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# Behaviour, biology and morphology of *Stephanus serrator* (FABRICIUS, 1798) (Hymenoptera: Stephanidae)

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**Abstract.** *Stephanus serrator* (F.) was observed for several years in situ on a woodpile and in captivity. The host-finding behaviour of females, which is linked to a characteristic posture, and the highly similar behaviour of males during the hatching of females are described. Mating of *S. serrator* is documented for the first time. Intraspecific aggressive behaviour was observed in both sexes. It was attempted to document the actual duration of the parasitization process. The present observations suggest that *S. serrator* is able to repeatedly locate the same drill hole. A new host, *Clytus arietis* (L.), is mentioned. Egg and pupa of *S. serrator* are illustrated for the first time. It is attempted to relate the host-finding behaviour of females to the sensoric features of the ovipositor, sheath, legs and antennae.

**Zusammenfassung.** *Stephanus serrator* (F.) wurde über mehrere Jahre im Freiland an einem Holzstoß und in Gefangenschaft beobachtet. Das Wirtfindungsverhalten der Weibchen, das mit einer charakteristischen Körperhaltung verbunden ist und das ganz ähnliche Verhalten der Männchen während der Schlüpfphase der Weibchen werden beschrieben. Paarungen von *S. serrator* werden erstmals dokumentiert. Bei beiden Geschlechtern wurde intraspezifisches Aggressionsverhalten beobachtet. Es wurde versucht, die tatsächliche Dauer des Parasitierungsprozesses zu dokumentieren. Die Untersuchungen deuten darauf hin, dass *S. serrator* imstande ist, ein bereits existierendes Bohrloch wiederzufinden. Ein neuer Wirt, *Clytus arietis* (L.), wird genannt. Ei und Puppe von *S. serrator* werden erstmals abgebildet. Es wird versucht, einen Zusammenhang zwischen dem Wirtfindungsverhalten der Weibchen und der sensorischen Ausstattung des Ovipositors, der Legebohrerscheide, der Beine und der Antennen herzustellen.

**Key words.** *Stephanus serrator*, host-finding process, host larvae, mating, drilling activities, ultramorphology, Styria, Austria.

## 1. Introduction

Most species of the family Stephanidae inhabit the tropics and subtropics. In the Palearctic region, including China and Japan, a total of 19 species exist (AGUIAR 2004). In Europe four species, *Stephanus serrator* (FABRICIUS), *Megischus anomalipes* (FÖRSTER), *Afromegischus gigas* (SCHLETTERER) and *Foenatopus turcomanorum* SEMENOW are known (MADL & SCHWARZ 2014). While *M. anomalipes* is distributed from the Mediterranean to the Middle East, *A. gigas* and *F. turcomanorum* are only known from Kreta. In Austria so far only the central and southern European species *S. serrator* has been found.

The biology and behavioural mechanisms of stephanid wasps are largely unknown. Most information on the life history and ontogeny of the Stephanidae (RODD 1951, TAYLOR 1967) concern *Parastephanellus* sp. (cf. VILHELMSEN et al. 2008) in Australia and the Nearctic species *Schlettererius cinctipes* (CRESSON) which characterize the group as idiobiont ectoparasitoides of wood-boring insect larvae. Hosts include species from the families Cerambycidae and Buprestidae, in addition to some other beetles, Symphyta (Siricidae) and solitary bees (AGUIAR 2006). For *S. serrator,* at present, the following cerambycid beetles have been listed as host species: *Xylotrechus arvicola* (OL.) (BLÜTHGEN 1953), *Pogonocherus eugeniae* GANGLB. (PAGLIANO 1986), *Rhopalopus femoratus* (L.), *R. macropus* (GERMAR) (LUKAS 1989), *Saperda similis* LAICHARTING (GEORGIEV et al. 2004) and *Xylotrechus antilope* (SCHÖNHERR) (P. Svacha, pers. comm.).

Few data on the behaviour of *S. serrator* exist. Only JANSEN et al. (1988) and REDER (2011), while discussing the species's distribution in Germany, report some occassional observations on host detection and drilling activity. A more detailed description of the drilling behaviour of *S. serrator* was published by BLÜTHGEN (1953).

## 2. Materials and methods

For the present long-term study of the reproductive biology and behaviour of *S. serrator* the Universalmuseum Joanneum Graz provided a stack of well-seasoned firewood in Tyrnau (650 m a.s.l.), near Frohnleiten, Styria, Austria which consisted of mid-sized and smaller corticated branches of maple (*Acer* sp.) and European beech (*Fagus sylvatica*) with diameters usually ≤ 10 cm or ≤ 6 cm (Fig. 1). The social and drilling behaviour of the species at the stapel was investigated in situ during summer (June–August) from 2008-2011.

Additionally, the behaviour of caged individuals was studied in a 61 x 33 x 51 cm large terrarium in the first author's flat in the city of Graz and in the field with the help of a smaller, transportable cage (30 x 20 x 20 cm). To provide breeding substrates, branches of firewood from the stack in Tyrnau were put into the larger terrarium. For studying population numbers, life span and behaviour, free-ranging as well as caged individuals were colour-marked on the thorax.

During drilling activities in the terrarium some injection sites were marked nearby. Potential host larvae within the marked wood were collected after the insects had been drilling for several hours by sawing out and carefully splitting the respective section of the branch. Immediatley following extraction, larvae were conserved in 70 % alcohol and passed on to Petr Svacha (Czech Republic) for identification of the host species.

The observations of living wasps were made by the first author and documented by digital photography and video footage.

In addition, sensory adaptations of the species were studied by preparing electronic-microscopic photographs (second author) of the shaft and sheath of the ovipositor, antennae and all legs (tibiae and tarsi) of a dried female specimen. The samples were mounted on an Al sample holder with a double-sided adhesive conductive carbon tape and gold-coated. Secondary electron (SE) images were done on a Jeol 6610 LV electron microscope equipped with an Oxford Instruments 50 mm$^2$ energy dispersive detector at 10 kV accelerating voltage. Additionally the content of heavy metals in the ovipositor was studied. Elemental mapping was done with 20 kV and a beam current of 30 nA.

*Fig. 1:* Stack of wood, Tyrnau, Frohnleiten, 650 m, Styria, Austria.

# 3. Results

## 3.1 Behaviour

### 3.1.1 Behaviour on the woodpile

Despite being well capable of flying, the insects generally moved on the substrate by crawling or rarely performing short “jump flights” between adjacent branches. Before jumping their bodies moved back and forth for some while. Females were usually busy tracing host larvae, they moved slowly and only for a few centimeters across the wood before pausing and freezing in their characteristic posture. Apart from this search behaviour also quickly running individuals with alternatingly moving, erect antennae at different parts of the woodpile were observed. After drilling, the females rested for a longer period of time. Males moved by crawling slowly with a bobbing abdomen and also rested frequently. They were more agitated when several males came together.

Most individuals of *S. serrator* observed in situ avoided direct sunshine and preferably stayed in shady parts of the woodpile. On a cool and overcast day more than 40 males were observed on the visible parts of the woodpile.

### 3.1.2 Mating

Mating did not take place immediately after the insects hatched in the terrarium. Freshly hatched individuals showed no interest in one another.

Mating was observed only once on the woodpile (24.6.2010). Further matings only were seen in a terrarium that had been brought to a field trip and contained recently caught females and males. They had been mating for several minutes after being put into the terrarium (14.6. and 24.6.2010). No preceding mating rituals were observed. In fact, the approach was rather sudden: the male quickly ran up to the female and mounted it. During mating the ovipositor of the female was held vertically upwards or sideways. The couple had not been moving for several minutes when the female started walking and the male dropped off backwards. The male remained in that position with angled legs and was dragged along by the female. In some cases it even hung down vertically from the body of the female (Fig. 2a). When mating was over, the male was dropped and fell to the ground. In another mating the couple positioned their bodies away from each other (Fig. 2b). Matings were observed both with larger males and dwarf males and lasted between 5 and 25 minutes. Both partners performed cleaning movements while mating and the abdomen of the female showed conspicuous pumping motions. Females that were unwilling to mate and were hassled by males fended off the approaches by raising their abdomen, kicking with mid and hind legs or quickly running to-

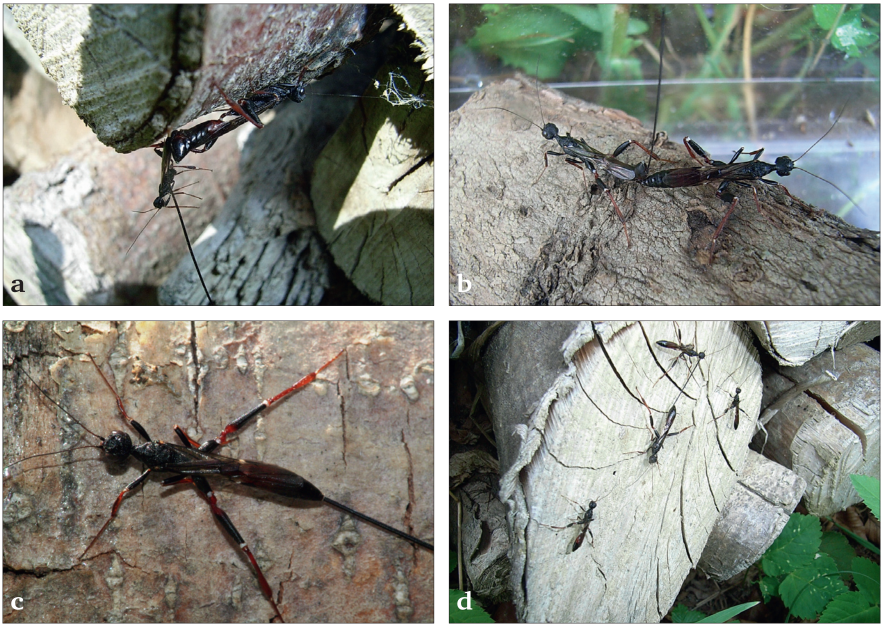

*Fig. 2:* (a) Mating between a female and a dwarf male, field observation; (b) mating, observation in portable terrarium; (c) search position of the female at the breeding habitat, field observation; (d) search position of three males and one female at the breeding habitat, field observation.

wards them. Sometimes females were even repeatedly hassled by males while they were drilling.

### 3.1.3 Host-finding behaviour in females

The females slowly walked across the wood and paused frequently, then the hind legs were spread sideways in an angle greater than 110°, the mid legs were tightened to the body, the fore legs were stretched diagonally forward and the tip of the sheath was pressed onto the wood following repeated movement of the abdomen (Fig. 2c). The female stayed in that position without moving for several seconds. Afterwards, this process was repeated a few centimeters away from the initial place. In this search position, the antennae were lowered either to the side or in front of the head until the tips were in contact with the wood surface. The antennae tips were not observed beating the substrate.

Once a potential oviposition location was found, the female barely moved off of it. At most, the insect turned 180°, got in the search position described above and eventually started drilling.

On several branches of the woodpile, nests of wild bees (*Chelostoma* sp.), closed with loam, were present. Neither search behaviour of *S. serrator* nor drilling of the ovipositor could be observed in an area close to the nests.

### 3.1.4 Search behaviour in males

Search behaviour similar to that of females was observed in males during the hatching of females. The insects paused at certain locations, widely spread the hind legs sideways and tightened the mid legs to the body (Fig. 2d). It was not observed that the antennae contacted the wood. It seems likely that this behaviour helps in searching for hatching females.

### 3.1.5 Intraspecific aggressive behaviour in females

In captivity and in situ sometimes drilling females were hassled by other females (Fig. 3a, b). However, the drilling individuals showed no signs of disturbance. Occasionally fierce fights for certain drill spots took place in the terrarium, lasting several seconds. During these fights the females kicked each other mainly with the hind legs (Fig. 3c). In the field, larger females chased away smaller individuals by quickly running up to them with raised abdomens.

### 3.1.6 Intraspecific aggressive behaviour in males

Aggressive behaviour towards conspecifics has only been observed in a few males in situ. Some individuals that had been waiting for hours in groups at the same location (likely the future hatching spots of females) approached other males sideways with raised abdomens and tried to push them away with their hind legs. The attacked males likewise raised their abdomens. Fights repeatedly occurred between three males that had been staying close to each other on the same branch for one and a half hours. One male was particularly aggressive (Fig. 3d). Dwarf males were simply overran or bumped from the front by quickly approaching bigger individuals. Most males, however, showed no aggressive behaviour in close proximity and they sometimes even crawled on top of each other (Fig. 4a). Twice an encounter of two males was observed that was interpreted as a mating attempt: one male attempted to mount the other one, which tried to fend off this approach.

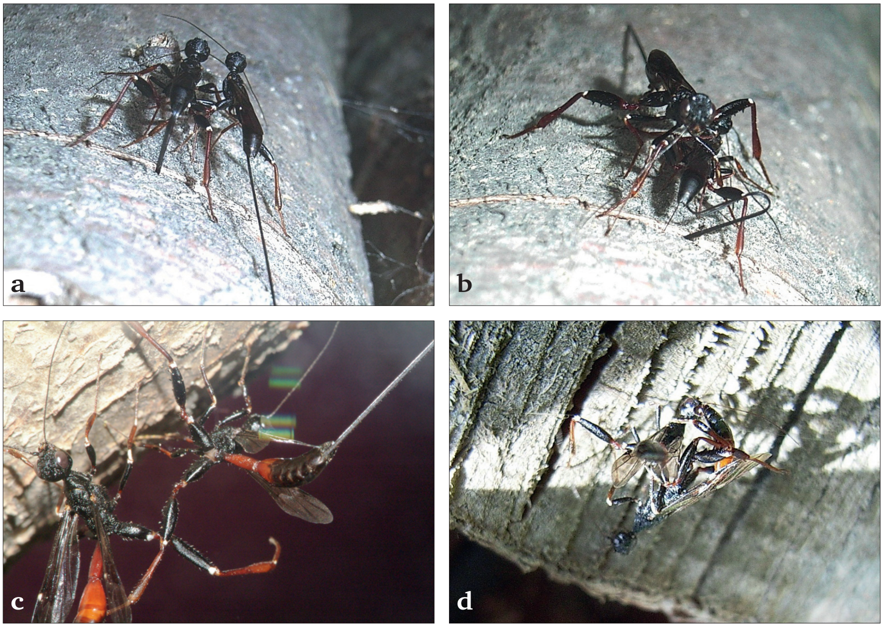

*Fig. 3:* (a, b) Intraspecific aggressive behaviour between females, field observation; (c) fighting females, observation in captivity; (d) fighting males, field observation.

### 3.1.7 Drilling activity

Usually the wood was drilled from underneath in hanging position. It was rarely observed that females drilled at the upperside of the wood. Already present cracks or crevices in the wood were usually not used for drilling.

At the start of the drilling process the female raised its legs and abdomen until the ovipositor could be put on the wood surface in an oblique angle (Fig. 4b). The antennae were oriented diagonally forward and downward with the tips touching the wood. The ovipositor penetrated the wood very slowly while the sheath curved backwards in an increasingly large loop (Fig. 4c). The drilling activities apparently took place repeatedly at the same spot and lasted for several hours, albeit not continuously as they were interrupted by lengthy breaks. Before drilling resumed, the sheath was again put on the wood surface in an oblique angle close to the previous marked drill hole and pulled over the wood surface for a short distance. Afterwards, the sheath was aligned almost vertically to the wood surface and a poking (searching) movement of the sheath tip was observed. One female had been poking the surface for more than four minutes closely to the marked drill hole but did not start drilling. In most cases, however, the ovipositor penetrated after a lengthy search – in fact, it penetrated relatively quickly to a certain depth. During the process of deeper penetration slow right-left movements or right-middle-right and left-middle-left movements of the abdomen tip including ovipositor were observed (Fig. 4d). These movements were sometimes interrupted by jerky thrusts into the wood. During this process the ovipositor was sometimes pulled out a short distance before penetrating even deeper. At the end of each drilling phase the ovipositor was pulled out within a few seconds, no matter how deeply it was drilled into the wood. For a short period of time the ovipositor of some individuals seemed to be bent or kinked. One female that had apparently been drilling at the same location for more than 55(!) hours (including breaks) had a conspicuously deformed ovipositor that had the form of a big "Ω" and repeatedly fell over to the side. This individual was found dying in the terrarium two hours later.

Tab. 1 shows the drilling process of a female, which had apparently been drilling repeatedly at the same spot over a period of more than 14 hours. Altogether this female had been drilling for more than 7 hours with at least equally long periods of rest.

| **Drilling duration (minutes)** | **Rest period (minutes)** |
|---|---|
| 40 | 10 |
| 65 | 25 |
| 70 | 33 |
| 40 | 32 |
| 65 | 28 |
| 62 | 45 (including search behaviour) |
| 47 | 73 |
| 42 | at least 183 (observation discontinued) |

*Tab. 1:* Example of a repeated drilling process of a female. Comparison of the duration of the individual drilling phases with the duration of the rest periods.

First drilling activities of females were observed in the terrarium only about 17 days after hatching. The same is true for the characteristic search behaviour on the wood. Any previous mating activities were not observed but they might have occurred. A certain timeframe was definitely present between mating and the first drilling activities. One female mated on June 14 and had not yet shown any search movements as of June 24.

### 3.1.8 Post-drilling behaviour

After the ovipositor had been retracted it was covered again by the sheath. Afterwards, it was moved up and down for several minutes. Following a period of rest the ovipositor was grabbed by the tibiae of the hind legs and slowly pulled through them by raising the abdomen (Fig. 5a). At the end of this process the ovipositor rebounded backwards like a streched spring. Two females briefly bent their ovipositors forward across the dorsal side of their bodies. The antennae were cleaned for several minutes using the fore legs.

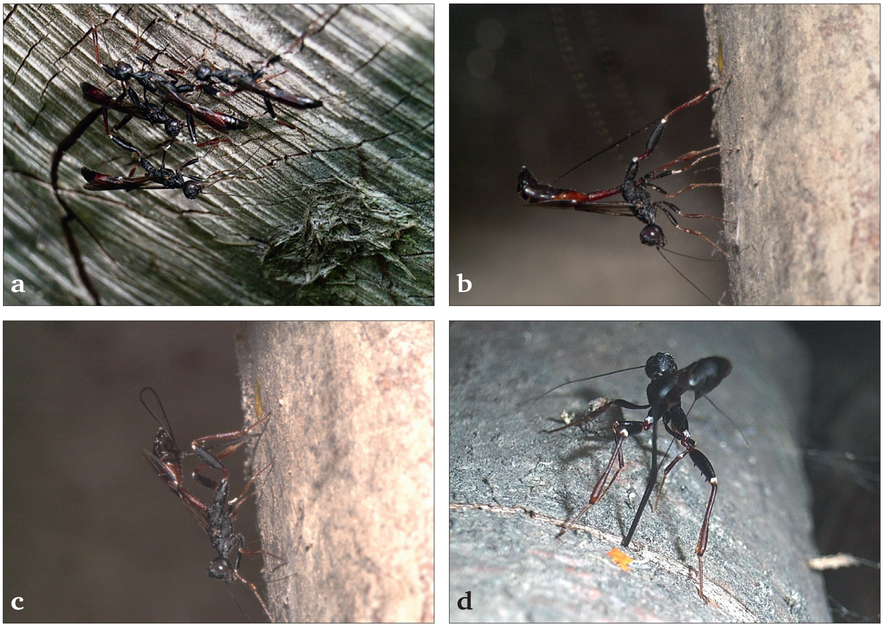

*Fig. 4:* (a) A group of males, field observation; (b) female, start of the drilling activity, observation in captivity; (c) female, advanced drilling activity, sheath building a loop, observation in captivity; (d) female, advanced drilling activity, lateral movements of the abdomen and the ovipositor, observation in captivity.

### 3.1.9 Feeding behaviour

Most individuals in the terrarium did not feed any of the provided food which consisted of flowers of different plants found near the woodpile (*Hieracium*, *Ranunculus*, *Trifolium* and *Taraxacum*) as well as diluted honey and banana pulp. Water was frequently provided in the form of spray. Only one or two days before dying some individuals were observed feeding on the banana pulp (Fig. 5b). In the field individuals feeding on flowers or any other sources of food were never observed.

## 3.2 Biology

### 3.2.1 Phenology

*Stephanus serrator* was observed from the beginning of June to the end of August at the studied location. Males appeared first, in the terrarium they hatched about 10 days before the females (protandry).

### 3.2.2 Sex ratio

In every year of the study considerably more males than females were present. However, only the individuals found on the visible parts of the woodpile (cut surface and visible surface of the stacked branches) could be counted, which makes reliable conclusions about the sex ratio impossible.

### 3.2.3 Development

During the extraction of the beetle larvae an egg was found inside the borehole of a *Clytus arietis* (L.) larva. Unfortunately, the larva was squashed and fragmented into two parts during the process of wood splitting, which made it impossible to reconstruct the original position of the egg on the host. The egg is elongate (1 mm long) and has two appendages of different sizes (one is long and thin, the other one is shorter and thicker) (Fig. 5c). Larval stages were not found during this study. However, a female (Fig. 5d) and a male pupa of *S. serrator* were extracted while splitting branches from the woodpile. The pupae lay freely and without a cocoon inside the slightly widened larval burrow. Rather heavily chitinized body parts of the hosts like the head capsules are not eaten by the parasitoid larva and are usually found laying in close proximity to the pupa (P. Svacha, pers. comm.). In the present study, however, no such remains could be found.

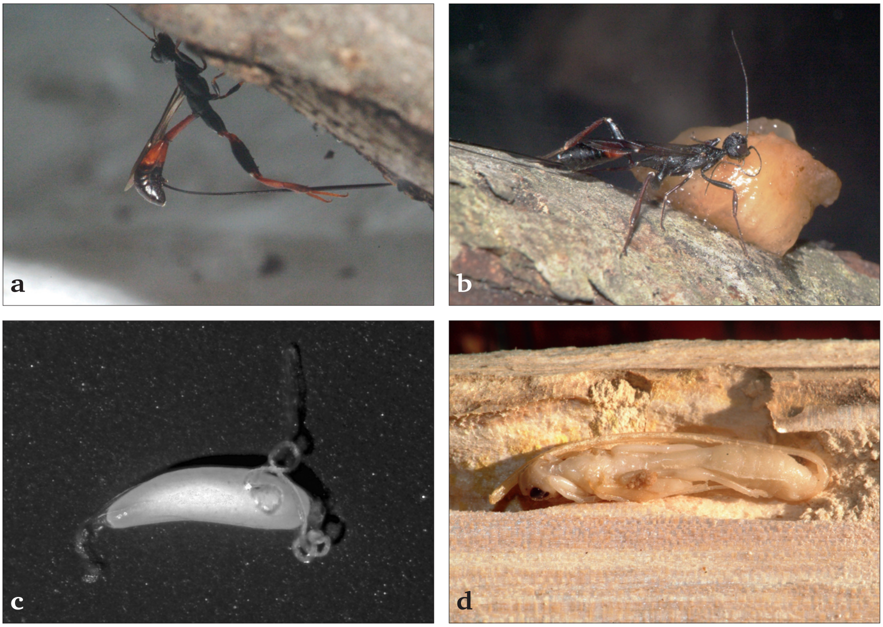

*Fig. 5:* (a) Female, cleaning of the ovipositor, observation in captivity; (b) female, feeding on banana pulp, observation in captivity; (c) egg, original size 1 mm; (d) female pupa.

### 3.2.4 Lifespan

One female which hatched in the terrarium lived for 21 days. Three large females, which were caught in the field, lived for 21 days (2 individuals) and 24 days (1 individual), respectively, in captivity. A large male (also caught in the field) showed the longest observed lifespan of 30 days in the terrarium.

### 3.2.5 Sleeping pattern

Periods of nightly rest of the individuals kept in the terrarium could not be observed apart from breaks during drilling activities. The insects drilled even in total darkness.

### 3.2.6 Predators

The spiders *Nuctenea umbricata* (Clerck) and *Parasteatoda* sp. wove numerous webs between the branches and were observed as predators of *S. serrator*. However, only few individuals of *S. serrator* actually got caught in the webs as they usually circumvented or crawled through them. The woodpile was also used as a perching site by the robber fly *Laphria flava* (L.) but it was never observed preying on *S. serrator*. The same is true for an individual of the common wall lizard *Podarcis muralis* (Laurenti) which was resting on the pile repeatedly.

## 3.3 Morphology

### 3.3.1 Size differences

Males and females show great variation in size. The body length of males (head without antennae to caudal end of abdomen) varied between 0.5 cm (dwarf male) and 1.5 cm. Large females were 1.8-1.9 cm long (head without antennae to caudal end of abdomen), the smallest female measured only 0.8 cm in length. Most females were medium-sized (1.2-1.5 cm) to large-sized, whereas in males many medium-sized (app. 1 cm) and many very small individuals were observed. The ovipositor of large females measured up to 2.2 cm, in medium-sized individuals it measured about 1.2-1.4 cm and in the smallest observed female it measured only 0.8 cm.

### 3.3.2 Ultra-morphology of the ovipositor

In hymenopterans the ovipositor consists of two pairs of basal valvifera and three pairs of valves. The first and second valves are closely and flexibly linked and form the actual ovipositor. The third valves form the sheath. The first (ventral) valves originate from the valvifera of the eighth segment and are almost completely isolated from one another over their entire length. The second (dorsal) valves originated from fusion of the valvifera of the ninth segment and are more or less grown together or membranously connected in the vast majority of hymenopterans. The dorsal valves are connected with the ventral valves through two lateral T-shaped ridges (rhachis), which are linked to two T-shaped grooves (aulax) on the ventral valves (Fig. 6a). The combination of rhachis and aulax is called olistheter. This flexible mechanism enables the backwards and forwards movement of the valves when sawing or drilling (Oeser 1961, Quicke et al. 1999, Quicke 2015).

Several coeloconical sensillae were found in the area of the apically serrated dorsal valves (Fig. 6b, 7, 8). The observed depression of a ventral valve might be the opening of a gland (Fig. 7) but histological examinations are needed to confirm this (L. Vilhelmsen, pers. comm.). Scattered coeloconical sensillae are also present on the ventral valves (Fig. 7, 8).

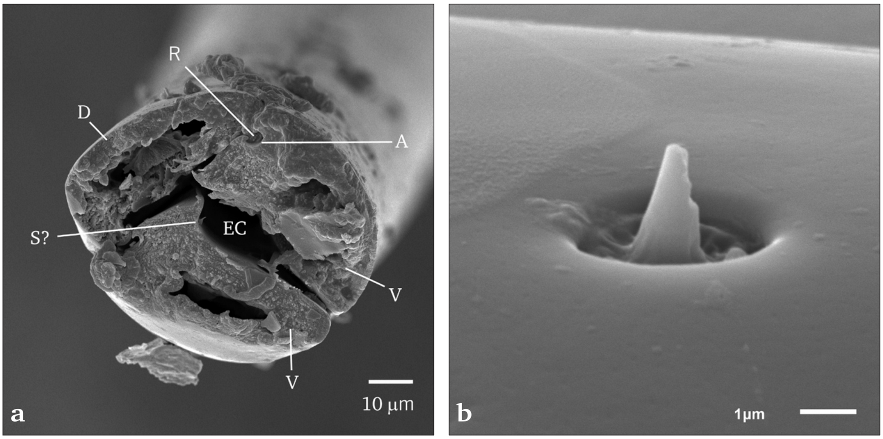

*Fig. 6:* (a) Ovipositor, transverse section, ventral valves (V), dorsal valves (D), rhachis (R), aulax (A), egg canal (EC), sensilla (?) (S?); (b) coeloconical sensilla at the tip of the ovipositor.

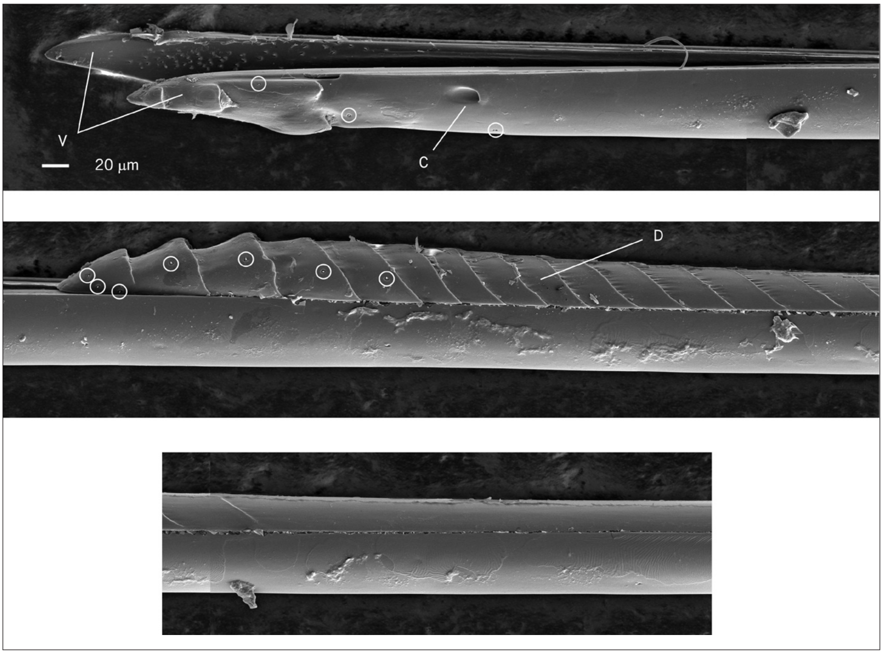

*Fig. 7:* Tip of the ovipositor, lateral view, ventral valves (V), dorsal valves (D); encircled: coeloconical sensillae; cavity (C): probably gland opening.

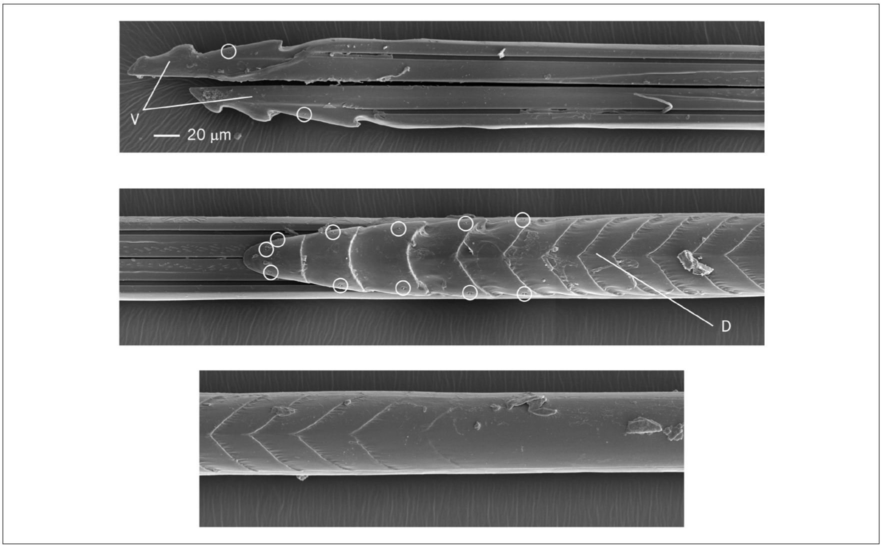

*Fig. 8:* Tip of the ovipositor, dorsal view, ventral valves (V), dorsal valves (D); encircled: coeloconical sensillae.

Three types of trichoid sensillae are found on the outer surface of the sheath tip (Fig. 9a). Type 1 is shorter than the other types (2 µm diameter, 45 µm long) and ends in a slightly bent, sharp tip. Type 2 is longer (2 µm diameter, 60 µm long) and also pointed. Type 3 is even thicker and longer (5-6 µm diameter, 120 µm long) and ends in a slightly bent but obtuse tip. Two pairs of type 3 trichoid sensillae are present close to the caudal end of both valves which form the sheath. Transversal segmentation of the sheath (Fig. 9b) allows the necessary flexibility when drilling (Vilhelmsen 2003).

The inside of the sheath contains subapical structures (Fig. 9c), which probably represent campaniform sensillae or broken trichoid sensillae (L. Vilhelmsen, pers. comm.). The inner surface of the sheath is additionally lined with thin, slightly bent, crest-like structures (Fig. 9d).

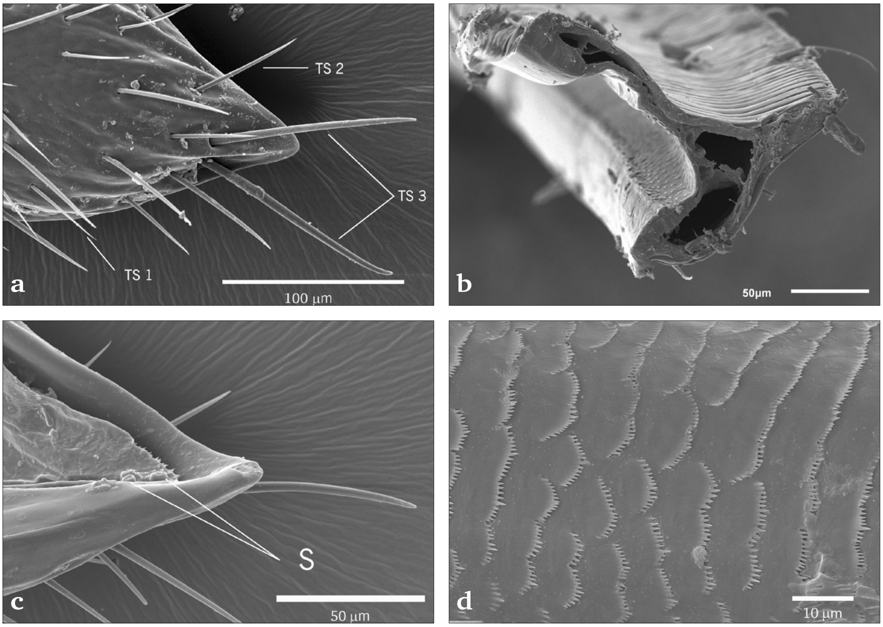

*Fig. 9:* (a) Tip of the sheath, outer surface, trichoid sensilla type 1 (TS 1), trichoid sensilla type 2 (TS 2), trichoid sensilla type 3 (TS 3); (b) sheath, transverse section; wall finely transversely subdivided; (c) tip of the interior of the sheath, probably campaniform sensillae or the base of trichoid sensillae, where the main sensillae have broken off (S) (Vilhelmsen, schriftl. Mitt.); (d) sheath, inner surface; crest-shaped cuticular structures.

### 3.3.3 Ultra-morphology of the legs

The legs of *S. serrator* (Fig. 10) are morphologically similar to the legs of *Schlettererius cinctipes* and are described by Vilhelmsen et al. (2008). The swellings on the tibiae, which are particularly conspicuous on the hind legs (Fig. 11a), probably contain subgenual organs. Striking apically duckbill-shaped sensory hairs (Fig. 11b) are intermixed within the trichoid sensillae and are mainly found on the apex of the hind tibiae (Fig. 11c). The distal ends of the fore and mid tibiae contain a cone-shaped sensillum (Fig. 11d).

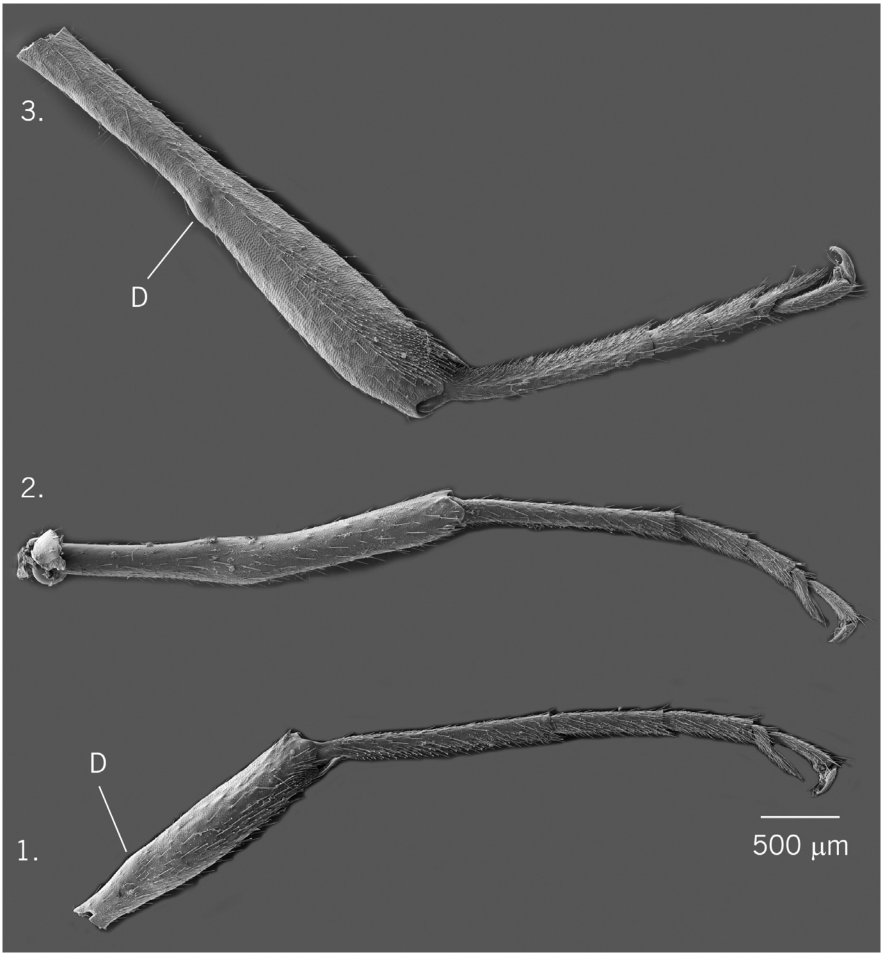

*Fig. 10:* Tibiae and tarsi of the fore (1.), mid (2.) and hind (3.) leg; dome (D) probably concealing subgenual organ.

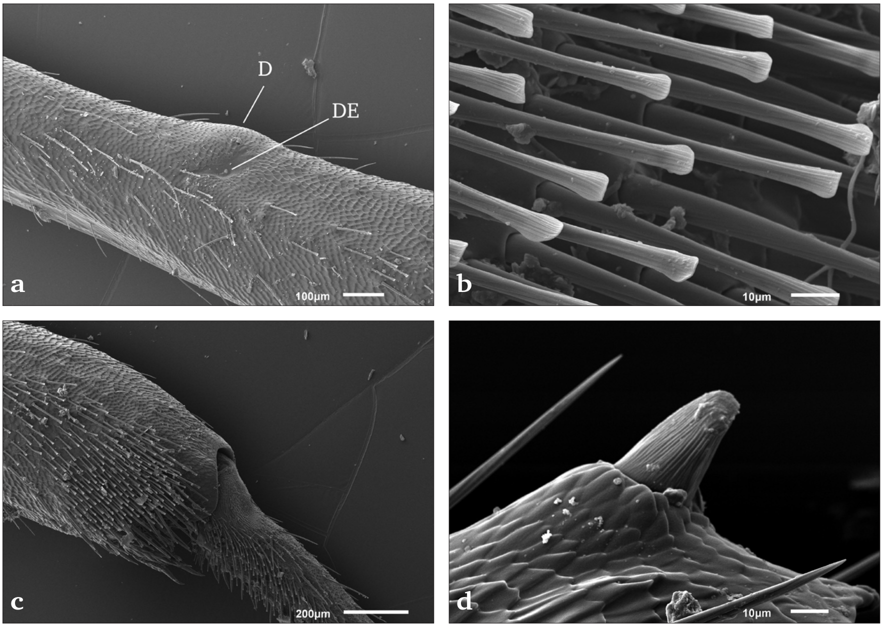

*Fig. 11:* (a) Tibia of the hind leg, probably at the level of the subgenual organ. Dome (D), depression (DE); (b) tibia of the hind leg, apically duckbill-shaped sensory hairs; (c) tibia of the hind leg, apically duckbill-shaped sensory hairs concentrated at the apex; (d) tibia of the fore leg, cone-shaped sensillum at the apex.

### 3.3.4 Ultra-morphology of the antennae

The segments of the antennae are densely fringed with trichoid sensillae. Most of these sensillae are relatively small and short (1-1.5 µm in diameter, 20 µm long) and tapering into a sharp tip, which is sometimes apically bent. Longer and thicker trichoid sensillae (2 µm in diameter, 30 µm long) are intermixed. They are denser on the last antenna segment, especially towards the tip (Fig. 12a). Placoid sensillae are present on the ventral side of the antennae (Fig. 12b), but they are missing on the distal two segments.

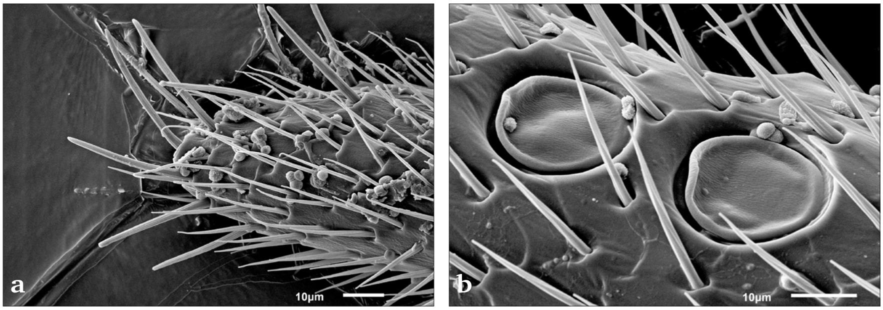

*Fig. 12:* (a) Last antenna segment; (b) placoid sensillae on the ventral side of antenna segments.

### 3.3.5 Heavy metals in the ovipositor

The ovipositor of *S. serrator* contains zinc in the area of the apically serrated valves (Fig. 13a-d). Chlorine is one of the zinc ligands. High zinc areas of the serrated valves correspond with elevated chlorine content. No other elements with an atomic number higher than eleven were detected in the tip of the ovipositor.

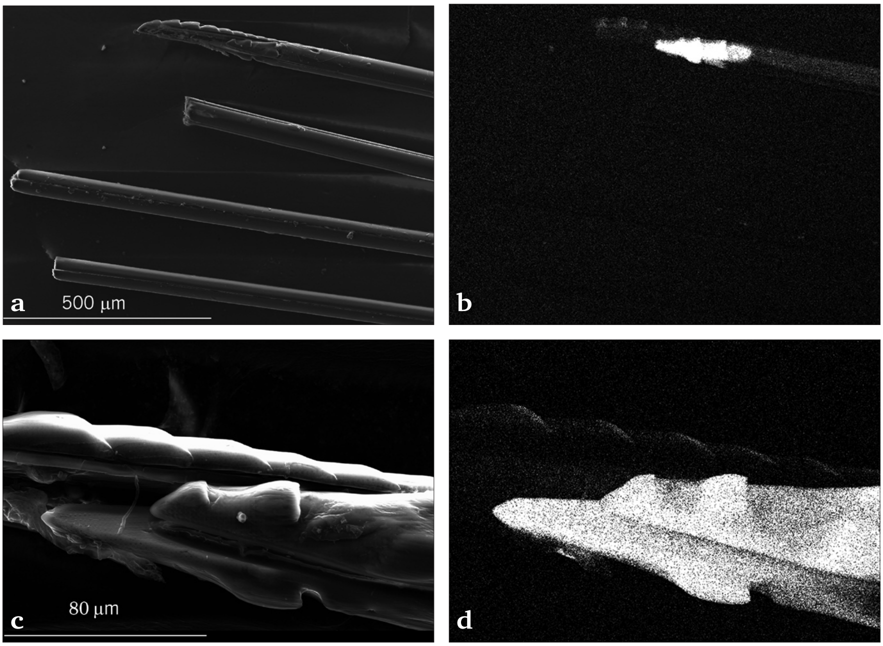

*Fig. 13:* Energy dispersive elemental mapping (Zn ka) of the ovipositor; 100 frames were added to an image; light zones correspond to areas with high Zn content. (a) SE image of the ovipositor; (b) Zn distribution of the same region; (c, d) are a zoom of the tip region.

### 3.4 Potential and confirmed host species

In 2011, 18 beetle larvae were carefully extracted from marked branch parts at which longer drilling activities had previously been taking place. Of all the examined larvae only on a single larva of *Clytus arietis* (L.) an egg was found.

| **Beetle species/family** | **Tree species and branch diameter** | **Depth of the burrow inside the wood** | **Larval size** | **parasitized** |
|---|---|---|---|---|
| *Clytus arietis* (L.) | *Fagus sylvatica*, 4 cm | 0.8 cm | 1.0 cm | no |
| *Clytus arietis* (L.) | *Fagus sylvatica*, 6 cm | 0.5 cm | 0.6 cm | no |
| *Clytus arietis* (L.) | *Fagus sylvatica*, 5 cm | 1.5 cm | 1.0 cm | no |
| cf. *Anaglyptus mysticus* (L.) | *Fagus sylvatica*, 5 cm | 0.9 cm | 1,5 cm | no |
| *Rutpela maculata* (PODA) | *Fagus sylvatica*, 4 cm | 0.6 cm | 0.8 cm | no |
| *Anaglyptus mysticus* (L.) | *Acer* sp., 3.5 cm | 1.4 cm | 1.0 cm | no |
| *Anaglyptus mysticus* (L.) | *Acer* sp., 4 cm | 1.0 cm | 1.1 cm | no |
| *Clytus arietis* (L.) | *Acer* sp., 3.5 cm | 0.8 cm | 0.8 cm | yes |
| *Anaglyptus mysticus* (L.) | *Fagus sylvatica*, 5.5 cm | 1.5 cm | 1.0 cm | no |
| *Anaglyptus mysticus* (L.) | *Fagus sylvatica*, 5 cm | 2.6 cm | 0.9 cm | no |
| *Anaglyptus mysticus* (L.) | *Fagus sylvatica*, 3.5 cm | 1.4 cm | 0.8 cm | no |
| *Anaglyptus mysticus* (L.) | *Fagus sylvatica* 5 cm | 1.2 cm | 0.8 cm | no |
| Anobiidae | *Fagus sylvatica*, 5 cm | 2.5 cm | 0.5 cm | no |
| *Anaglyptus mysticus* (L.) | *Fagus sylvatica*, 5 cm | 2.0 cm | 1.0 cm | no |
| *Anaglyptus mysticus* (L.) | *Fagus sylvatica*, 5 cm | 1.5 cm | 0.9 cm | no |
| Anthribidae | *Fagus sylvatica*, 9 cm | 1.7 cm | 0.6 cm | no |
| Anthribidae | *Fagus sylvatica*, 9 cm | 0.8 cm | 0.7 mm | no |
| Anthribidae | *Fagus sylvatica*, 8 cm | 1.0 cm | 1.0 cm | no |

*Tab. 2:* List of the extracted beetle larvae and their breeding substrate with information on the depth of the burrow and the parasitization by *S. serrator*.

## 4. Discussion

The slow movement and reluctance to fly at the oviposition place, both of which have been described by previous authors (BLÜTHGEN 1953, JANSEN et al. 1988, REDER 2011), are vital factors to avoid predators which are specialized on quickly moving or flying prey.

Slower movement also reduces the chance of getting caught in spiderwebs. The avoidance of sunlight is also mentioned in other papers (Jansen et al. 1988, Reder 2011), and the same behaviour is reported from *Parastephanellus* sp. (Rodd 1951).

The females of *S. serrator* show a characteristic repeated leg posture while searching for potential hosts: the hind legs are widely spread and the mid legs are tightened to the body. This behaviour is also described and illustrated by Reder 2011. We can only try to explain this behaviour by studying the SEM photographs, however, histological preparations are needed to classify most of the externally visible structures. Many Stephanidae show conspicuous leg modifications, e.g. distally widened tibiae or modified tarsi, especially on the hind legs. Ultramorphological studies of the legs of *Megischus* spp. showed that the tibiae of all legs contain subgenual organs (Vilhelmsen et al. 2008). The subgenual organ is a chordotonal organ that functions as a mechanoreceptor and is usually found in the legs of winged insects. It seems likely that some hymenopteran parasitoids detect vibrations from the inside of the substrate with their tarsi from where the vibrations spread to the subgenual organs inside the tibiae (Vilhelmsen et al. 2008). The legs of *Schlettererius cinctipes* and *S. serrator* were only studied externally by Vilhelmsen et al. (2008) but due to morphological similarities with *Megischus* spp. the authors suspect that both species possess subgenual organs at least in the front legs. As in most Stephanidae, the mid tibiae of *S. serrator* are inconspicuous and unlikely to possess any subgenual organs. *Schlettererius cinctipes* and *Megischus* spp. have a cone-shaped sensillum at the apex of the fore and mid tibiae (Vilhelmsen et al. 2008). This study revealed the presence of a similar sensillum on the fore and mid tibiae of females of *S. serrator*. Whether these sensillae play any role in host detection needs to be clarified in future studies.

The distal enlargement of the hind tibiae of Stephanidae could potentially contain strengthened musculature. Higher pressure on the tarsi would facilitate the perception of vibrations (Vilhelmsen et al. 2008). The tarsi of *S. serrator* are ventrally densely hairy even though they do not have the dense hair tufts on the hind legs, which are present in most other Stephanidae. The hind tibiae of *S. serrator* might contain a subgenual organ in the area of the dome-shaped bump. If the legs of *S. serrator* contain subgenual organs, the characteristic leg posture while searching for a host would allow for these organs to be as close to the wood surface as possible in order to detect potential vibrations produced by the host.

The observed posture of males, which is similar to the posture of females when searching for hosts, might be used to locate hatching females. In Stephanidae where the females have strongly modified hind tibiae, such tibiae are also found in males (Vilhelmsen et al. 2008) and the same is true for *S. serrator*. It is possible that the hind tibiae of males contain subgenual organs, which detect vibrations produced by hatching females. The fact that mating does not take place immediately after hatching makes one wonder about the purpose of this behaviour.

During the characteristic search posture of the female the tip of the sheath is in contact with the substrate, which suggests that the sheath plays an important role, apart from the legs, in the search for host larvae.

The outside of the sheath is fringed with trichoid sensillae of different types. Each valve possesses two trichoid sensillae at the apex, which are considerably longer and thicker compared to the other sensillae and clearly project above the end of the sheath. Even though sheaths with sensillae are present in many hymenopteran parasitoids, little is known about their function and only few studies have been published on this topic. Removing the sheath of *Diadromus pulchellus* WESMAEL (Ichneumonidae) causes it to stop searching for hosts (cf. QUICKE et al. 1999). PAXTON & BURKHARDT (1970) applied various chemicals to the sheath of *Bruchophagus roddi* GUSSAKOVSKII (Eurytomidae), successfully initiating the oviposition process. Trichoid sensillae on sheaths are usually considered to be mechanoreceptors but this function might also be linked to a chemoreceptive function (NÉNON et al. 1997). Regarding parasitic behaviour, VILHELMSEN (2003), DWECK et al. (2008) and XIAOYI & ZHONGQI (2008) found that the ovipositor is as important as the antennae. In *S. serrator* the long trichoid sensillae at the end of the sheath could potentially detect vibrations produced by the host and might thus be useful for detecting it. Similar suspicions are published by ALVES et al. (2014) regarding the parasitic behaviour of *Bracon vulgaris* ASHMEAD (Braconidae). A comparison with *Megischus coronator* (F.) shows that this species does not possess any trichoid sensillae at the apex of the sheath (VILHELMSEN 2003).

In the present study of *S. serrator* an involvement of the antennae in the host-finding process by beating the substrate was not observed. It seems more likely that the females passively detect vibrations produced by the wood-inhabiting larvae. In contrast, JANSEN et al. (1988) report a female of *S. serrator*, which had been examining the cut surface of a *Fagus* branch for several minutes using its antennae and a similar way of examining potential host locations with vibrating antennae is described for *Parastephanellus* sp. (RODD 1951), whereas *Schlettererius cinctipes* reportedly spends little time antennating the bark surface (TAYLOR 1967). Female Stephanidae do not possess modified antenna tips used for beating the substrate, unlike the Orussidae or some Ichneumonidae (VILHELMSEN et al. 2008). While searching for a host, the females of *S. serrator* lower their antennae until the tips are in contact with the wood. Trichoid sensillae are located at the tips of the antennae and likely detect vibrations produced by the host. The segments of the antennae (except for the last two) also contain placoid sensillae. These olfactory receptors are easily stimulated by volatile plant products and are found on the antennae of many parasitoids. They are likely involved in the search for and selection of potential hosts (ALVES et al. 2014).

The results of the present study indicate that it takes several hours until the ovipositor reaches a larva and an egg is laid (longest observed drilling activity: 55 hours including rest). These observations suggest that the drilling activity is interrupted several times, which raises the question if and how it is possible for *S. serrator* to repeatedly locate the same drill hole. Observations of marked drill holes showed that females perform poking movements with the ovipositor vertically to the surface in close proximity to the holes and that they were able to penetrate relatively quickly to a certain depth. Electron-microscopical photos of the inside of the sheath revealed subapical structures, which probably represent campaniform sensillae or broken trichoid sensillae (L. Vilhelmsen, pers. comm.) and might facilitate the repeated locating of the drill hole together with the apical trichoid sensillae on the outside of the sheath. Several publications contain potential observations of this searching and finding of drill holes: “nach mehreren Ansätzen fing das Weibchen an, seinen Legebohrer in das Holz zu treiben” (JANSEN et al. 1988: 424), “der Bohrer drang ziemlich rasch und stetig ein” (BLÜTHGEN 1953: 233).

The long duration of oviposition might be an explanation for the observed low ovipositional success rate (5.5 %). The larvae have likely been extracted too early or were too deeply concealed in the wood. However, similar low rates of successful oviposition are reported by QUICKE et al. (1998) for certain Ichneumonidae (Rhyssinae) with 10 %, and by TAYLOR (1967) who extracted 172 larvae of *Sirex noctilio* F., of which 22 were parasitized by *Schlettererius cinctipes* (13 % success rate).

Both mechanoreceptors and chemoreceptors (usually located at the tip) can be expected to be present on the ovipositor of hymenopterans (QUICKE 1999). The commonest sensillae near the ovipositor tip are coeloconical sensillae (QUICKE 2015). This type of sensillae has been found on ovipositors of *Trybliographa rapae* WESTWOOD (Figitidae) (BROWN & ANDERSON 1998), *Leptopilina heterotoma* NORDLANDER (Figitidae) (VAN LENTEREN et al. 2007), *Aprostocetus procerae* (RISBEC) (Eulophidae) (NACRO & NÉNON 2009) and *Venturia canescens* (GRAVENHORST) (Ichneumonidae) (SHAH 2012). Apart from their mechanoreceptive role these sensillae could have a chemoreceptive or thermo-hygroreceptive function (TOFAEL et al. 2013). Coeloconical sensillae were also found at the tip of the ovipositor of *S. serrator*. The subapical cavity existing on one ventral valve might be the opening of a gland, however, histological examinations are needed to confirm this (L. Vilhelmsen, pers. comm.). *S. serrator* belongs to the idiobiont parasitoids (AGUIAR 2004, 2006), which disrupt the development of their hosts by injecting poison into them. NÉNON et al. (1997), who studied the sensoric features of the ovipositor of *Megarhyssa atrata* (F.) (Ichneumonidae), suspect that additional wood-dissolving substances can be released through the ovipositor.

The inside of the sheath is covered with crest-like structures, which might serve for the cleaning of the ovipositor and its sensillae between drilling phases. Similar structures are also found in the ichneumonid *Venturia canescens* (GRAVENHORST) (SHAH et al. 2012), the megalyrid *Megalyra fasciipennis* (WESTWOOD) and the braconid *Doryctes leucogaster* (NEES) (VILHELMSEN 2003).

Many hymenopteran parasitoids that drill into hard wood incorporate zinc or manganese into their ovipositors. In the case of *S. serrator* as well as in most other parasitoids these deposits are restricted to the area of the serrated valves. In contrast, the ovipositor of *Diastephanus* sp. contains high traces of zinc over its whole length (QUICKE et al. 1998).

In this study the ability of *S. serrator* to retract the ovipositor relatively quickly was observed, which is also described for *Parastephanellus* sp. (RODD 1951). However, some authors doubt this ability due to the morphology of the valve apices (QUICKE 1999, L. Vilhelmsen, pers. comm.).

The egg of *S. serrator* is similar to the egg of *Schlettererius cinctipes* (TAYLOR 1967) and seems relatively big compared to the hair-thin ovipositor. It is known from various parasitoid wasps, especially those with large eggs and long ovipositors, that the eggs are considerably deformed during oviposition. This deformation might stimulate the initiation of embryogenesis (QUICKE et al. 1999). Appendiculate eggs are commonly found in idiobiont parasitoids (QUICKE 2015). The appendages of the egg might anchor it to the host larvae. The observed pupae of *S. serrator* lay freely and without a cocoon inside the larval burrow. Similarly, the last larval stage of *Schlettererius cinctipes* pupates without cocoon in the chamber in which it fed the host larva (TAYLOR 1967).

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