* (Iwanishi et al. 2003). This species forms new colonies by fission according to two modalities. First, after nest relocation, a subset of the workers may abandon the mother colony with young female larvae and produce a new gyne which becomes the queen after fecundation (Ledoux 1971, 1973, 1976; Boulay et al. 2007). Second, fission may occur in very large colonies which rear a few gynes because of the dilution of the queen pheromone, and one new fecundated gyne leaves the mother colony with workers and the brood in order to found a new nest (Boulay et al. 2007). Under queenless conditions, workers lay haploid eggs, which can develop into normal males (Ledoux 1976). Therefore, worker conflicts for reproduction can occur regardless of relatedness asymmetries.

First, we observed aggression between workers. Generally, experiments are performed by reuniting queenless and queenright groups of the same colony, or by reintroducing queenless workers into queenright colonies. To avoid a possible confounding effect of the separation on the diversification of the chemical profile, we observed aggression only in cohabitating workers. Second, as cuticular hydrocarbons are generally considered to be responsible for nestmate and caste recognition (see Lenoir et al. 1999; Howard and Blomquist 2005; Hefetz 2007; and as verified in *A. senilis*, see Ichinose et al. 2005), we performed chemical analyses of these substances. Third, we measured morphological and anatomical parameters in the attacker/attacked pairs of workers. We expected that

dominant workers would be larger, as this is generally an advantage in conflicts, and they would have more developed ovaries.

## Methods

### Preparation of ants

All experiments were conducted on four colonies of *A. senilis* collected in Doñana National Park, Andalusia (Southern Spain) in 1999 and kept in the laboratory. The ants were maintained in the laboratory at 28°C with a 12:12 h photoperiod and fed twice a week with live maggots, oranges and a commercial syrup for bumblebees. On 8 October 2002, two groups were prepared from each colony: one contained the queen and 250 workers (queenright nest), and the other only 250 workers (queenless nest). The workers were taken randomly; half were inside the nest with the brood and half were actively foraging. We removed all of the brood from these nests in the first two days after the separation, and after then counted the numbers of eggs and larvae once a week in each nest over eight weeks.

The behaviour of workers in the nest was observed every day from 10 October 2002 to 10 December 2002 during 10 min periods for each nest (i.e. for a total of 80 h). Observations took place between 10 am and 4 pm. When an aggression was observed (generally biting), both the attacker (A) and the attacked (E) workers were carefully picked up and marked with a dot of paint on the abdomen, using a different colour for each category. Ants that were involved in two successive interactions were marked again: when they behaved first as attacker and second as attacked, they received a different second mark and were called AE. Thus, we had four categories: AA, AE, EA and EE. The ants were not generally involved in many aggressions, so we did not use new categories. We divided the two months into five periods (the first week, the second, the third and fourth, the fifth and sixth, the seventh and eighth weeks after separation). Since marked ants were not marked individually, their behavioural ranks were not directly known. Instead, we evaluated the dominance of workers with the following assumption: if only one or a few workers behaved dominantly, workers that had been marked previously as attackers (A) would be less likely to be attacked later (E), and workers that had previously been attacked (E) would not attack later. Under this situation, the proportion of AA workers would be higher than AE, and that of EE would be higher than EA. This hypothesis was tested by contrast tests using a repeated measures ANOVA applied to the frequencies of AA, AE, EA, and EE workers over the five periods. No ant was observed in three

aggressions. After this first set of observations, we reared queenless groups for a longer time. Nine new groups of 250 workers from nine different colonies were constituted in the same conditions and followed for four months to observe brood rearing.

### Morphological and anatomical analyses

One pair of workers involved in aggression that were observed in the first, third, and fifth weeks after the separation was randomly taken from each nest. These workers were dissected to measure head width and to observe the teeth of the mandibles, as they abrade with age [see for example Smeeton (1982) for *Myrmica rubra* or van Wilgenburg et al. (2005) for *Iridomyrmex purpureus*]. In *A. senilis*, 80% of the workers in queenright field colonies have fully developed ovaries (Boulay et al. 2007) which produce mainly trophic eggs, as in other *Aphaenogaster* (Iwanishi et al. 2003). Ovaries regress in older ants. Ovarian development was determined by the number of eggs with yolk, and the total volume of these eggs was calculated via the maximum length, assuming a spherical shape. The colour of the poison gland changes with aging (unpublished data); it was recorded in four colours: (1) white in callow ants that are less than ten days old, (2) yellow, (3) orange, and (4) brown in the oldest forager ants. Combining these measures, we distinguished four age stages in workers: stage 1, the youngest (complete mandibular dents, no eggs with yolk, and white poison gland); 2 (complete dents, one egg with yolk, and pale yellow poison gland); 3 (little polished dents, two or more eggs with yolk, and dark yellow to orange poison gland); and 4, the oldest (polished dents, no eggs, and brown poison gland). For anatomical measures, see the figures in the “Electronic supplementary material”. By comparing with ants with known ages, workers at stage 4 were estimated to be older than 60 days.

### Chemical analyses

The thorax with legs of each worker involved in aggression and taken for morphological and anatomical measures was immersed in 1 ml of pentane for 5 min. The thorax was removed and the solvent was evaporated. These samples were kept in a refrigerator at  $-21^{\circ}\text{C}$ . For the analysis, the dry extract was dissolved in 50  $\mu\text{l}$  of pentane containing 12.5 ng of C20 as internal standard, and 5  $\mu\text{l}$  of the dissolved extract was analysed with gas chromatography (VGM250Q) using a DB-5 fused silica capillary column. The temperature was kept at  $150^{\circ}\text{C}$  during the initial 2 min, raised from 150 to  $300^{\circ}\text{C}$  at  $5^{\circ}\text{C}/\text{min}$  for the

following 30 min, and held at  $300^{\circ}\text{C}$  for the last 12 min. The hydrocarbons had been identified previously (Lenoir et al. 2001; Boulay et al. 2007), and a total of 32 peaks were used in our study. The areas of the peaks were estimated by peak integration using a TurboChrome Workstation, and the quantities were calculated from the internal standard.

To search for chemical differences between the attacker and attacked workers, we measured for each individual both the quantities and proportions of cuticular hydrocarbons. For the profile comparisons, we distinguished four categories: attacker and attacked workers in queenright nests and in queenless nests. The origin of the colony was included in the analysis as a variable. Discriminant analyses were performed both on the quantities and the proportions of cuticular hydrocarbons. Univariate tests were performed on the absolute and relative amounts of each substance to examine those that differed significantly between the attacker and attacked workers.

## Results

### Occurrence of worker aggression

The first eggs in queenright nests appeared in one week, probably due to the stress induced by the nest changing. Later on, more than 50 eggs per group were always present in each of the queenright nests. At the end of the experiment we counted 50 larvae which would have developed into workers, as in control colonies. In queenless nests, the eggs appeared in two weeks, the time necessary to produce male eggs. The number of eggs was 15–20, and some of them developed into small larvae, indicating that workers began to lay reproductive eggs. All eggs and larvae disappeared in three weeks. In these nests, eggs were laid again and they developed into larvae, but most disappeared, indicating repeated brood execution. Eggs laid in the last period (weeks 7 and 8) of this study would have probably developed into males if the study had been continued, as has been observed in complementary experiments: after four months we obtained male pupae ( $9.4 \pm 8.8$ , range 0–30) and adult males ( $12.3 \pm 13.5$ , range 0–50).

Attacks increased tenfold in queenless nests in comparison to control queenright groups (48 attacks per nest for the entire study, range 36–54, vs. 4.75 per nest, range 3–6;  $G$ -test,  $G_1 = 91.376$ ,  $P < 0.001$ ). This corresponds to a mean of 5.15 attacks per hour in queenless nests versus 0.12 in queenright ones. There was no difference in attacks between the four colonies ( $G$ -test, adjusted  $G = 1.059$ ,  $df = 3$ ,  $P = 0.787$ ). Thus, the queen’s presence considerably reduced worker aggression. The AA workers were

always more numerous than any other (Fig. 1,  $F_{(3,12)} = 9.914$ ,  $P = 0.001$ , with the differences between other types being nonsignificant  $P > 0.4$ ). The frequencies are different among periods: attacks increase and reach a maximum during the second week ( $F_{(4,12)} = 15.854$ ,  $P < 0.001$ ). The results indicate that aggressive workers tended to stay aggressive. There may have been hierarchies during the study period but they were not linear and/or stable.

#### Worker characteristics

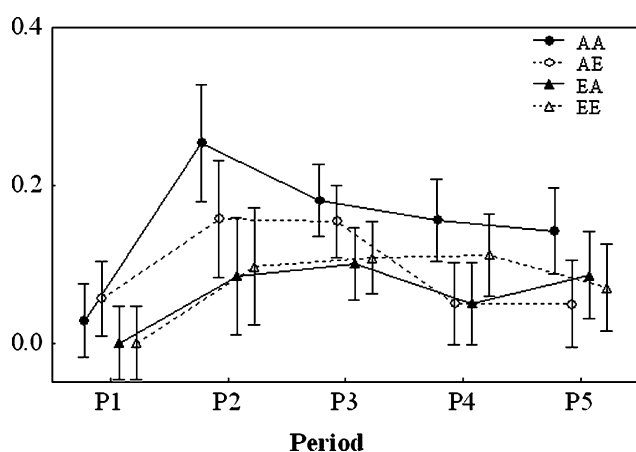
Head width, egg number, total egg volume, and age stage were compared between the attackers and attacked workers by paired  $t$ -tests. The mean ( $\pm$ SE) of head width and egg numbers were significantly larger in the attackers than in the attacked workers ( $1.24 \pm 0.02$  and  $1.20 \pm 0.02$  mm,  $t_{23} = 2.261$ ,  $P = 0.033$ , i.e. a 3.3% increase in linear measure, corresponding to a 10.2% increase in surface;  $6.46 \pm 0.65$  and  $4.96 \pm 0.63$  eggs,  $t_{23} = 2.213$ ,  $P = 0.037$ , respectively), while their egg volume or age stage was not significantly different ( $0.102 \pm 0.015$  and  $0.077 \pm 0.011$   $\mu$ l,  $t_{23} = 1.874$ ,  $P = 0.074$ ;  $2.67 \pm 0.17$  and  $2.42 \pm 0.16$ ,  $t_{23} = 1.362$ ,  $P = 0.186$ ). Age stages of between 2 and 3 indicate that the ants were neither young nor old. However, the poison gland was darker in attackers (stage  $2.58 \pm 0.18$  vs  $2.12 \pm 0.14$ ,  $t_{23} = 0.018$ ). This is also indicated by the frequencies: in 18 cases it was darker, three similar, and two paler in the attacker, (adjusted  $G_2 = 9.126$ ,  $P = 0.010$ ). This indicates that workers involved in aggression are from the same middle-aged stage, the fertile period, but attackers are slightly more

advanced physiologically (darker poison gland) and more fecund.

#### Chemical profile

The attackers and attacked workers had the same hydrocarbon blend and were not differentiated by any specific substances. The total quantity of HCs was 45.8% higher in the attacker ( $6525.1 \pm 1003.0$  ng) than in the attacked ( $4474.2 \pm 552.0$  ng) (paired  $t$ -test,  $t_{23} = 2.803$ ,  $P = 0.010$ ). Both the quantities and the proportions of the different HCs were significantly different between attacker and attacked ant in the queenright or queenless nests (MANOVA;  $F_{(31,2170)} = 13.228$ ,  $P < 0.001$ ;  $F_{(31,2170)} = 26.007$ ,  $P < 0.001$ , respectively). Univariate tests showed that many substances were significantly different in both quantity and proportion between these workers. The quantities of almost all substances with more than 27 carbons were higher in attacking workers than in attacked workers, while the proportions of individual substances were generally similar between these workers. Only four peaks—all branched alkanes—differed in proportion: 11 + 13 + 15 MeC27, 9, 13DiMeC27 + 3MeC27, 5MeC29 and 7, 11 + 11, 15DiMeC29 + 3MeC29 (see the table in the “Electronic supplementary material”).

Discriminant analyses on the quantity and proportion of cuticular hydrocarbons clearly discriminated attacking workers from attacked ones in both the queenright and queenless nests (Fig. 2). These results indicate that the chemical profiles of the attackers and the attacked ants in the colony were slightly different: as indicated above, some substances differed in relative proportions in both queenright and queenless conditions.

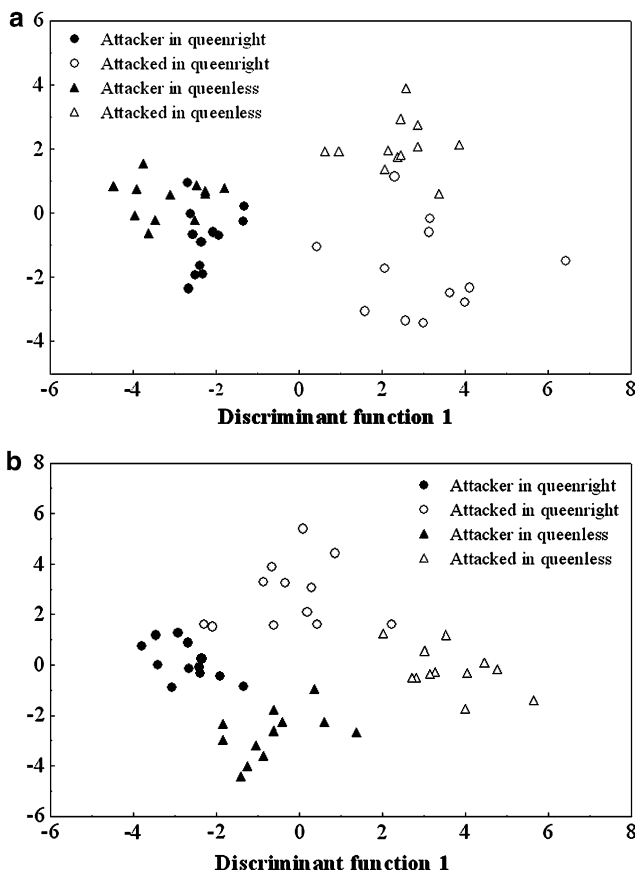


**Fig. 1** Mean numbers per colony per observation ( $\pm$ SE) of attacking workers (designated by circles) that had attacked or had been attacked by others previously (AA and AE), and attacked workers (triangles) that had attacked or had been attacked by others (EA and EE) for each of five periods: the first, second, third to fourth, fifth to sixth, and seventh to eighth weeks

#### Discussion

##### The significance of intranidal aggression

In *A. senilis*, nonfatal, intranidal worker aggression was observed between workers in queenless nests, but was rare in queenright nests. This phenomenon has been observed in *Cataglyphis cursor* queenless nests with a frequency of 2.2/h in the studied colony comprising 350 workers (Clémencet et al. 2007), and it is suggested to be a simple competition for producing males. Aggression events are more frequent in *A. senilis* (5.15/h in groups of 250 workers). This is similar to what was observed in *A. japonica* (4.5/h) (Iwanishi et al. 2003). Two explanations are possible: worker policing and egg-laying competition. If it is worker policing, it must involve an indirect fitness benefit to the aggressive worker, whereas the benefit would be direct in dominance interactions. Policing can be defined as



**Fig. 2** First and second discriminant functions obtained by discriminant analyses performed on the quantity (a) and proportion (b) of cuticular hydrocarbons for four categories of workers: attacker and attacked workers in queenright nests (closed circles and open circles), and attacker and attacked in queenless ones (closed triangles and open triangles)

“coercive actions that reduce direct reproduction by other individuals” (Monnin and Ratnieks 2001). As *A. senilis* and *A. japonica* are monoandrous, the workers cannot attack workers of other patrines; therefore, policing is not expected. Nevertheless, policing may exist also in monogynous and monoandrous ants like *Diacamma* (Kikuta and Tsuji 1999) when the laying workers reduce the fitness of the colony. In our study on *A. senilis*, attacked workers were less fertile and smaller than the attackers; therefore, aggression between workers is probably dominance. On the other hand, the brood is destroyed in the first few weeks, and all of the larvae of the first cohorts disappeared. We did not directly observe brood execution by most aggressive workers. Though some of these eggs may be trophic, it is indeed probably policing by egg eating. Therefore, we probably have a mixture of dominance and policing. Nevertheless, this period of conflicts finishes after two months, and then workers are able to rear a male brood and males emerge after four months. In *A. japonica*, the same results were observed after 4–5 months (Iwanishi et al. 2003).

## Role of the queen

In *A. senilis*, the queen strongly suppresses worker reproduction, and they lay male eggs in queenless groups (Ledoux 1976; Boulay et al. 2007). Nevertheless, in the field, male production has been observed in the presence of the queen in large colonies (Boulay et al. 2007), and also in large colonies in the laboratory (unpublished data). This indicates that queen’s inhibition in this species may break down if the colony becomes large and some workers happen to get out of the effective concentration range of the inhibitor substances of the queen. These compounds may be a primer pheromone. It may also indicate that workers actively try to escape the queen’s inhibition and begin to lay their own eggs in the context of a queen–workers conflict. It may also simply indicate that small colonies are in the growing phase and invest in worker production, while larger colonies can afford to reproduce. This can explain the occurrence of some rare intranidal aggression events between workers in large queenright colonies where the workers can escape the queen’s inhibition.

## Proximal mechanism of aggression

Chemical analyses revealed that intranidal aggression in *A. senilis* is not correlated to any cuticular substance that characterises either the attacker or attacked worker, but to the differences in the general profile and the quantity of hydrocarbons. In ants, it is now well known that the egg-laying queen presents a different chemical profile that is a signal of fertility (Monnin 2006). In some ponerine species this queen signal is just one type of molecule (Monnin et al. 2002; D’Ettorre et al. 2004). In *A. senilis*, the queen signal may be more related to Dufour gland secretion than to cuticular hydrocarbons (Boulay et al. 2007), so it should also be interesting to compare the glandular contents of workers involved in aggression. In our study, Fig. 2 clearly shows that attackers from queenright and queenless colonies group together and thus share the same hydrocarbon profile. This shows that workers that started to lay fertile eggs instead of trophic eggs did not acquire a new fertility signal; they already had this odour under queenright conditions. This in turn indicates that this odour depends on how many eggs are produced, no matter whether these eggs are fertile or not. Therefore, the role of odour in the regulation of conflicts may be limited and not comparable to a fertility signal.

## Size effect

The other characteristic trait of dominance in *A. senilis* is the role of size in aggression: workers attacked smaller



ants. This has been observed in many cases; for example, in *Cataglyphis cursor*, the larger workers are more likely to reproduce and attack smaller nonlaying ones (Clémencet et al. 2007). In *C. niger*, larger workers are also more aggressive in intercolonial conflicts unless it is in a different context (Nowbahari et al. 1999). The larger quantities of hydrocarbons in attackers may be simply due to their larger size, but a 10% increase in surface probably cannot explain a 50% increase in the quantity of cuticular hydrocarbons. The difference in the hydrocarbons of larger workers is more likely to be correlated to a difference in fecundity. In *C. floridanus*, the low fecundity of laying workers is not associated with changes in hydrocarbon profiles, and so aggressions occur only rarely (Enderl et al. 2007).

#### Age and egg-laying

Orphaned workers are able to lay fertile eggs that develop into males. Our data confirm previous findings: in queenless groups of *A. senilis*, 30 males were obtained on average after 14 weeks (Boulay et al. 2007). We never obtained thelytokous parthenogenesis, as indicated by Ledoux (1984), probably because this author exposed the workers to a cold stress. In field and laboratory queenright colonies of *A. senilis*, 80% of the workers have fully developed ovaries. They probably only lay trophic eggs, but are ready to lay haploid eggs (Boulay et al. 2007). This explains why workers begin to lay reproductive eggs after two weeks when the queen's inhibition ceases, and why they produce males in large colonies. This suggests that the queen/worker conflict over male production is latent in this ant species. In the dominance interactions, the attackers have more developed ovaries and so are more fecund than the attacked ants. The consequence of conflicts between laying workers is drastic: all of the first eggs are destroyed. Unpublished observations showed that *A. senilis* workers develop their ovaries in 50–60 days after emergence under queenless conditions and then produce eggs for only a very short time. In *A. senilis*, the rapid senescence for egg-laying observed after 60 days does not permit any worker to be dominant for long periods, and probably no stable hierarchy is possible. The situation is very different in *Camponotus floridanus*, which forms large colonies with workers of low reproductive potential. Egg-laying orphaned workers are not policed when reunited in the mother colony, and they do not have different hydrocarbon profiles (Enderl et al. 2007). On the contrary, in *A. senilis*, there is an excess of male egg-laying, and egg-laying workers compete to eliminate the surplus. Later on, the worker progeny is preserved; the later egg cohorts are not destroyed and are reared to adult males. This suggests that the conflicts in queenless colonies reach an equilibrium

phase, as prolonged periods of conflict should be selected against (Ratnieks et al. 2006).

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## Electronic supplementary material

Below is the link to the electronic supplementary material.

[Ovaries of \*A. senilis\* workers; \*top\*: a laying worker with ovocytes; \*bottom\*: an old worker with atrophied ovaries and traces of yellow bodies coloured in red \(JPG 1554 kb\)](#)

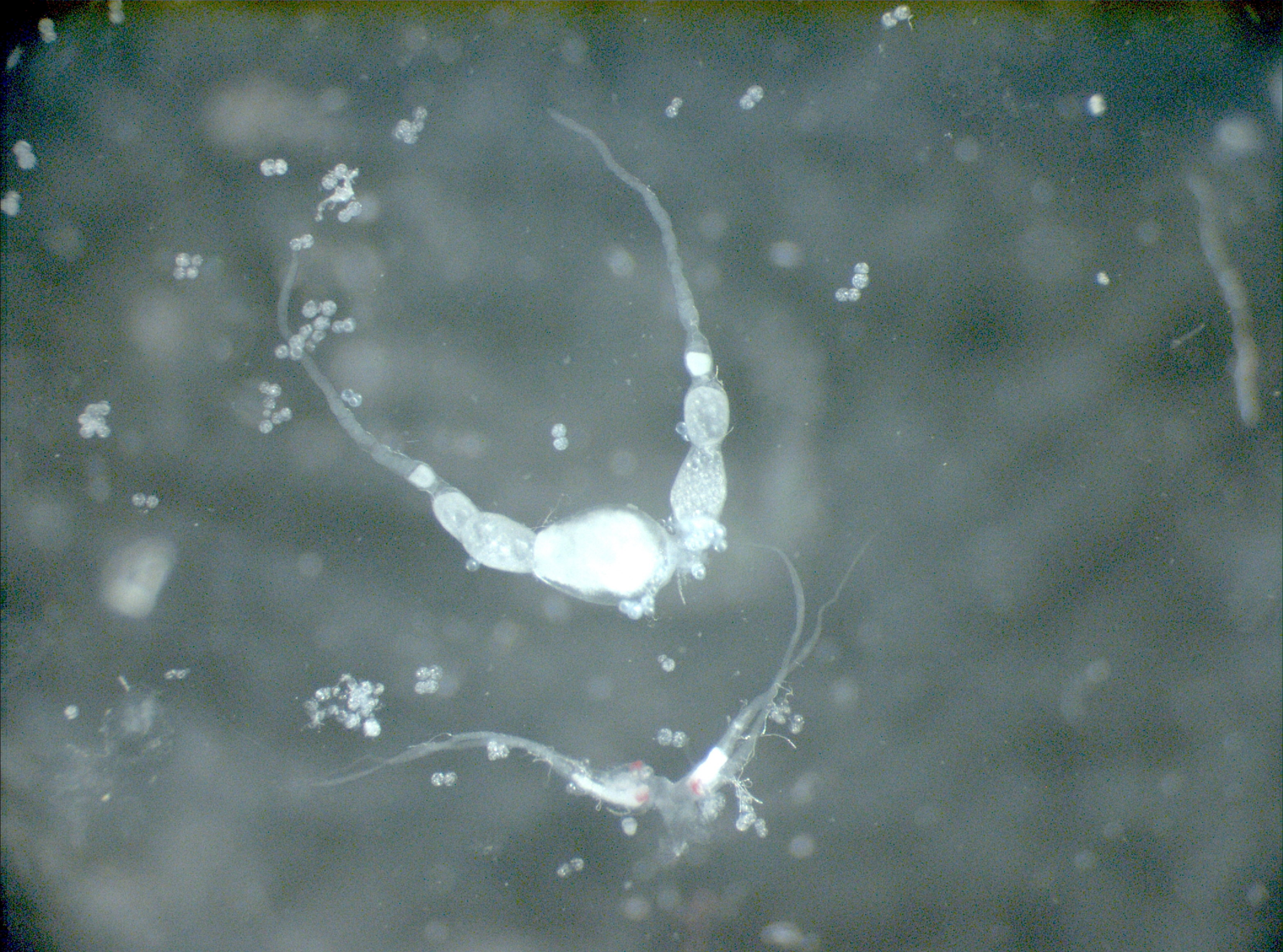
[Dufour \(\*left\*\) and poison \(\*right\*\) glands of young \*A. senilis\* worker. The glands are empty \(JPG 1586 kb\)](#)

[Poison gland of an old \*A. senilis\* worker \(JPG 1176 kb\)](#)

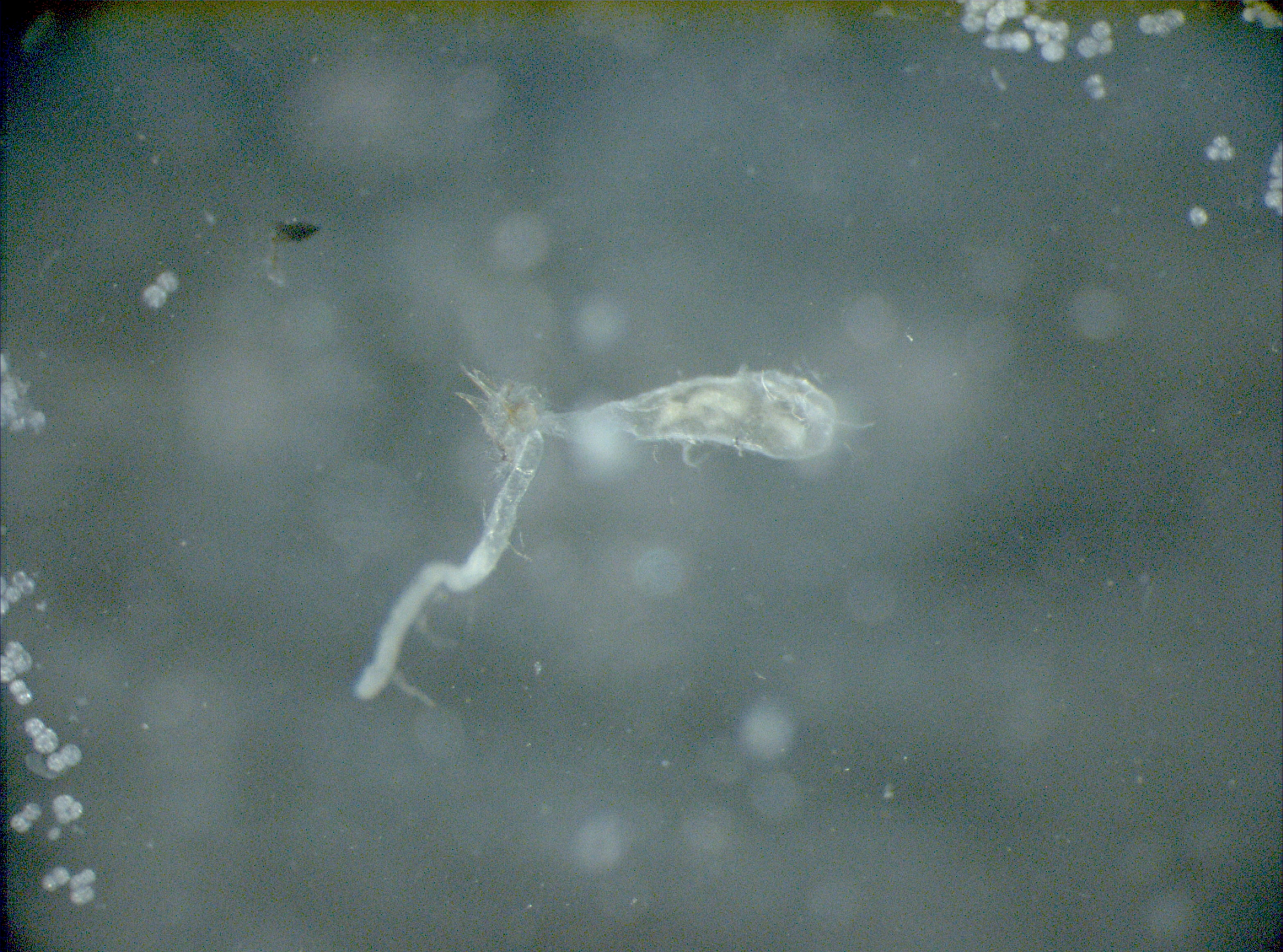
[Mandibles of \*A. senilis\* workers. \*Left\*: young worker; \*right\*: old worker \(polished mandibles\) \(JPG 728 kb\)](#)

[Means \( \$\pm\$  SD\) of the quantity and proportion of cuticular hydrocarbons in the pairs of attackers and attacked workers \( \$n=24\$ \). The quantity and proportion were square-root and arcsine-transformed, respectively, and compared by a paired  \$t\$ -test \( \$df = 23\$  in all tests\). Peak numbers are from Lenoir et al. \(2001\). Significant differences are shown in bold \(DOC 39 kb\)](#)





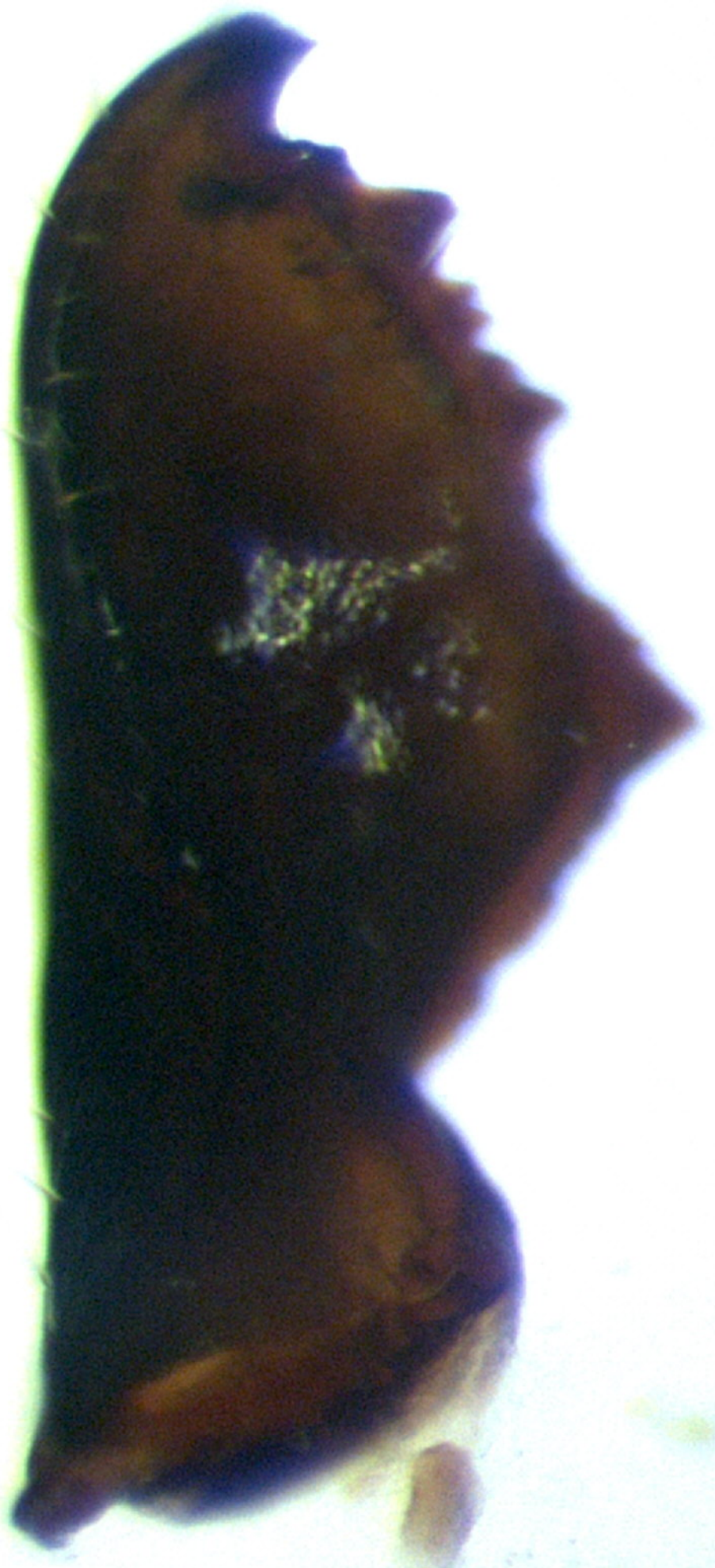
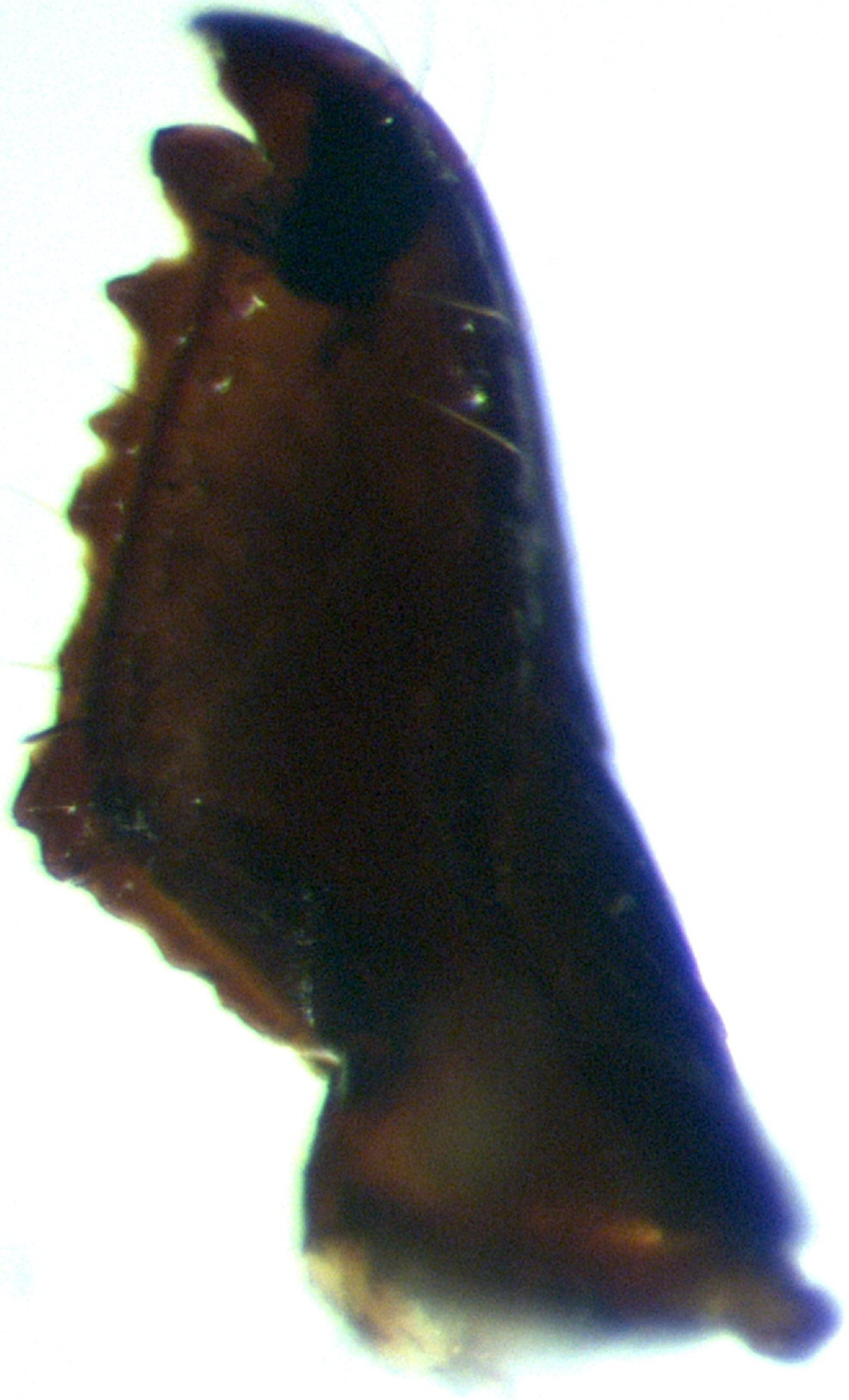














## Supplementary data

Table S1. Means ( $\pm$  SD) of the quantity and proportion of cuticular hydrocarbons in the pairs attacker and attacked worker (n=24). The quantity and proportion were square-root and arcsine transformed, respectively, and compared by a paired *t*-test (df = 23 in all tests). Peak numbers according to Lenoir et al. (2001).

In bold, the significant differences.

Peak	Substance	Quantity (ng)				Proportion (%)			
		Attacker	Attacked	<i>t</i>	<i>P</i>	Attacker	Attacked	<i>t</i>	<i>P</i>
1	C25:1	4.47 $\pm$ 2.23	3.94 $\pm$ 2.25	1.01	0.32	0.35 $\pm$ 0.20	0.39 $\pm$ 0.33	-0.41	0.68
2	C25	7.64 $\pm$ 2.27	6.41 $\pm$ 1.38	3.28	<b>0.003</b>	1.07 $\pm$ 0.33	1.09 $\pm$ 0.31	-0.30	0.77
3	11+13Me C25	4.03 $\pm$ 1.69	3.59 $\pm$ 1.48	1.73	0.10	0.27 $\pm$ 0.06	0.32 $\pm$ 0.15	-0.59	0.56
4	7 Me C25	4.42 $\pm$ 2.01	3.76 $\pm$ 1.40	1.88	0.072	0.33 $\pm$ 0.08	0.34 $\pm$ 0.11	-0.72	0.48
5	7,9 DiMeC25	1.54 $\pm$ 2.02	0.80 $\pm$ 1.44	1.41	0.17	0.06 $\pm$ 0.08	0.04 $\pm$ 0.07	1.04	0.31
6	3 Me C25	4.41 $\pm$ 1.91	3.77 $\pm$ 1.14	1.92	0.07	0.33 $\pm$ 0.06	0.35 $\pm$ 0.06	-1.10	0.28
7	C26	10.86 $\pm$ 4.24	9.72 $\pm$ 3.14	1.63	0.12	0.29 $\pm$ 0.16	0.25 $\pm$ 0.15	-2.03	0.05
8	10+12 MeC26	8.52 $\pm$ 4.17	7.19 $\pm$ 3.32	1.49	0.15	2.05 $\pm$ 0.52	2.31 $\pm$ 0.47	0.16	0.87
9	8 Me C26	8.52 $\pm$ 4.17	7.20 $\pm$ 3.32	1.49	0.15	1.29 $\pm$ 0.45	1.30 $\pm$ 0.47	0.16	0.87
10	8,10+8,12 Di Me C26	8.73 $\pm$ 6.26	7.65 $\pm$ 4.3	1.45	0.16	1.48 $\pm$ 1.24	1.68 $\pm$ 1.09	-1.09	0.28
11	6,10+6,12 DiMe C26	10.14 $\pm$ 5.67	9.123 $\pm$ 3.94	0.836	0.41	2.06 $\pm$ 1.33	2.31 $\pm$ .111	-0.52	0.61
12+13	4,8 DiMe C26 + C27	18.38 $\pm$ 7.68	15.03 $\pm$ 6.11	2.59	<b>0.02</b>	6.12 $\pm$ 2.90	5.85 $\pm$ .053	0.44	0.66
14	4,8,12 TriMe C26	13.22 $\pm$ 6.44	11.06 $\pm$ 3.9	1.90	0.07	2.99 $\pm$ 1.35	3.05 $\pm$ 0.91	-0.45	0.68
15	9+11+13 Me C27	27.45 $\pm$ 12.00	25.26 $\pm$ 8.38	1.17	0.25	12.72 $\pm$ 3.46	15.57 $\pm$ 3.10	-3.37	<b>0.003</b>

16	9,17 DiMe C27	7.45 ± 3.59	5.86 ± 2.78	1.950	0.06	0.96 ± 0.39	0.86 ± 0.34	0.76	0.46
17+18	9,13 DiMe C27 + 3 Me C27	32.79 ± 11.92	26.02 ± 9.36	3.14	<b>0.005</b>	18.78 ± 4.73	16.40 ± 4.06	2.39	<b>0.02</b>
19	C28	14.62 ± 5.57	11.46 ± 4.78	3.24	<b>0.004</b>	3.88 ± 1.68	3.38 ± 1.68	1.45	0.15
20	3,7+3,9 DiMe C27	10.58 ± 4.03	8.90 ± 2.42	2.54	<b>0.02</b>	2.05 ± 0.85	2.03 ± 0.54	-0.05	0.96
21	10 +12 Me C28	26.99 ± 9.52	23.44 ± 7.01	2.15	<b>0.04</b>	13.06 ± 3.76	13.83 ± 3.56	-1.14	0.27
22	6Me C28	5.81 ± 1.74	4.76 ± 1.32	2.85	<b>0.01</b>	0.62 ± 0.17	0.57 ± 0.13	1.27	0.22
23+24	4 Me C28 + 8,12 DiMe C28	20.35 ± 8.50	17.27 ± 5.51	2.26	<b>0.03</b>	7.26 ± 2.38	7.57 ± 2.22	-0.82	0.42
25	6,10 DiMe C28	11.50 ± 4.43	9.71 ± 2.84	2.55	<b>0.02</b>	2.30 ± 0.63	2.37 ± 0.48	-0.62	0.54
26	4,8 +4,10 DiMe C28	10.37 ± 4.18	8.69 ± 2.63	2.27	<b>0.03</b>	1.87 ± 0.52	1.90 ± 0.50	-0.36	0.72
27	C29	17.45 ± 6.14	13.74 ± 5.44	3.42	<b>0.002</b>	5.50 ± 1.99	4.82 ± 2.21	1.43	0.17
28	4,8,12 TriMe C28	8.93 ± 4.29	7.55 ± 2.68	1.90	0.07	1.37 ± 0.58	1.41 ± 0.39	-0.59	0.56
29+30	7+9+11 Me C29	15.34 ± 5.50	13.35 ± 4.30	2.45	<b>0.02</b>	4.14 ± 0.94	4.35 ± 0.79	-1.15	0.26
31	5 Me C29	10.30 ± 3.64	8.02 ± 2.73	3.38	<b>0.003</b>	1.88 ± 0.50	1.56 ± 0.33	2.49	<b>0.02</b>
32+33	11,15+7,11DiMeC29+3 Me C29	14.17 ± 5.25	10.87± 4.26	3.20	<b>0.004</b>	3.58 ± 1.25	2.87 ± 0.93	2.31	<b>0.03</b>
34	C30	2.350 ± 1.43	1.49 ± 1.51	2.96	<b>0.01</b>	0.12 ± 0.08	0.09 ± 0.10	1.81	0.08
35	10+12 Me C30	5.26 ± 2.56	4.36 ± 1.89	2.18	<b>0.04</b>	0.58 ± 0.49	0.53 ± 0.35	0.13	0.89
36	11+13 Me C31	1.14 ± 1.41	1.27 ± 1.57	-0.42	0.68	0.08 ± 0.11	0.09 ± 0.09	-0.57	0.57
37	11,19 DiMe C31	4.45 ± 1.06	4.01 ± 0.96	2.36	<b>0.03</b>	0.47 ± 0.35	0.53 ± 0.33	-1.17	0.25

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