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Remarks on the behaviour of the Velvet Ant *Nemka viduata viduata* (Pallas, 1773)

(Insecta Hymenoptera Mutillidae)

Abstract

The present paper reports observations of the Velvet Ant *Nemka viduata viduata* (Pallas) performed in nature and in the laboratory. Observations in nature were carried out in several Italian locations between 1984 and 2011. The observations concern aspects of the reproductive behaviour, the phases of pre-copulation, copulation and post-copulation. The examination of the female specimens reveals the presence of a morphological character, correlated with the mechanical action of the mandible of the male and represented by the propleuron that in the dorsal view presents a groove. Four different masculine behaviours and four female ones have been singled out in the pre-copulation phase, and the active role of the female is underlined, her sexual receptivity and the importance of her nuptial flight for successful mating. Other observations have examined the activity of body grooming enhancing/ highlighting new behaviour patterns in Mutillidae. Other observations have regarded the grooming activities, highlighting new behavioural patterns in Mutillidae. The morphology of the special organ for the grooming of the antennae, both male and female, located near the tibio-tarsal articulation of the foreleg is also described. Finally, observations in nature and in the laboratory indicate that the daily wandering of female exemplaries of *Nemka viduata viduata* is strongly dependent on solar radiation and the position of the sun.

Key words: mating and grooming behaviours, velvet ants, *Nemka viduata viduata*, Mutillidae.

Riassunto

[*Note sul comportamento del Mutillide* Nemka viduata viduata *(Pallas, 1773) (Insecta Hymenoptera Mutillidae)*]

Il presente lavoro riporta le osservazioni effettuate in natura ed in laboratorio sul Mutillide *Nemka viduata* v*iduata* (Pallas). Le osservazioni in natura sono state eseguite in tre diverse località italiane: nel 1984 in Puglia a ridosso della città di Vieste (provincia di Foggia), nel 1986 in Sardegna nei dintorni di Badesi (provincia di Sassari) e sempre in Sardegna, a Vignola Mare (provincia di Olbia-Tempio) nel 2010 e nel 2011. Altre osservazioni sono state effettuate presso la pineta costiera di Cecina (Toscana, provincia di Livorno) nel 2000 da Luciano Filippi (dati non pubblicati). Le osservazioni mettono in evidenza diversi moduli comportamentali non noti o poco conosciuti: che riguardano l'incontro tra i due sessi, le fasi di pre- e post- copula e la pulizia del corpo.
Viene identificato il primo segnale femminile, visibile all'osservatore, che autorizza il maschio a

trasportarla (in volo o a piedi) in un altro luogo per proseguire il percorso di copula. Si sottolinea il ruolo attivo della femmina nell'accoppiamento e l'importanza del volo nuziale.
Le osservazioni evidenziano che il girovagare quotidiano delle femmine è fortemente orientato dalla posizione del sole (fototassìa positiva).
Le osservazioni in laboratorio sono la prosecuzione delle campagne effettuate in Sardegna; gli esemplari sono stati osservati in contenitori trasparenti (140 x 75 e 70 mm di altezza) preparati con circa tre centimetri di sabbia (raccolta proprio nel luogo di cattura), rametti di *Ephedra distachya* L., alcuni sassolini, qualche pezzetto di corteccia per fornire nascondigli e sono stati alimentati con miele diluito in acqua ed acini d'uva tagliati a metà.
L'esame degli esemplari di sesso femminile evidenzia la presenza di un carattere morfologico correlato con l'azione meccanica di bloccaggio da parte delle mandibole del maschio, rappresentato dalla propleura, che in visione dorsale presenta una scanalatura.
Le osservazioni in laboratorio evidenziano come l'attività prevalente sia la pulizia delle antenne, del capo e del metasoma, con modalità simili per entrambi i sessi, eccezion fatta per le ali nei maschi. I comportamenti di pulizia del corpo sono stati osservati allo stereomicroscopio, sono stati anche provocati sporcando gli esemplari con farina e polline come già sperimentato da Bayliss & Brothers (1996, 2001). Per quanto riguarda le attività di pulizia del corpo sono stati osservati 18 dei 19 caratteri descritti da Baybuyuk & Quicke (1999) per i Mutillidi ed un ulteriore carattere noto per altre famiglie di Imenotteri; sono state riscontrate anche tre modalità evidenziate da Bayliss & Brothers nel 1996 più una nel 2001 sempre per i Mutillidi, inoltre sono state osservate due nuove modalità mai osservate prima nei Mutillidi e negli Imenotteri.
Infine viene descritta la morfologia dell'organo per la pulizia delle antenne, sia maschile che femminile, situato in prossimità dell'articolazione tibio-tarsale delle zampe anteriori.
Le risultanze delle osservazioni, riportate cronologicamente, vengono confrontate soprattutto con gli aspetti eco-etologici oggetto di studi in questa specie (Nonveiller, 1963; Alicata et al., 1974; Tormos et al., 2010) e di altre specie di Mutillidae (Ferguson, 1962; Brothers, 1996; Bayliss & Brothers, 2001).

Introduction

Publications on the behaviour of Mutillidae are rather limited, mainly due to the difficulties in meeting them under conditions allowing for ethological observations, especially with regards to reproductive behaviour; in particular it is very difficult to document the interaction between specimens of both sexes during the phases of pre-copulation, copula and post-copulation. In the Palearctic area, particularly in its western sector, one of the most studied species is *Nemka viduata viduata* (Pallas), taxon at chorotypus Eurasian-Mediterranean, rather common especially in sandy coastal areas; eco-ethological aspects have been the subject of various papers by: Nonveiller (1963), Alicata et al. (1974) and recently by Tormos et al. (2010). In the present paper, observations made by the author over a period of thirty years in several Italian locations have been reported, which integrate and enrich knowledge on the eco-ethology of this species.

Materials and methods

Observations have been performed both in nature and in the laboratory.

Observations in nature have been performed in three different Italian locations, in different years, as specified below:
- in the surroundings of the town of Vieste (province of Foggia, Apulia) in 1984;
- in the surroundings of Badesi (province of Sassari, Sardinia) in 1986;
- surroundings of Vignola Mare (province of Olbia-Tempio, Sardinia) in 2010 and 2011.

These observations were integrated by others made in 2000 in the coastal pinewoods of Cecina (province of Livorno, Tuscany) by Luciano Filippi (unpubl.). Ground temperatures were detected by a digital infra-red thermometer (resolution ± 0,1 °C). Observations in the laboratory are the continuation of the field studies carried out in Sardinia and are listed chronologically below: specimens were kept alive and observed in transparent terrariums (140 x 75 x 70 mm height) where the habitat of capture was partly rebuilt, they were fed with honey diluted in water and some grape halves. In 2011 the activity of females was monitored daily, highlighting walking directions and times on maps made by the station of Vignola Mare; those of June 30th are attached to this paper.

The different grooming behaviours were observed using an Optika SMZ2 light stereomicroscope, a large part of these events were recorded with a digital camera Aiptek HD 1080P connected to the trinocular microscope; video tapes were analyzed using an iMac computer and Quicktime Player software with variable speeds and frames by frame playback.

In order to study in further detail and better document the grooming phases, the specimens were smeared with flour and pollen, as was already experimented by Bayliss & Brothers (1996, 2001). The terminology used in describing and listing these behavioural patterns is that proposed by Baybuyuk & Quicke (1999).

Results

The observations carried out for *Nemka viduata viduata* are reported separately below, according to the three different locations investigated.

Vieste (Apulia).

In the last week of June 1984, in a sandy area separated from the sea by a coastline road, numerous specimens of both sexes and seven couples were collected during two consecutive mornings between 07:30 and 09:15 a.m.: five were in nuptial flight and two in copulation, one on a herbaceous stem and the other on the ground. As already reported (Matteini Palmerini, 1992) it was not possible to ascertain if the mating had taken place during the flight, because once in contact with the insect net the specimens separated immediately.

Badesi (Sardinia).

In the second half of July 1986, along the road that leads from Badesi to the seaside, in a large sandy area, several males flying low were captured individually and a several females; a few metres away a large number of males about sixteen, were competing for a female.

In the following days, some specimens from both sexes were placed into a glass

terrarium (prepared with a layer of sand of a few inches, a couple of pebbles and some small pieces of bark for shelter) and examined. Whenever specimens from both sexes came into contact head-on/face to face, they seemed to get frightened and then ran away in the opposite direction. The next morning a two-fold attempt at copulating was observed: the males, who were both on the ground, grabbed the females from behind their heads with their mandibles, but the females refused copulation, by fully folding the metasoma forward with their sting erected and partly evaginated; the males also had the metasoma folded forward with the copulatory apparatus turned outward in an attempt to reach the females'. This situation lasted several hours. In the following days, no other attempts at mating were reported.
In the early days of life in captivity, when honey or grapes were provided, all the specimens quickly rushed to lap at length: the labial and maxillary palps were always relaxed and immobile, antennae at rest, folded towards the body, while metasomal segments, from the third onwards, made small and continuous telescopic movements. When licking the cut grapes, they occasionally shifted onto the surface where the juice was more abundant. With the passing of days, the interest in the trophic source underwent a sharp decline.
The specimens from both sexes in the glass terrariums were strongly engaged in a grooming activity of all the parts of their bodies. During periods of inactivity (including night hours), the females buried under the sand or found refuge under a shelter, while the males stopped on a shrub or on the sand.
The males died in a few days, between late July and early August, one after the other, while the females died between the last week of September and the first week of October.

Vignola Mare (Sardinia), 2010.
In late June and early July 2010, observation of the behaviour of *Nemka viduata viduata* was carried out, in a glade sheltered from the wind and about 80 m² wide for a period of 9 days, for about 35 hours in total, both in the hours of the morning and the afternoon.
The activity of excavation of the *Stizus fasciatus* (Fabricius) (Hymenoptera, Crabronidae) and the transit of specimens like the *Pimelia angusticollis* Solier (Coleoptera, Tenebrionidae) was quite frequent in the station, as well as in neighbouring glades.
The activity of females was very different from that of males: the former moved quickly on the ground to explore every crevice, while the latter did not supervise a specific area but they flew over vast areas in search of females, especially in areas lacking or with scarce vegetation, at a variable height from the ground ranging from 8-10 to 30 cm. In the station, males seemed to come out before the females and remained out for more than 2,5 hours, both in the morning and the afternoon: in the morning between 6:45/07:00 and 09:00/09:15 (DST), while in the afternoon between 17:00/17:15 and 19:30/19:45. In the morning males began their patrolling activities in areas with good sun-exposure, while in the afternoon they turned to shady areas. This is to be correlated with the appearance of females, that started

their activities in the early hours of the morning after the solar radiations had warmed the substrate adequately, interrupting them during the hottest hours to resume them during afternoon hours, when air and substrate temperatures were remarkably lower. The morning activity in the station was reduced since a great part of the area remained in the shade. At 09:00, the substrate temperature fluctuated between 23.1 and 23.8 °C; between 09:00 and 09:15 females interrupted their research activity on the substrate, when the temperature was higher than 42 °C, while in the afternoon they reappeared when the temperature of the substrate was 44.8-45.9 °C in the sun and of 28.7-37.1 °C in the shade.
Males could locate females even underneath the surface of the substrate: numerous landings, including those of several males, were observed, in spots where a female had just buried under the sand, or surfaced after landing. Filippi (unpubl.) observed «males waiting immobile even for long periods of time, 10-15 minutes, for the coming out of a female». Having singled out a female, males reacted according to four different patterns of behaviour (Tab. I).
Even ♀♀ react to the approaches of ♂♂ according to four different modalities: three of avoidance and one of acceptance (Table II).

Table I. Behaviours of ♂♂ of *Nemka viduata viduata* when singling out and approaching ♀♀.

BEHAVIOUR	DEFINITION
Bm.1	They fly over ♀ and then continue the exploration flight.
Bm.2	They land near the ♀, get closer with their antennae vibrating strongly, and if the ♀ leaves, they follow her for a while; they often stop, groom their antennae with their forelegs and resume the flight.
Bm.3	They land with their wings erected and vibrating really strongly, run and often revolve on themselves, sometimes to the left and other times to the right, drawing a circle (Fig. 1a, 1b), with small leaps, resume their flying and then land again several times in an attempt to attract or stop the ♀; if she accelerates and continues her wandering, they resume their flight completely, and also in this case the grooming of the antennae was frequently noted.
Bm.4	They land near or directly onto the ♀ and try to grab her with their mandibles between the head and the pronotum.

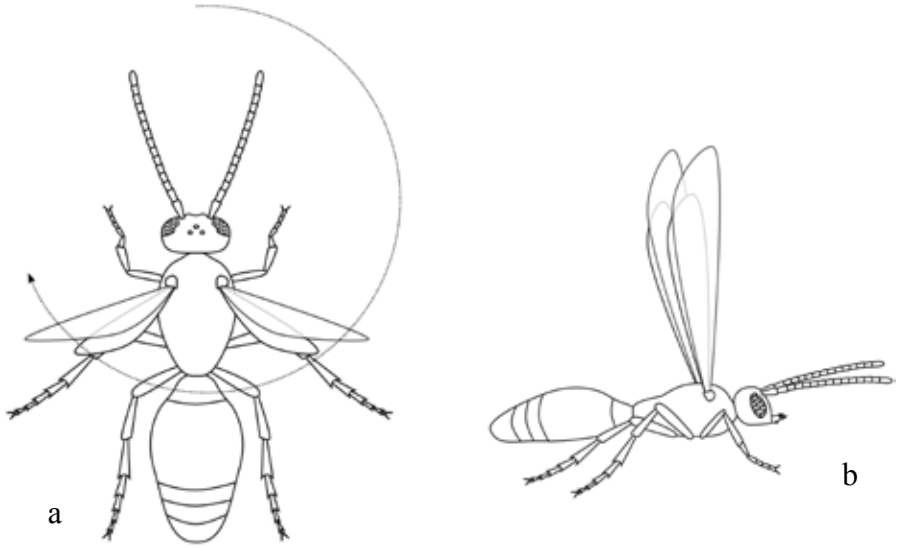


Fig. 1 - Male behaviour (Bm.3) of *Nemka viduata viduata*: a) dorsal view; b) lateral view.

Table II. Behaviours of ♀♀ of *Nemka viduata viduata* when they are approched by ♂♂.

BEHAVIOUR	DEFINITION
Bf.1	They get away quickly once the male lands.
Bf.2	They react to attempts at capturing them by rolling on their back and struggling furiously in an attempt to back out.
Bf.3	When grabbed, they try to reject the male with their legs, even passing under obstacles, they often add the folding of the metasoma forward with their sting partly evaginated and erected.
Bf.4	When grabbed, they stand immobile, bend their antennae and legs under or sideways along their body.

As mentioned in the Bf.2 and Bf.3 modalities, during attempts by males to grab the females with their mandibles and block them from behind their head, these try to back out by turning dorsally and defending themselves or attacking furiously with their legs and mandibles, at times even with their sting, in part evaginated and erected; during the struggle the ♂♂ often end up being under the ♀♀, always in dorsal position. With such reactions, males escape by flying away. The escape flight, however, is much faster, almost rectilinear, and higher than the exploratory one. In the nine days of research the nuptial flight of two couples (one in the morning and one in the afternoon) and a mating were observed. The two couples in nuptial flight got out of the station and locating them was not possible; the

Table III. Number of behaviours of ♂♂ (A) and ♀♀ (B) of *Nemka viduata viduata* registered in Vignola Mare during the period of observation.

N°	A	B	N°
> 100	Bm.1	Bf.1	> 100
c. 80	Bm.2	Bf.2	c. 30
c. 60	Bm.3	Bf.3	c. 25
c. 25	Bm.4	Bf.4	3

nuptial flight took place in the same way: after the male had grabbed the female with his mandible round her head, she had completely abandoned herself, folding legs and antennae, he kept her under his body with his legs, then carried her, flying, to another place. The mating took place in the afternoon: behaviours of males and females already described by ALICATA et al. (1974) and TORMOS et al. (2010) were observed, and are summarized in Table IV.

Considering all these phases, the mating observed lasted more than 2 hours, in line with what was highlighted by TORMOS et al. (2010); further male and female behaviours are reported below, observed during the pre and post-copulation. At about 18:10, at the base of a large bush (*Juniperus oxycedrus macrocarpa*), over a time frame of 30 seconds, about 6 males gathered, some that landed on the ground with their wings held high up were chasing a large-sized female, while others, without stopping, touched the ground and resumed their flight in an attempt to grab the female with their mandibles. All the males performed fast antennal movements. A male succeeded in grabbing her from behind her head, the female stood immobile and folded her legs, and immediately after that the male tried to walk away while trying to perform flights of a few centimetres carrying her under his body; he shifted from the base of the Juniper and walked along the branches of *Ephedra distachya* L., passing from a small plant to another in an attempt to get away from the other males, who instead were still chasing after her. Meanwhile the female was always immobile with her antennae and legs folded under her body. The male, in an almost vertical position, blocked the female under himself with his middle legs and at the same time held tightly onto the twigs of *Ephedra*; his wings were lying on the metasoma; subsequently with the forelegs, he began to rhythmically stimulate, from top to bottom, the lateral pubescence of the head, up to the front-lateral area of the pronotum of the female: 4-6 rubs that were repeated several times after a pause of several seconds. Movements of the forelegs were also accompanied by periodic movements of the antennae that hit the female on the top of her head; in the meantime the male, with his copulatory apparatus everted, sought the female's genital opening. The female evaginated her sting completely, folded it on the right side and upward parallel to the males' metasoma which joined his own apparatus with the female's. At this point the males that were continuing to explore the twigs where the couple had transited, resumed

Tab. IV. Male and female behaviours during the phases of mating of *Nemka viduata viduata.*

male behaviours	female behaviours
Capture of the ♀ with mandibles	Immobilization, folding of antennae and legs under the body
Departure from the meeting place with transportation of the ♀ under the male's body (in flight or on the substratum)	Lets her body be carried
Stimulation of the anterolateral area of the pronotum of the ♀ with rhythmic movements of the legs and antennae	Lateral evagination of sting
Union of sexual apparatus	Stridulation at the beginning of copulation
Telescopic movements of the last metasomal segments	
Separation of sexual apparatus	Stridulation at the end of copulation
	Invagination of sting
Liberation of the ♀	

their flight one by one, always with their antennae vibrating strongly. The use of a magnifier facilitated observation but on some occasions, unintentionally disturbed the couple by moving the twigs and even touching them. The male reacted to this annoyance by shifting a few centimetres onto another twig without interrupting the copulation. At about 8:00 p.m. the twig was cut off and placed in a large glass tube with the couple still in copula for observation in the laboratory; during the transport, at 8:12 p.m., the male extracted his copulatory apparatus, but kept his grip with his mandibles for a few more minutes, the female remained motionless and still hanging loose until the moment of liberation. At the end of copulation both specimens started grooming their bodies from the antennae and soon after began to explore the environment, meeting and then fleeing in the opposite direction. The male spent the night amid the branches while, the female took refuge between the little plant and the glass. The following morning, both lapped ripe grape halves repeatedly and at length; they ignored each other throughout the day, in the afternoon the couple were recorded in the station: the male flew out of the clearing immediately; while the female started to cross it at full speed, but within seconds, she was subject to the harassing attention of five males that chased her all the way into a big and thick Juniper bush, where it was not possible to observe her. The males with their antennae vibrating fast, continued to look for her traces for several minutes going in and coming out of the Juniper.
Some females were captured (10 on the first day and 7 on the second) and

placed in vials closed with tulle and then buried in sand, up to their edge, on two consecutive days; the vials were completely ignored by the males. On the second day only the females that had attracted the males a few moments before, were gradually inserted, without being touched by fingers or tweezers, but this time once again with no positive outcome. The vials and the jars were buried, in a row, at a distance of about 30 cm, in an area illuminated by the sun (5 during the first day and 4 in the second) and in the shaded area of a Juniper (5 during the first day of observation and 3 on the second).
Twice, first in the morning and then in the afternoon, a few grape halves were distributed in the station. Despite the large number of males specimens and females that had transited there, no-one reached them; in captivity, the grapes were much appreciated by both sexes. In the early afternoon, only one specimen of *Pimelia angusticollis* stopped to lick for a few minutes.
During the research two attempts by predaceous larvae of Myrmeleontidae (Neuroptera) at attacking just as many females of *N. v. viduata* were observed; these attacks were almost rectilinear and right under the surface of the sand with within a stretch of 20-25 cm. The first attack was interrupted by the fast and random parting of the female that had backed out from the attentions of a male, but in the second attack the mandibles of the larva and the head emerged from the sand right under the abdomen of the female: both remained perfectly still for ten seconds, then the female moved away undisturbed.
On the last day of permanence in the station (July 8th) all the specimens who had visited it were captured: 72 ♂♂ and 11 ♀♀; 11 males and all the females were detained, while the rest were freed. The specimens captured were arranged according to their sex and placed in glass terrariums for follow-up observations in the laboratory. During the early days of captivity, all the males died except for one (most likely due to air conditioning during transport); the females buried themselves for the night or when the temperature became lower, the males remained almost always uncovered on the layer of sand or among the branches of seedlings and, presumably, suffered due to temperature swings. The male survivor was inserted in the same compartment as the females and also in this case, the encounter between the two sexes was followed by an escape in opposite directions, especially when the meeting was frontal. On July 31st, after more than 20 days of life in captivity, copulation occurred on a grassy support, at around 12 p.m. (copulation was already taking place) and ended at 12:45 p.m.. The two specimens were placed into a smaller container together with the branch for observation under the stereomicroscope. The male was even shifted, using the fingers to hold him by his wings, but indifferent to manipulations, he continued the copula. The mandibles of the male blocked the female squeezing on the propleuron, on both sides. The mating was similar to those observed in nature. The copula took place in a vertical position and when the female was released, she fell with her ventral parts upwards and in this position she groomed her antennae, first one at a time, then simultaneously with both forelegs, then keeping them in a folded position for over a minute; she subsequently turned over and started to explore the substratum. After specimens were stored in the original container, the

female started to dig, but the male licked the honey for several minutes. The next day, an entire inflorescence of *Foeniculum vulgare* was inserted in the container, attracting the male, and within a few seconds he visited many flowers, and also a female, although with little interest. In the following days other inflorescences of *Foeniculum vulgare* were included, and the male licked them promptly.
The specimens preferred the more illuminated side of the terrarium, whenever the terrarium was rotated to 90° or 180°, in a few seconds moving towards the side exposed to light. In the terrarium, the main activity seemed to be, for both sexes, grooming of the antennae, the head, mesosoma, metasoma and legs; males often also groomed the wings, from both sides. The male survivor found shelter in the slot of a rock riddled with holes until the day of death after 63 days from capture. In the last three days, while continuing to feed, he began to lose strength and balance frequently, before he died, he tried to mate grabbing the neck of a female who fought back and in the struggle the male turned on his side remaining there for more than four hours moving the legs and antennae from time to time. When he died, in the same terrarium females of other species caught in Sardinia were included. A female of *Mutilla quinquemaculata* Cyrillus chose and maintained that shelter for nearly a month and a half, until his death after 102 days from capture [however, the longevity record is held by a female of *Dasylabris carinulata maura* (Dalla Torre), that died after 124 days]. More specimens shared the same shelter although belonging to several species and different size.

Vignola Mare (Sardinia), 2011.
During the summer of 2011 observation was also carried out in the station, intentionally, between the end of June and the first week of July, for nine days and for a total of approximately 35 hours (including the mornings and afternoons). The situation in the station, however, had completely changed from the previous year: the number of males on patrol had drastically reduced, both in the station and in the surrounding areas; every day, between morning and afternoon, only 6 or 7 males explored the territory of the station. Despite this, it was possible to observe the same behavioural mode of the previous year between the two sexes and the numerical relation between transactions was proportionally similar to the one in 2010. In nine days only one type of behaviour Bf.4 was observed, immediately followed by nuptial flight: the two specimens, both of small size, separated during the transfer from the butterfly net to vials.
The females moved mainly in two different directions, in apparent correlation with the period of the day: morning to the east and afternoon to the west. These modes are supported by the observations made on four consecutive days, as reported by records of movements made in the morning (Fig. 2a) and in the afternoon (Fig. 2b) on 30th June. Instead it was not possible to verify, if the movements of the males followed a specific direction or if they were random; often, especially in the afternoon, in the surrounding clearings the flight was strongly disturbed by the wind and in those areas there was no migration, detected at other times.
To continue observations in the laboratory, during the last three days 17 males and 12 females were caught in the station, 5 additional males and 9 females in nearby

areas; a male was collected while licking the inflorescences of *Echinophora spinosa*. As during the previous year, the specimens were inserted, separating the two sexes, in terrariums where the habitat of capture was rebuilt and supplied with honey diluted with water and grapes cut in half. During transport to the laboratory the specimens were protected from temperature fluctuations. At the end of the first week only four males survived in spite of the fact that all were fed every day. The male captured during nuptial flight died during transport. On arrival in the laboratory the female was placed in a Petri dish with another small-sized male. After a few minutes, at approximately 3:00 p.m., the male blocked the female with his mandibles and stimulated her several times on her forelegs using his own, but the female rejected mating and reacted with all her legs in order to escape. This process continued uninterruptedly until about 8:30 p.m. when the male released the female. The two specimens remained one at a few millimetres distance from the other and on the following morning they were agonizing and died, one a few minutes after the other. The remaining males lived for between two and three weeks and during this period, whenever they were inserted in the same container with a female, they made several attempts at mating, always followed by avoidance reactions from the females. These observations were made by inserting two specimens of the opposite sex, once in the morning and once in the afternoon, in a Petri dish (60 x 30 mm) equipped with a substrate of paper towelling and a small stone in the centre; alternatively the males or the females were inserted first, and for one hour all behaviours were recorded: from the attempts at mating to the grooming of the body. The latter seems to be the prevailing daily activity for both sexes. There are no substantial differences in grooming techniques betwen sexes (except for wings): the antennae are groomed with the appropriate organ (antenna cleaner or strigilis) located in the proximal end of the basitarsus of the foreleg, while the head (including mouth parts, eyes and ocelli), the mesosoma, the metasoma and wings are groomed by rubbing or scraping with the tarsi and tibial spurs.

Discussion

From the observations, recorded at different times and different places an overview of many ♂♂ and ♀♀ behaviours of *Nemka viduata viduata* can be outlined.

During research in 2010 in Vignola Mare, hundreds and hundreds of encounters between males and females were observed, three couples, two in nuptial flight and one mating. In 2011, still in Vignola Mare, only one nuptial flight was observed. In 22 days of research Alicata et al. (1974) were able to see two matings and in three weeks of study, still with regard to *Nemka viduata viduata*, Nonveiller (1963) reported the observation of a nuptial flight; Tormos et al. (2010) reported the observation of 16 copulations in a total time of 120 days, divided between two summers. The ratio between the number of days of observation and copula-nuptial flight, however, seems to be similar for all the authors compared. Despite

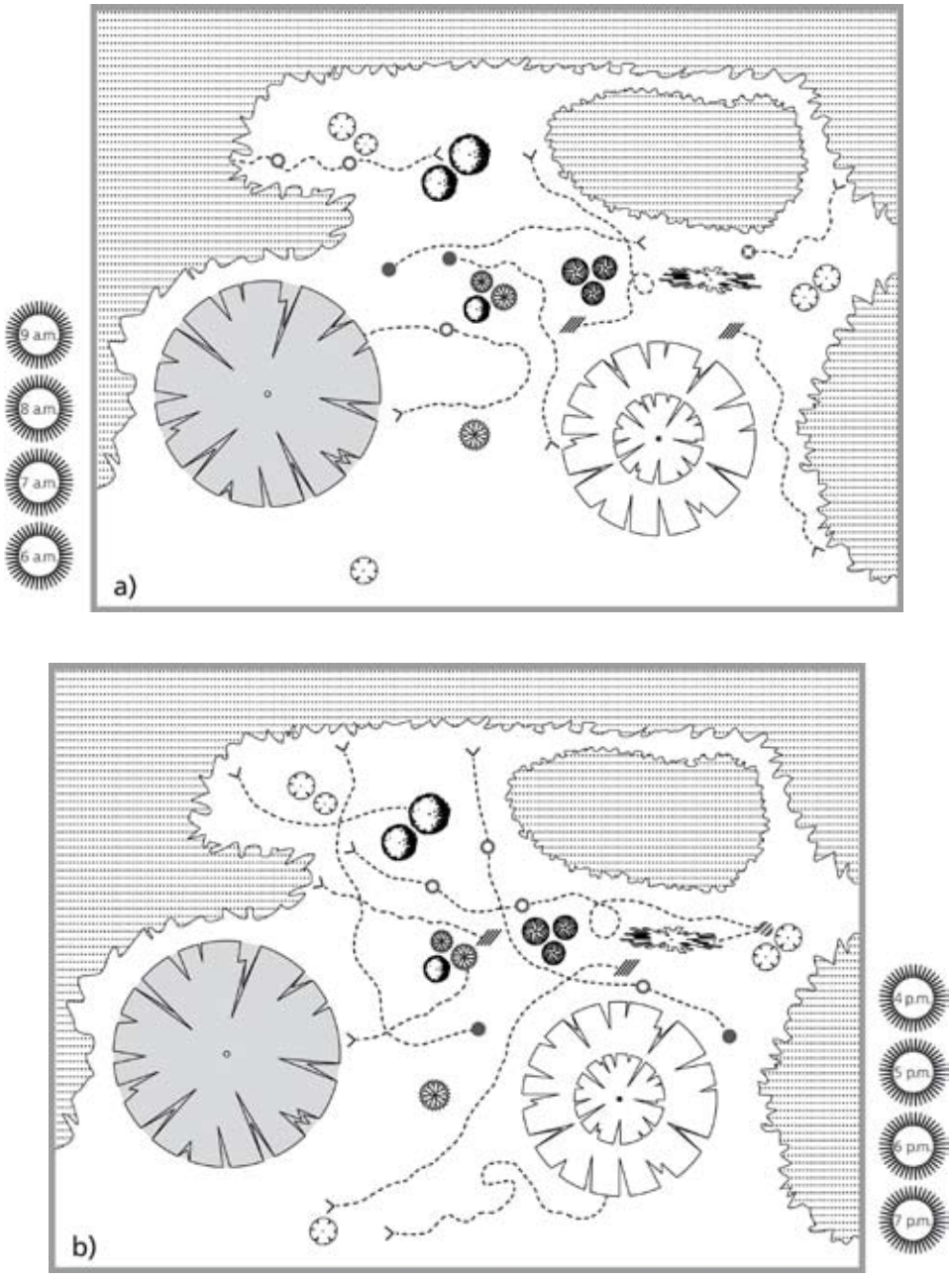
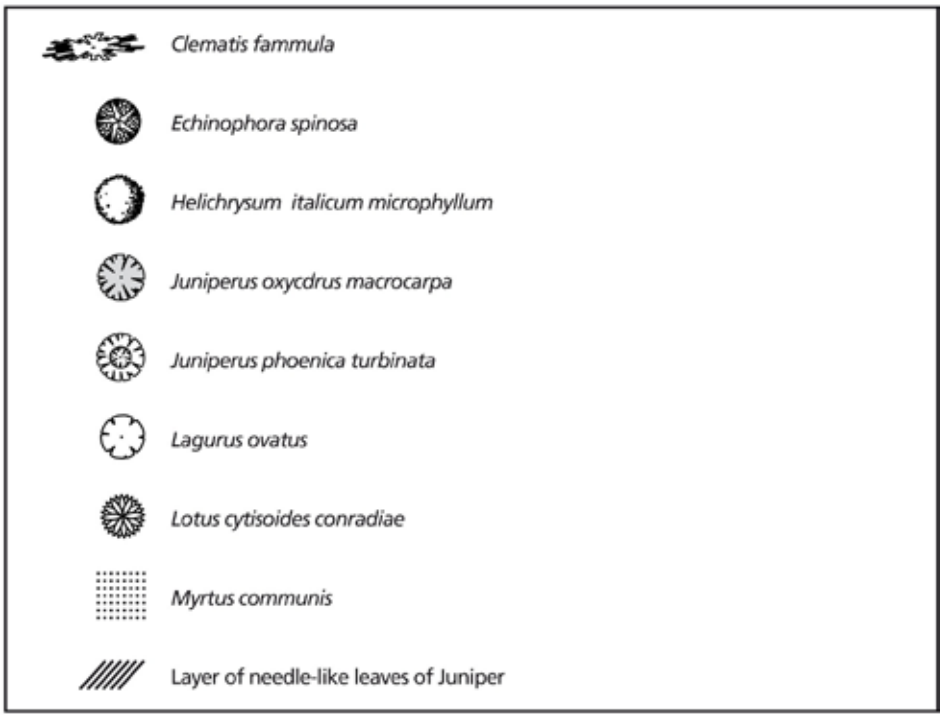
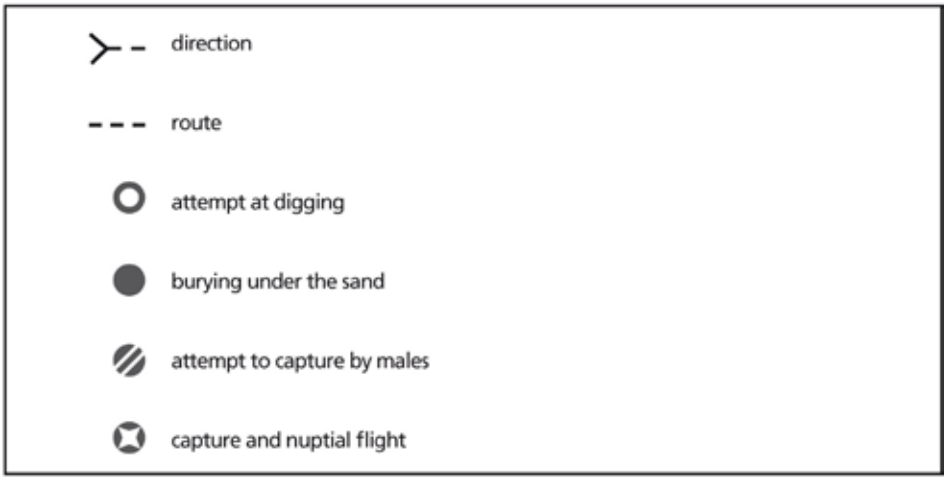


Fig. 2 - Movements of ♀♀ of *Nemka viduata viduata* in station Vignola Mare on 30th June: a) in the morning; b) in the afternoon.



Legend to figs 2a and 2b: Flora present in station Vignola Mare.



Legend to figs 2a and 2b: Activity of ♀♀ of *Nemka viduata viduata* in station Vignola Mare on 30th June.

the large number of males involved in the patrolling in search of females for at least five hours a day and the several days of observation, mating on the premise was observed on few occasions. The capture of 5 couples in nuptial flight and 2 in copula on the substrate, on two consecutive mornings (MATTEINI PALMERINI, 1992), therefore seems an extraordinary event.
The data collected seems to prove that mating depends on the availability of females, surely related to their sexual receptiveness. According to SAUTER & BROWN (2001) this is the most important factor for successful mating and it is due to this behaviour that females have an active role in reproduction (RINGO, 1996). Females surely emit sexual signals to different degrees of intensity, in fact, some of them attract numerous males, but a great part of them does not seem to attract the attention of males (Bm.1), others escape from close encounters (Bf.1) or even oppose attempts at being tackled by males (Bf.2, Bf.3). These females may not be sexually mature and not yet ready to mate, or have already been fecundated. With regard to behaviour Bf.3, it can be assumed that the males did not grab the females at the exact point or were not skillful enough in tactile stimulation or, they simply misinterpreted signals from the females. RINGO (1996) points out that males may try to mate even without receiving the appropriate signal of acceptance; in this case females struggle furiously in order to escape. A hypothesis to explain the attempt by females to free their backs of males refers to a mechanism of sexual selection, a method of testing the sexual fitness of the male, as happens in other groups of insects (eg the Diptera Sepsidae: BAENA & EBERHARD, 2007). Notwithstanding, from the 30 Bf.3 type of avoidance behaviours observed, none of them became a Bf.4, on the contrary, as has already been reported, males were forced to give up and flee, in nature, often flying off precipitously. It was also observed that, at the end of the behavioural mode Bm.2, Bm.3 and sometimes Bm.4 and before resuming their flight and continuing their inspection, males almost always groom their antennae for a long time, probably in order to optimize efficiency in the reception of signals emitted by the female. Avoidance reactions on the part of females may also depend on mechanisms of sexual selection in the choice of the male, as in the documented case of a female captured in Vignola Mare in 2011 and subsequently placed in the laboratory together with another male, chosen by the observer taking into account its size, but refused until death.
The rare cases of acceptance of the male by the female observed (Bf.4) and the large number of males on patrol, show the importance of transferring the female to another place for the success of the copula for this species. The nuptial flight or shifting is almost certainly aimed at defending the potential mating by subtracting the female available from the competition of males that abound in meeting areas. The nuptial flight is made possible, quite blatantly, by the dimensional ratio between male and female, therefore when the ratio is unfavourable for the male, he looks for a safer place for copulation transporting the female under him. The larger size of a male compared to a female seems to be a reproductive advantage, a positive factor in the competition with other smaller males, when subtracting the female during an attempted copulation. The chemical trace released by receptive females seems to stop when the male starts the copula, in fact all the other males

interrupt their search and fly away; with regard to this Filippi (unpubl.) noted that at the moment of copula «males had promptly disappeared».
The observations reported by Alicata et al. (1974) and those mentioned in this paper show that an ongoing mating never interrupts, not even when remarkably disturbed; the male, at the most, seeks a new position by shifting onto herbaceous supports or shifts to another place with longer or shorter flights, never giving up the female or interrupting copulation. The specimens captured in flight in July 1984 (Matteini Palmerini, 1992), to be immediately separated inside the insect net, suggest that they were not yet in copula.
The various and complex behaviours observed by Alicata et al. (1974), Tormos et al. (2010) and by author of pre-copulation and copulation, including their duration and summarized schematically in Table IV, make it difficult to assume that the mating of this species happens in flight, unaffected by the size and proportions between male and female.
While trying to copulate, and especially during copulation, the mandible of the male blocks the female squeezing her propleuron [outer edge of the proepisternum (Reid, 1941)]; in a dorsal view this part presents itself as a groove, quite blatantly correlated with the mechanical action on the part of the locking of mandibles of the male. Referring to the Coleoptera Cicindelidae, Freitag (1974), called «coupling sulcus» a similar structure located between the elytra and prothorax and suggested that this female characteristic may function as a mechanism to isolate the species and/or select the male. In fact if he does not perform a strong hold (with his mandibles), he may be thrown by the reactions of reluctance of the female.
Examining the propleuron of females of *Nemka viduata viduata* and comparing it with that of the species present in Italy that, with certainty, are grabbed from behind their head by males for mating [*Mutilla europaea* (Drewsen, 1847; Hoffer, 1936), *Smicromyrme rufipes* (Crevecoeur, 1930), *Myrmilla calva* (Pagliano, 1983, Monastra, 1990), *Myrmilla erytrocephala* f. *bison* (Monastra, 1990), *Tropidotilla litolaris* (Miceli, 2008), *Pseudomutilla capitata*, *Ronisia barbara* and *Ronisia ghilianii* (Matteini Palmerini, unpublished personal observations)], are all well-characterized and differentiated between them. The males of *N. viduata viduata* have mandibles with three teeth and sharp apex, with a very pronounced tooth at the middle of the outer side, while the mandibles of the females are sickle-shaped, long and sharp. The observations on the field and in the laboratory and what can be read in literature have not singled out diversifed functions of the mandibles between the two sexes, except that males grab females with their mandibles, functionally for the copula. The mandibles of males therefore participate in the mating and represent the secondary sexual structures or non-genitalic mating structures.
The reactions of females to attempts at copula by males were reported by Crevecoeur (1929) for *Smicromyrme rufipes*, while there are no notes for *Nemka viduata viduata*, even though these reactions on the field resulted almost twenty times more frequent than those of Bf.4 type. The latter mode seems to represent the appropriate signal of acceptance and also represents the first sign of pre-copulation visible to the human observer: the behaviour that allows the male to

carry the female (in flight or ambulating) to a more suitable place for continuing the process aimed at copulation.
It was possible to observe the post-copulation behaviour of only two couples, one in nature and one in the laboratory; no aggressive behaviours on the part of the males towards the females were recorded, even in the couple that was kept in the same vial for over 20 hours. In both the copulas observed, the males did not try to further mate with the same female and no aggressive behaviour was noticed, even towards other males in the pre-copulation, at least with regard to the event observed in nature. Filippi (unpubl.), wrote that «males chase females alone or in groups of 3-4, but do not attack each other, on the contrary it seems that there is some sort of collaboration, it seems to be teamwork». Alcock (1979) reported that there may be intraspecific differences in the male reproductive behaviour, both in the same population and in a population separated by space or time. The behavioural differences observed might be determined by a different numerical relationship between sexes in the areas of observation or by the higher or lower number, of sexually receptive females at a certain time, or the intraspecific behavioural differences of males can be linked to the ability to adopt more than one behavioural role (Alcock, 1979).
The females placed in vials and jars did not attract any males, although seven of them had been doing just that a few seconds before capture. Did the laboratory conditions cause females to stop the emission of sexual signals? The female, set free in the station of Vignola Mare, after more than 20 hours from copulation, was immediately subjected to repeated attempts at copulation by other males. This event seems in contrast with what was reported by Brothers (1972), Bayliss & Brothers (2001) and Tormos et al. (2010).
Observations in captivity and paths recorded in the station of Vignola Mare show that the daily wanderings of females are strongly oriented by the position of the sun (positive phototaxis): to the east in the morning and to the west in the afternoon. Alicata et al. (1974) also reported two different directions of daily movement: one in the morning and one in the afternoon; these shifts were attributed to trophic purposes, especially for the search of nectar on the inflorescences of *Echinophora spinosa*.
As reported, the attack by the predator larva of Myrmeleontidae (Neuroptera) was not completed; the larva probably recognized a potential danger in the female of *Nemka viduata viduata*. Alicata et al. (1974) also reported an interrupted attack on a female of *N. viduata viduata* by a larva of Neuroptera which «then fled precipitously going backwards».
18 of the 19 characters of grooming behaviours described for Mutillidae were observed and a further character was noted in other families of Hymenoptera by Baybuyuk & Quicke (1999); furthermore, four characters that had already been highlighted in Mutillidae by Bayliss & Brothers (1996, 2001) were observed, together with two new modalities of grooming behaviours never observed before.
All these characters of grooming behaviours (g.b.) are reported in the form of a matrix where the absence and presence of each character are represented by 0 and 1 (Tab. V).

Table V - Grooming behaviours repertoire.

	Baybuyuk & Quicke, 1999																																	Bayliss & Brothers, 1996 and 2001				Vicini, 1997	new g.b.	
Hymenoptera g.b. repertoire	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Mutillidae g.b. repertoire	1	0	0	0	1	1	1	0	0	1	1	0	1	1	0	1	0	0	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0	1	1	1	1	0	1	1
Nemka v. viduata g.b. repertoire ♂	1	0	0	0	1	1	1	0	0	1	1	0	1	1	0	0	0	0	1	1	1	1	0	1	0	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1
Nemka v. viduata g.b. repertoire ♀	1	0	0	0	1	1	1	0	0	1	1	#	#	#	#	#	#	#	1	1	1	1	0	1	0	1	1	1	1	1	0	1	0	1	#	1	1	0	#	#

Explanation of symbols: (0) absent; (1) present; (#) not applicable.

The antennae are groomed with the special apparatus located near the articulation tibiotarsal articulation of the forelegs: the antenna, respecting laterality, is inserted into the notch and blocked with the movable spur (calcaria) of the distal end of the tibia and then the leg is pushed forward and outwards while the head seems to be pushing in the opposite direction (g.b. 1); the antennae are often groomed simultaneously by both the antennal groomers and the head is pushed backwards (g.b. 5); these two modalities, have frequently been observed in sequence. The tarsi, the tibiae and the mobile spur of the forelegs, groom mouthparts from behind to forward (g.b. 19): with the mandibles open wide, the head, the eyes and the ocelli (g.b. 6); during the grooming the head is rotated to the right and to the left; the tarsi and tibias of the forelegs also groom the upper and lateral part of mesosoma, tegulae and the first part of the wings in the resting position (g.b. 7 and 10). The forelegs are also rubbed against one another, while elongated forward (g.b. 20) or folded under the body; furthermore they groom the sternal area (g.b. 34) and the middle legs, respecting laterality either individually or simultaneously (g.b. 22, 24 and 26): «fore leg-fore leg-middle leg rubbing» (g.b. 21) was not observed. Middle legs then groom hind legs (g.b. 27, 28 and 29) and the latter are groomed by mutual rubbing (g.b. 30). The dorsal and ventral side of the metasoma are groomed with the hind legs (g.b. 11 - Fig. 3); sometimes a foreleg is involved in the grooming of the metasoma which is elongated completely (g.b. 32 - new for

Mutillidae). This g.b., named «fore leg-metasoma-hind leg grooming on ventral» (BAYBUYUK & QUICKE, 1999) and found in Platygastidae, Scelionidae, Bethylidae and Pompilidae, has been observed repeatedly in both sexes: the ventral part of the metasoma is groomed by the hind legs with the help of a foreleg that is fully elongated backwards, and on this, the hind legs remove the debris in the direction of the pygidial area. The hind legs groom themselves onto the foreleg periodically, the body is lifted slightly and titlted in the opposite direction of that of the fore leg that is being used, the balance being mainly supported by the foreleg and the middle contralateral to the fore leg used. Both hind legs participate in the ventral grooming of the metasoma alternating rhythmically with the hind legs (g.b. 36), sometimes a middle leg participates in the lateral grooming of the metasoma (g.b. 37). During the lateral and dorsal grooming of the metasoma with hind legs, the wings, which are normally in resting position, are often parted from the body (g.b. 39 - a new beahviour for Mutillidae and for Hymenoptera - Fig. 4). The hind legs also groom the wings (first the forewings, then the rear ones) which are oriented ventrolaterally to the metasoma and are rotated inwards or outwards in order to groom respectively the dorsal part or the ventral part, with movements of the hind legs which go backwards and from inside to outside. During the grooming a part of the wings is also pushed under the metasoma while the hind legs continue to groom them (g.b. 13, 14) sometimes, the fore wings are pushed outwards, perpendicular to the body, during grooming of the hind wings (g.b. 35 - Fig. 5). The g.b. 16 «ichneumonoid type wing grooming» has not been observed. By a rubbing movement, the wings groom the last sternite while the segments of the metasoma are tilted rhythmically, to the right and to the left (40 g.b. - new for Mutillidae and for Hymenoptera - Fig. 6a, 6b).

Grooming activities may last a few seconds up to several minutes. Some grooming activities take place simultaneously in several parts of the body (as for example those carried out with the fore legs and with the hind legs) while others are sequential (for example the different grooming modalities of the antennae, the head and the mesosoma or the grooming of the body or the wings), most can be observed many times during the day and others more rarely, others still, have only occasionally been recorded, such as g.b. 40.

The antennae grooming apparatus, on the ventral side consists of a semi-circular groove in the proximity of the tibio-tarsal articulation of the forelegs and a movable spur inserted at the end of the tibia. The groove has a smooth surface at the front and on its back it is coated with about one hundred short and tight-locked bristles; the spur instead is characterized by a cuticular velum with a smooth edge terminating in an apex with two rows of thorns: a ventral and an apical one, the latter one with longer thorns than the first. The structure differs considerably between the two sexes. In females the apical and ventral rows bear 6 thorns each (Fig. 7a, 7b), males instead more than twenty tiny thorns on the ventral line and 9 on the apical side of the spur. Furthermore in males the apex is longer in length (Fig. 8a, 8b).

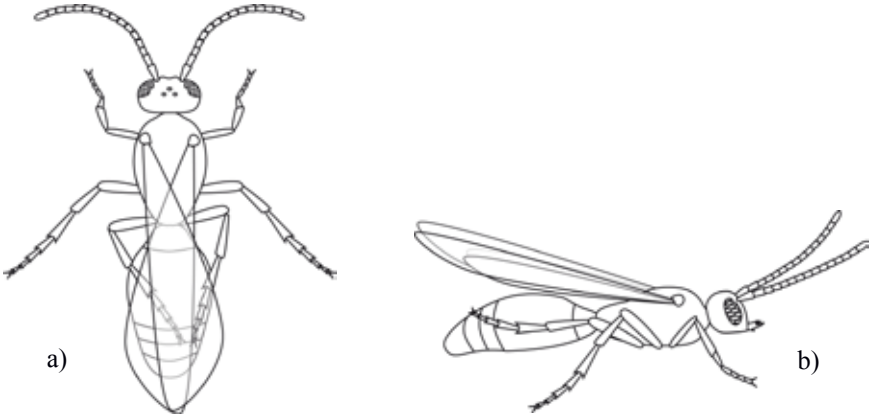


Fig. 3 - Grooming behaviour 11: «hind legs-metasoma», a) dorsal view; b) lateral view.

Fig. 4 - Grooming behaviour 39: «hind legs-metasoma-diverged wings».

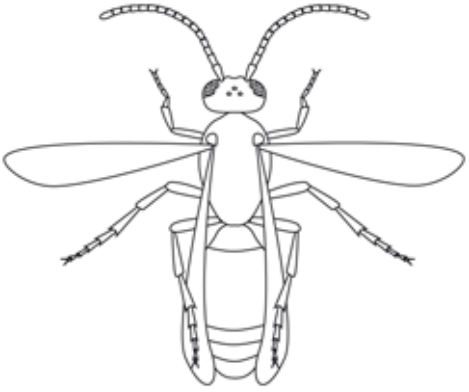


Fig. 5 - Grooming behaviour 36: «hind legs-hindwings, forewings perpendicular».

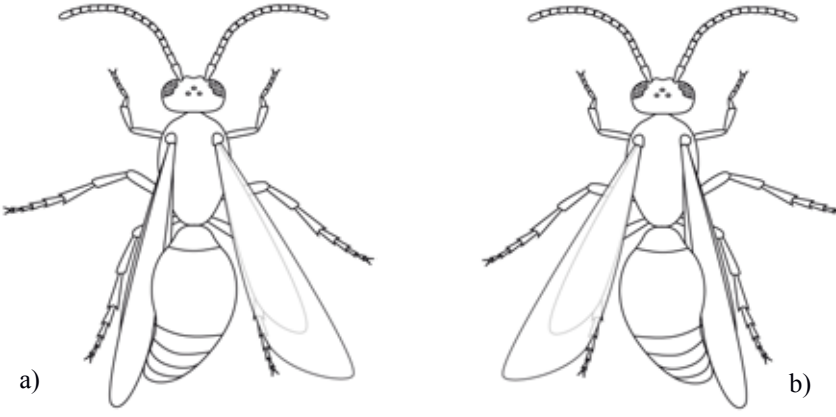


Fig. 6 - Grooming behaviour 40: «wings-last sternite and tergite», a) left cleaning; b) right cleaning.

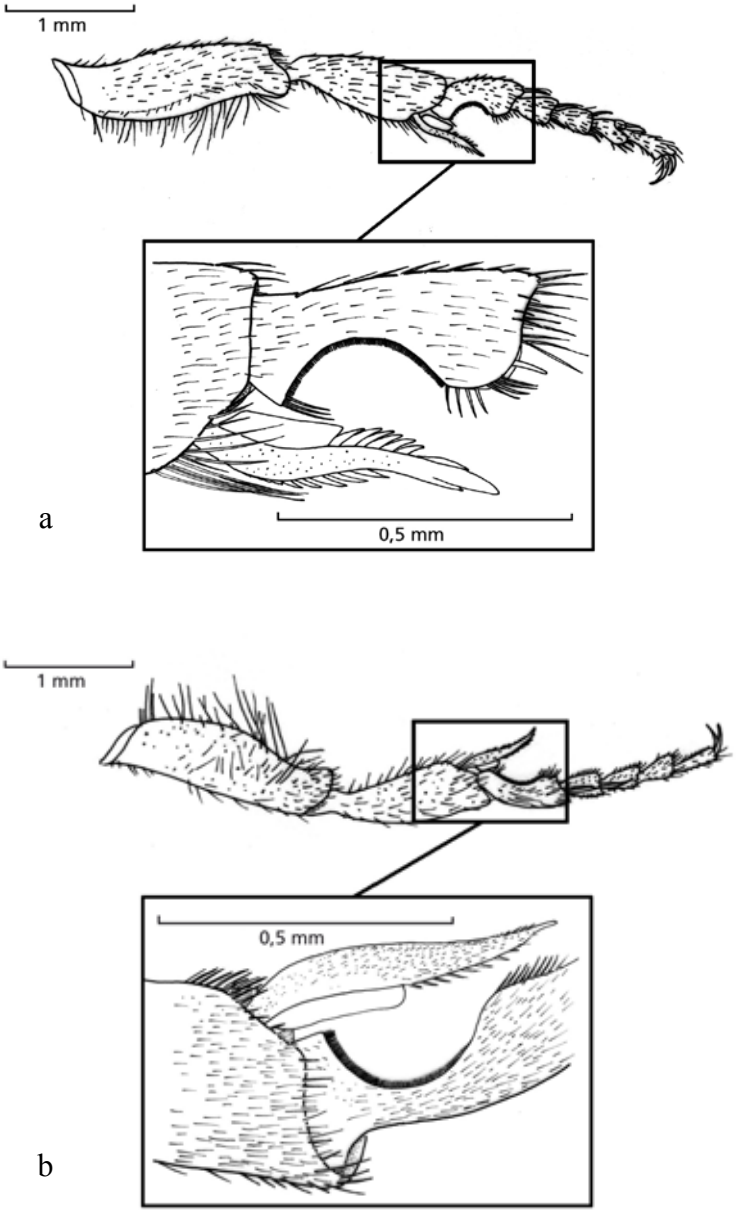


Fig. 7 - Left foreleg and detail of antenna cleaner of *Nemka viduata viduata* ♀: a) from anterior; b) from posterior.

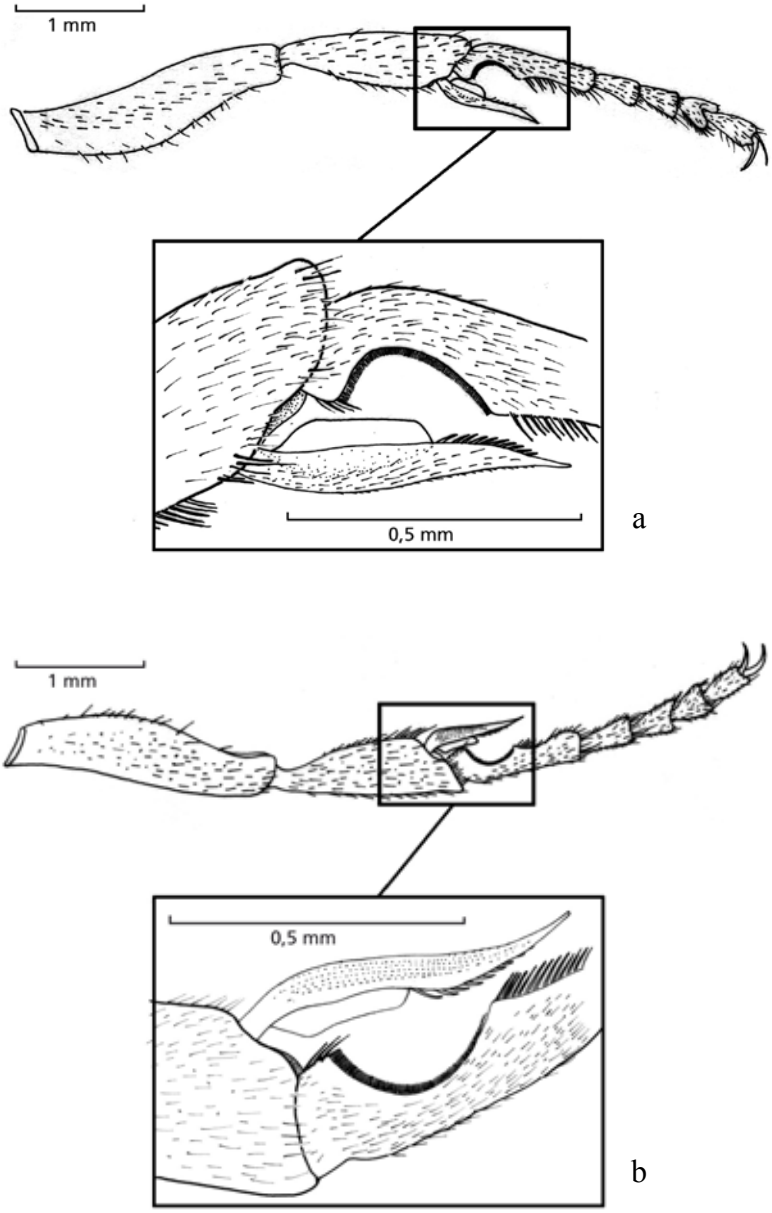


Fig. 8 - Left foreleg and detail of antenna cleaner of *Nemka viduata viduata* ♂ : a) from anterior; b) from posterior.

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