Voracious larvae of the Asian hornet *Vespa velutina nigrithorax* (Hymenoptera: Vespidae)

Michel Asperges & Zoë Vanstraelen

Abstract. In June 2023 several primary nests of the Asian hornet *Vespa velutina nigrithorax* Buysson, 1905 (Hymenoptera: Vespidae) were found in Landen, a municipality in the province of Flemish Brabant. Later, during October and November, secondary nests were found 300 m from the primary nests, the larvae and pupae of which were examined microscopically. A part of their anatomical structure, in particular the head, is described.

Samenvatting. In de loop van de maand juni 2023 werden verscheidene primaire nesten van de Aziatische hoornaar *Vespa velutina nigrithorax* Buysson, 1905 (Hymenoptera: Vespidae) gevonden in Landen, een gemeente in de provincie Vlaams-Brabant. Later, in de loop van oktober en november werden secundaire nesten gevonden op 300 m van de primaire nesten. De larven en poppen van de hoornaars werden microscopisch onderzocht. Een deel van hun anatomische bouw, meer bepaald de kop wordt beschreven.

Résumé. Au cours du mois de juin 2023 plusieurs nids primaires de frelons asiatiques *Vespa velutina nigrithorax* Buysson, 1905 (Hymenoptera: Vespidae) ont été découverts à Landen, une commune de la province du Brabant flamand. Plus tard, aux mois d'octobre et de novembre, des nids secondaires ont été trouvés à 300 m des nids primaires. Les larves et les pupes de frelons ont été examinées au microscope. Une partie de leur structure anatomique, plus spécialement la tête, a été décrite.

Key words: Invasive species — Morphology — Predatory behaviour — Hymenoptera — Wasp — Belgium.

Asperges M.: Universiteit Hasselt, Agoralaan gebouw D, 3590 Diepenbeek, Belgium. asperges.michel@uhasselt.be

Vanstraelen Z.: Bezoekerscentrum Lieteberg, Zuurbroekstraat 16, 3690 Zutendaal, Belgium. zoe@lieteberg.be

DOI: https://doi.org/10.6084/m9.figshare.26385460

Life cycle

The Asian hornet has an annual life cycle. The queen mates in the autumn and then goes into hibernation. In spring, she founds a new colony. Nest construction and protection, food collection and nursing the brood, the queen carries out all these tasks until the emergence of the first workers. The nest which the queen builds is known as a primary nest. With the production of workers, the colony develops rapidly. When the nest of the colony becomes too cramped, thereby hindering the expansion of the nest, workers begin to search for a new site in the vicinity. When a suitable site is found workers settle at the site. After a few days, the queen leaves the primary nest and the workers and brood in it. She checks out potential candidate locations and settles on the most favourable site. The workers at the queen-chosen site start constructing a new nest (secondary nest). Startled by the absence of their queen, the workers in the primary nest go searching for their queen, while workers from the secondary nest return to the primary nest to take care of the brood. The relocation of the nest usually lasts for a month that is until all the eggs larvae and pupae have matured and migrated to the secondary nest. The queen never returns to the primary nest. Between late summer and autumn reproductive individuals are produced, and the queen dies. The new queens and drones leave the nest. Young fecundated queens search for a suitable place to hibernate and the workers will die.

Primary nest

All primary nests were found between the beginning of June and the end of July. At the beginning of June, the start of a primary nest was reported and destroyed in Ezemaal-Landen (province Flemish Brabant). On July 9th, a primary nest with three paper combs containing larvae, pupae and hornets was observed in a bird's nest box and wall cavities (Fig. 1). In the same period, four primary nests were destroyed in Neerwinden-Landen: one in a chimney that was cleared by the fire brigade and the other three in cavity walls. The residents of a house in Ezemaal only noticed the presence of Asian hornets in their garden when the hornets suddenly attacked them. The Asian hornets had occupied a birds' nest box (Fig. 2a) near a place they passed several times a day. Fig. 2b shows an ear 2 minutes after a painful sting. At the same location, a new nest was found in an air brick of a wall cavity on 11th July. On the 22nd of June, a primary nest was found in an old woodpecker's nest hole in an old, dead willow (*Salix*). Both colonies were destroyed.

In the primary nests, we did not find the queens of the colonies. So, they were either dead or they had already started secondary nests. They could not have escaped during the extermination. In addition to workers, we also found drones in the primary nest. These so-called early males have been described in several vespine wasp species and are, in most cases, a consequence of inbreeding (Darrouzet *et al.* 2015; Budge *et al.* 2017).

Genetic research on these early males revealed that most of them are diploid (Arca 2012; Darrouzet *et al.* 2015). They develop from fertilised eggs giving them two sets of chromosomes in their cells, while males produced in the reproductive period are haploid. Haploid males develop from unfertilised eggs, therefore having only a maternal copy of the chromosomes in their cells. Multiple genes at a single locus typically determine sex in the Asian hornet as well as in many other wasps. Sex locus heterozygotes develop as diploid females, hemizygotes as haploid males and homozygotes as diploid males (Darrouzet *et al*. 2015). Inbreeding increases the chance of homozygosity at the sex locus.

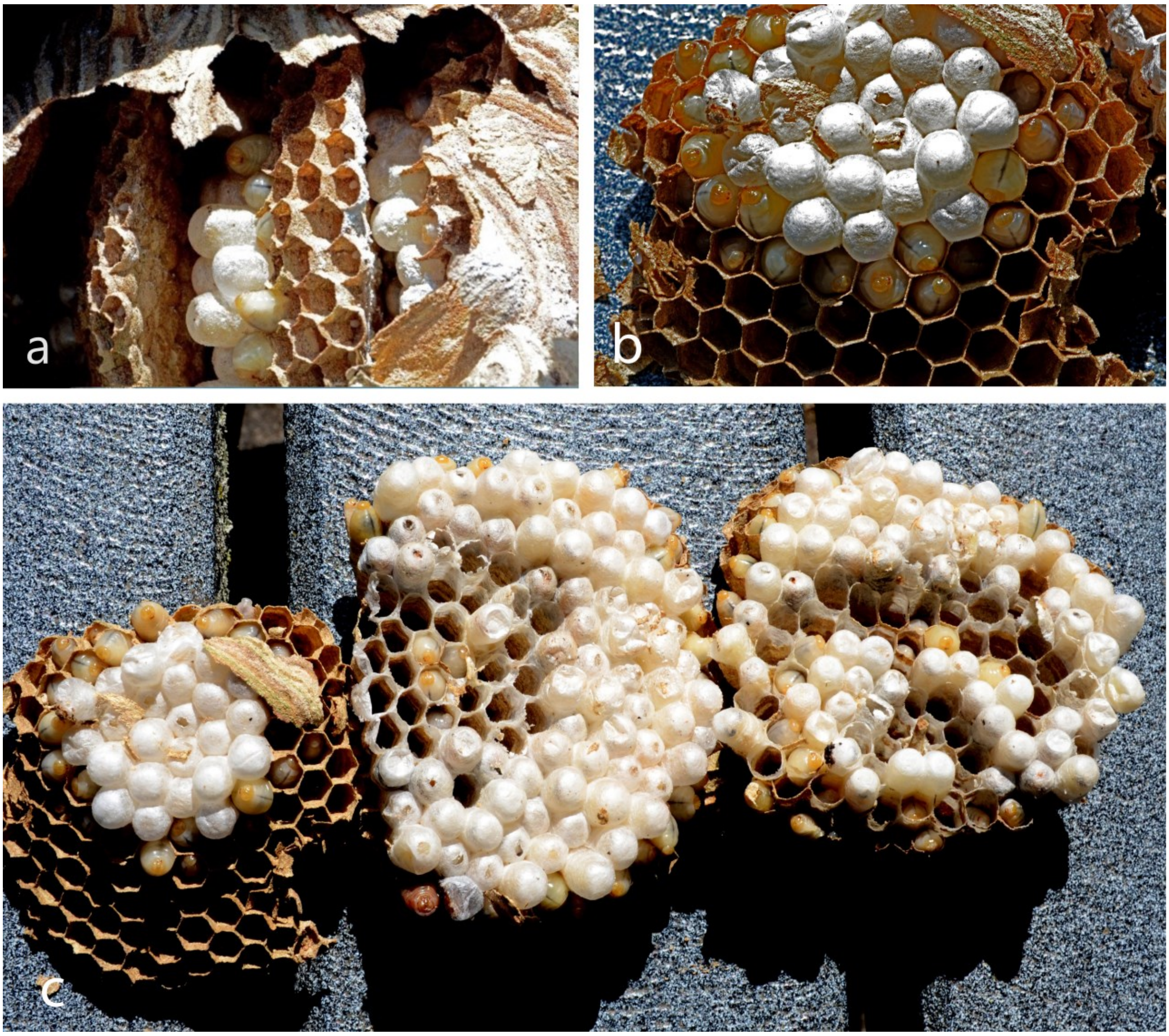

Fig. 1. The primary nest found on July 9; a, the multi-layered outer shell covering the combs; b, a more detailed photo of the larvae and pupae; c, the three paper combs of the nest. © Michel Asperges.

Fig. 2. a, a primary nest in a bird nest box; b, an ear two minutes after a painful sting. © Michel Asperges.

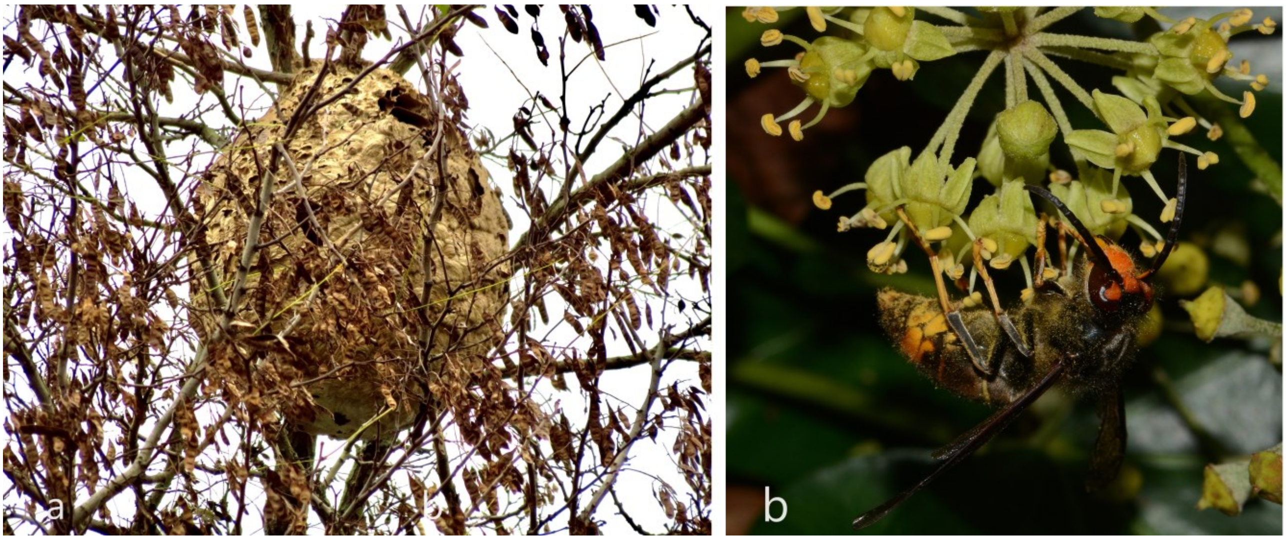

Fig. 3. a, a secondary nest in a black locust (*Robinia pseudoacacia*); b, an Asian hornet on a flower of ivy (*Hedera helix*). © Michel Asperges.

The vast majority of diploid males are sterile, inviable or father sterile triploid female progeny (Heimpel & de Boer 2008). The result is a fitness cost to their parents, the females they mate with, and an additional cost: diploid males, who do not contribute to the colony productivity, are produced at the cost of female workers. Although the populations of the Asian hornet in Europe exhibit a strong founder effect, this genetic impoverishment did not preclude the successful population establishment and spread of the species. Two conditions increase the genetic variability of the Asian hornet: the introduction of gynes from genetically distinct populations and multiple mating by the gynes (Otis *et al.* 2023). Gynes of the Asian hornet mate on average with more males than other Vespine wasps to reduce the likelihood of producing diploid males (Darrouzet *et al.* 2015; Herrera *et al.* 2023).

We ask ourselves what the reproductive function of these early males is and if they are involved in alternative reproductive strategies. Insight into the genetics and the pathways of introduction of the Asian Hornet can greatly benefit the development of effective management.

Secondary nests

At the end of October we found, at about 300 m from a primary nest, a secondary nest in a pine (*Pinus sylvestris* L.) and another on the top of a black locust (*Robinia pseudoacacia* L.) at the beginning of November (Fig. 3a) again at 300 m from a primary nest. At the same time, several hornets were seen nectar feeding on ivy (*Hedera helix* L.) (Fig. 3b).

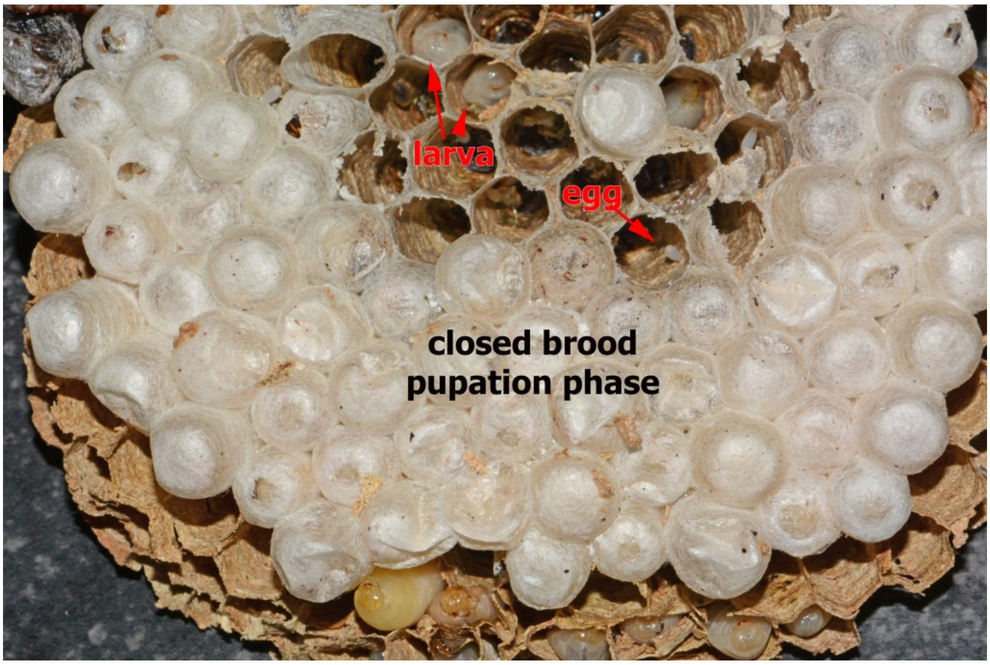

Fig. 4. Different stages in the development: egg, larvae, and pupae as closed brood. © Michel Asperges.

ISSN 0771-5277

Fig. 5. The transformation of a matured larva to pupa. © Michel Asperges.

Observations of larvae and pupae in a primary nest

Before the destruction of the primary nest in a birds' nest box, the Asian hornets were removed from the nest with an insect net. The combs contained pupae of different ages (Fig. 4) and quite a lot of larvae. Several larvae were collected, and the others were offered to tits on a feeding shelf; but strangely enough, the birds did not pick them up. The thick, fleshy larvae are white to off-white and are stretched out in the cells. (Fig. 6). The larvae protrude with their heads slightly above the surface of the cells and they constantly beg for food while turning their heads. Once out of the cells, they curve their bodies. Strikingly, on the head are two brownish-yellow points next to the mouth opening. The intestinal system is apparent on the ventral side. The intestinal system is coloured black by the food within it. When the larvae pupate, the black intestinal content is no longer visible. Very fine white channels, the tracheae, run all over the body of the larvae. At the stigmata there are two very short projections that are used to attach themselves to the paper cell after spinning a cocoon.

Fig. 6. White adult larvae with their creamy white heads, reddish brown points that mark the mandibles and the filled intestine. © Michel Asperges.

Fig. 7. a, An overview of the mouth with the mandibles and a salivary gland 10×4; b, a detail of the mouth, suction throat (pharynx) and jaws with part of a salivary gland in the front 10×40; c, a detail of part of a salivary gland 10×60. © Michel Asperges.

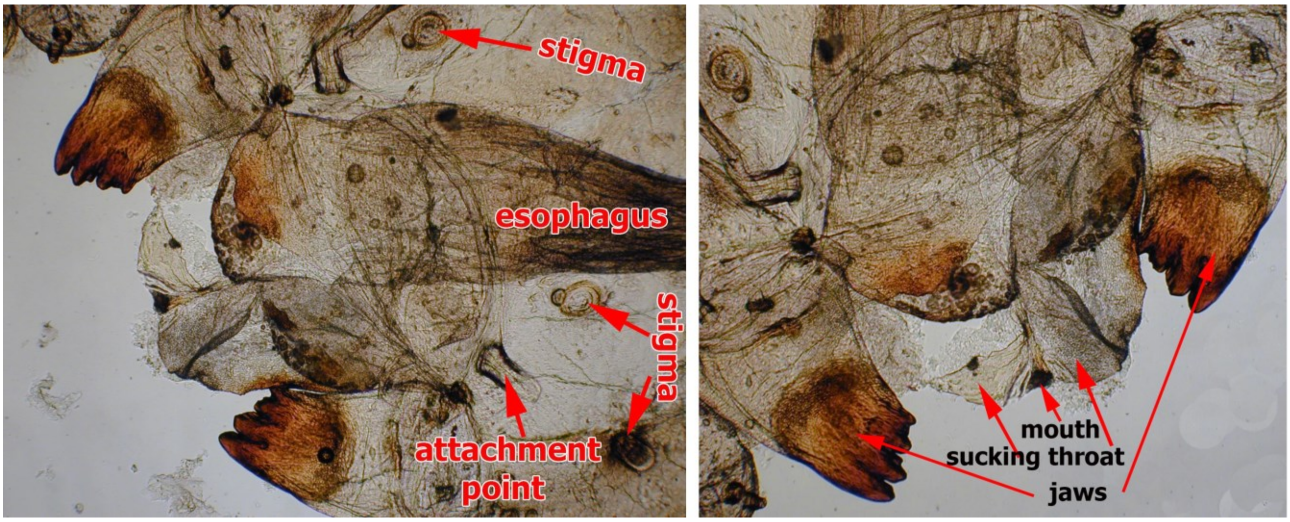

Fig. 8. An overview of the suction throat (pharynx) with the oesophagus, the attachments points and the three stigmata 10×10. © Michel Asperges.

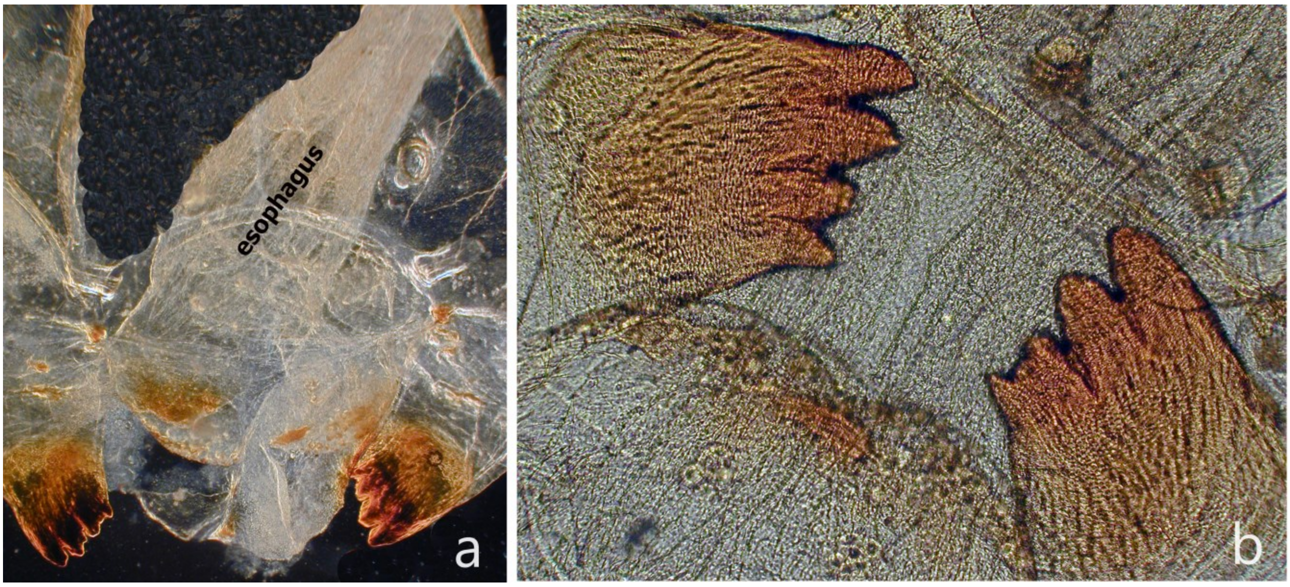

Fig. 9. a, The mouth with the mandibles and the pharynx with the oesophagus 10×10 dark field; b, a detail of the mandibles and part of the pharynx 10×20. © Michel Asperges.

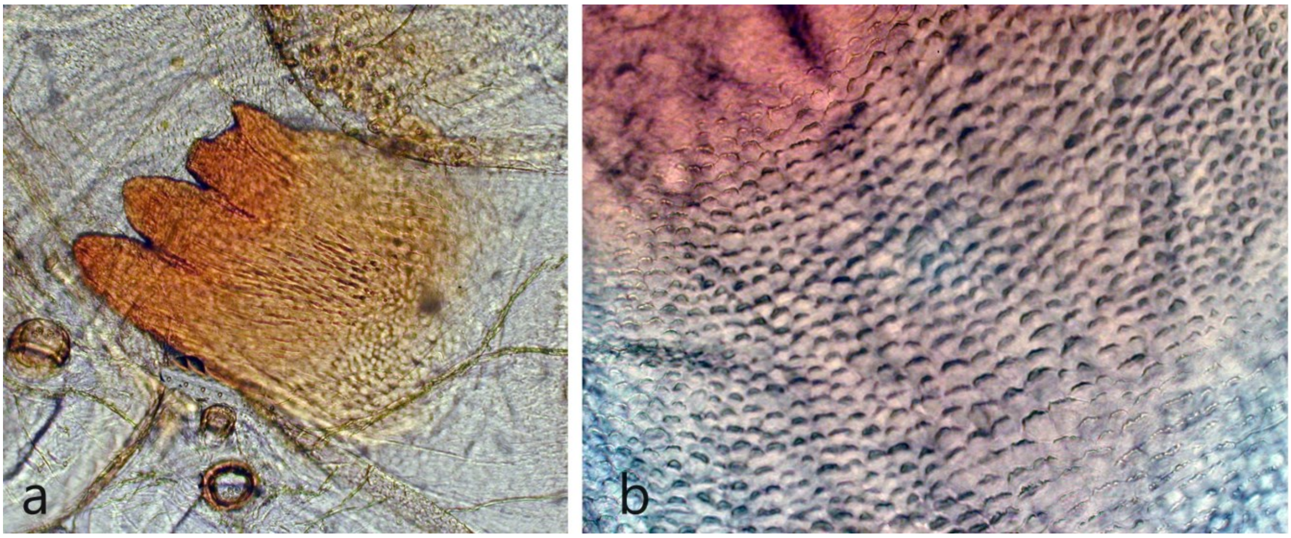

Fig. 10. a, The mandibles with four teeth, at the bottom the circular opening of a stigma 10×40; b, a detail of the scaly surface of the mandibles 10×60. © Michel Asperges.

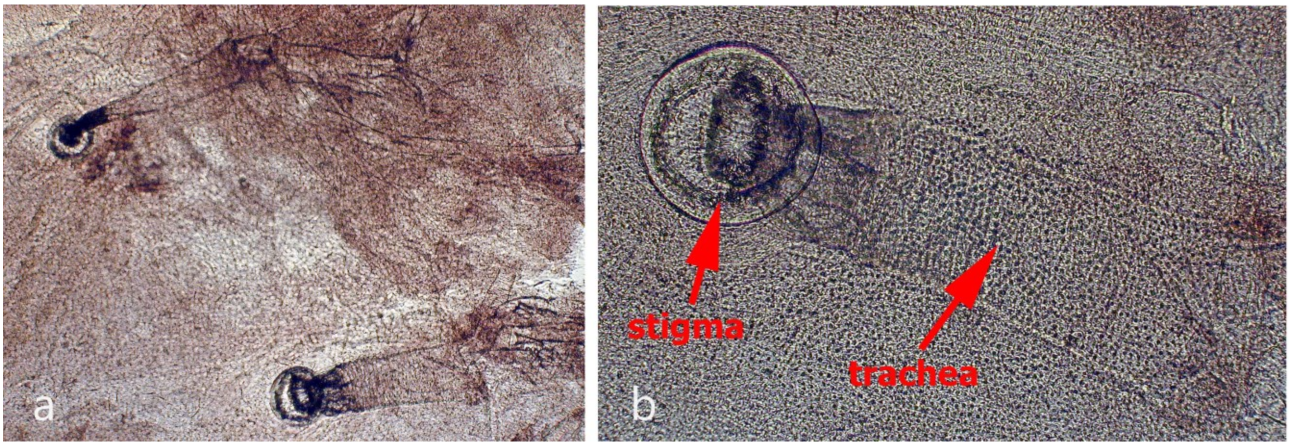

Fig. 11. Two stigmata with their trachea; a, 10×40; b, 10×60. © Michel Asperges.

Microscopic observations of the mouthparts of larvae

After establishing that tits did not want to pick up the live larvae to give to their young, we were curious about the reason for this behaviour.

Preparation: To study insects microscopically, the chitinous cuticle has to be made more or less transparent. We do this by placing them in a 10% KOH solution. After a day, the brown solution is replaced, and we repeat this until the insects have cleared up sufficiently. However, the larvae of the Asian hornet turned black and not transparent. The young pupae remained white but did not become transparent either. The larvae have little chitin in their skin, in contrast to the pupae. For the microscopic observations, we used an Olympus CH2 and a Zeiss Primo Star, objectives with magnifications of 10×, 40× and 60× and an eyepiece with magnification of 10x. Sometimes dark field was used to get a clearer image.

Observations (Figs 7, 8, 9 & 10): A crush preparation (with water) of the larvae makes it clear that the larvae have quite large mandibles with four teeth with which they chew the food (muscle mass) offered to them by the workers. Furthermore, the large sucking throat (pharynx), located centrally between the mandibles and surrounded by salivary glands, is striking. The salivary glands consist of a series of nicely arranged, egg-shaped glandular cells. These produce enzymes that break down proteins in the food. The suction throat (pharynx) is quite large and bag-shaped and merges into the actual small oesophagus. Two lateral attachment points hold the pharynx. Next to the oesophagus, we found two stigmata.

The females of the Asian hornet catch insects with rather thick flight muscles, such as honeybees and hoverflies. They bite the thorax with the thick flight muscles into pieces and give them to the larvae, which grind them further and mix them with salivary enzymes. This protein-rich food is essential for the development of the larvae. We rarely see, except in autumn, hornets foraging on sugary food such as nectar or ripe fruits, but this is also important for the development of the larvae. Beekeepers who use traps to catch hornets use liquid, sugar-rich substances.

The surface of the four or five-toothed mandibles is not smooth but scaly, which gives them a better grip on the food offered. The mandibles with which they bite are the reason that the tits did not want to pick up the live

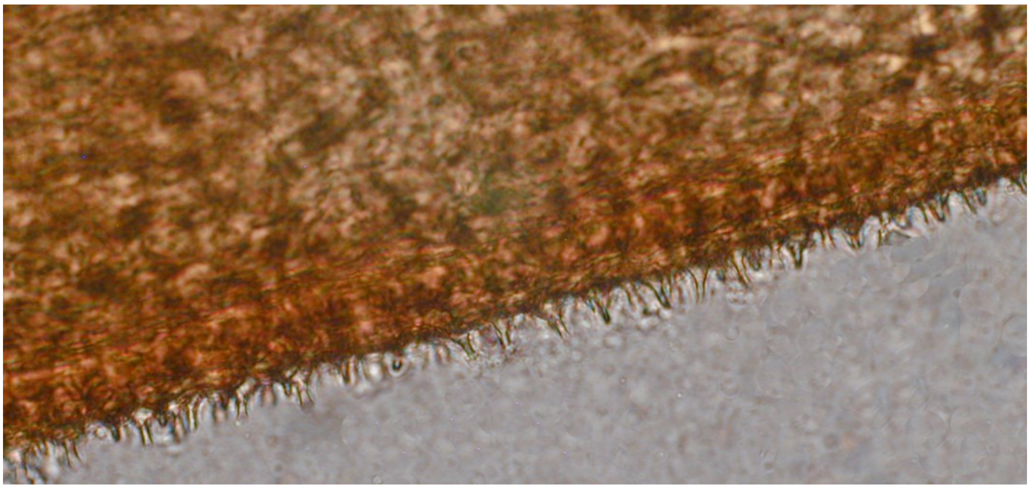

Fig. 12. Part of the skin with chitin spines 10×40 dark field. © Michel Asperges.

larvae. Only after a few days, when the larvae are dead, the tits extracted this protein-rich food from the combs. Throughout the body of the larvae there are many round openings of the stigmata that are connected to the trachea. Remarkably, the tracheae were tubular without spiral chitinous reinforcements (Fig. 11).

It was also noticeable that the tits had to pull quite hard to get the larvae out of the cells. Usually, the larvae turned around and were taken in pieces. What then was the cause? Microscopically it became clear that the thin chitinous cuticle is completely covered with very small chitin spines (Fig. 12). As a result, the larvae stand nicely upright in the comb cells, but it is difficult to pull them out.

Acknowledgments

We would like to thank Mrs. Mieke Van den Wijngaert for rereading and correcting the text. Finally, a word of thanks to Roeland Vaes for updating the text and numbering of the figures so that they became didactically readable.

References

Arca M. A. 2012. Caractérisation génétique et étude comportementale d'une espèce envahissante en France: *Vespa velutina* Lepeletier (Hymenoptera, Vespidae). — PhD thesis, University Pierre et Marie Curie, Paris, 106–111.

Budge G. E., Hodgetts J., Jones E. P., Ostojá-Starzewski J. C., Hall J., Tomkies V., Semmence N., Brown M., Wakefield M. & Stainton K. 2017. The invasion, provenance and diversity of *Vespa velutina* Lepeletier (Hymenoptera: Vespidae) in Great Britain. — *PLoS One*, **12**(9): e0185172. https://doi.org/10.1371/journal.pone.0185172

Darrouzet E., Gévar J., Guignard Q., & Aron S. 2015. Production of early diploid males by European colonies of the invasive hornet *Vespa velutina nigrithorax*. — *PLoS One*, **10**(9): e0136680. https://doi.org/10.1371/journal.pone.0136680

Heimpel G. E. & De Boer J. G. 2008. Sex determination in the Hymenoptera. — *Annual Review of Entomology* **53**: 209–230.

Herrera C., Ferragut J. F., Leza M. & Jurado-Rivera J. A. 2023. Invasion genetics of the yellow-legged hornet *Vespa velutina* in the Westernmost Mediterranean archipelago. *Journal of Pest Science*: https://doi.org/10.1007/s10340-023-01680-y

Otis G. W., Taylor B. A., & Mattila H. R. 2023. Invasion potential of hornets (Hymenoptera: Vespidae: *Vespa* spp.). — *Frontiers in Insect Science* **3**: https://doi.org/10.3389/finsc.2023.1145158

Received: 28 December 2023
Accepted: 09 January 2024