

Simultaneous percussion by the larvae of a stem-nesting solitary bee – a collaborative defence strategy against parasitoid wasps?

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Abstract

Disturbance sounds to deter antagonists are widespread among insects but have never been recorded for the larvae of bees. Here, we report on the production of disturbance sounds by the postdefecating larva (“prepupa”) of the Palearctic osmiine bee *Hoplitis (Alcidamea) tridentata*, which constructs linear series of brood cells in excavated burrows in pithy plant stems. Upon disturbance, the prepupa produces two types of sounds, one of which can be heard up to a distance of 2–3 m (“stroking sounds”), whereas the other is scarcely audible by bare ear (“tapping sounds”). To produce the stroking sounds, the prepupa rapidly pulls a horseshoe-shaped callosity around the anus one to five times in quick succession over the cocoon wall before it starts to produce tapping sounds by knocking a triangularly shaped callosity on the clypeus against the cocoon wall in long uninterrupted series of one to four knocks per second. Sound analysis revealed that the stroking sounds consist of several syllables, which are very similar to the single syllables of the tapping sounds: both last about 0.5 ms and spread over 40 kHz bandwidth from the audible far into the ultrasonic range. The production of stroking sounds by a prepupa induces other prepupae of the same nest to stroke and/or to tap resulting in a long-lasting and simultaneous albeit unsynchronized percussion by numerous prepupae along the whole nest stem. We hypothesize that these disturbance sounds serve an anti-antagonist function and that they have evolved to disturb the reflectance signals that parasitoid wasps use to localize concealed hosts during vibrational sounding.

Keywords

Anthophila, Apiformes, chorusing behaviour, echolocation, *Hoplocryptus*, ichneumonid wasps, Megachilidae, Osmiini

Introduction

Innumerable insects from many different taxa produce vibrational signals (“sounds” hereafter), which are propagated through air, water or solids and perceived by the recipients with tympanal ears or near-field receptors including sensory sensillae, subgenual organs or the antennae (Gullan and Cranston 2000; Yack and Hoy 2003; Drosopoulos and Claridge 2005). Depending on the insect taxon, these sounds are produced in very different ways encompassing i) vibration of thorax, abdomen or wings, ii) percussion of body parts against each other or against a substrate, iii) deformation of modified cuticular areas by contraction and relaxation of special musculature, iv) expulsion of air through spiracles or proboscis, v) stick-slip movements of adjacent body segments and vi) true stridulation, which involves the movement of two specialized body parts against each other in a regular patterned manner (Ewing 1989; Claridge 2005; Dolle et al. 2018). The majority of these air-, water- or substrate-born insect sounds serve for intraspecific communication, such as species recognition, mate finding, courtship, male competition, recruitment to feeding sites or warning against antagonists.

However, there are other insect sounds, which are not aimed at conspecifics, but which are assumed to be predominantly defensive since they are mainly produced when the insects are disturbed or attacked. In fact, experiments with a species each of ground beetles (Carabidae), tiger beetles (Cicindelidae), water scavenger beetles (Hydrophilidae), scolytid beetles (Scolytidae), mutillid wasps (Mutillidae), cicadas (Cicadidae) and butterflies (Nymphalidae) revealed that such disturbance sounds deter predators like spiders, predatory beetles, mice or birds (Bauer 1976; Smith and Langley 1978; Masters 1979; Lewis and Cane 1990; Olofsson et al. 2012). Three mechanisms are discussed, which might underlie the effect of the disturbance sounds: they may either serve i) to alert the antagonist to the harmfulness of the sound producer (“acoustic aposematism”), ii) to startle the attacker or iii) to render the localization of the sender by parasites or predators more difficult (Masters 1979; Kočárek 2009; Corcoran et al. 2010). Disturbance sounds are widespread among insects and known for example from i) larvae of Blattodea (Blaberidae, Ectobiidae) and Coleoptera (Cerambycidae), ii) pupae of Coleoptera (Scarabaeidae) and Lepidoptera (Arctiidae, Callidulidae, Hesperiiidae, Lycaenidae, Lymantriidae, Noctuidae, Notodontidae, Nymphalidae, Phalaenidae, Pyralidae, Riodinidae, Sphingidae, Zygaenidae) and iii) imagines of Auchenorrhyncha (Cicadidae), Blattodea (Blaberidae), Coleoptera (Cerambycidae, Cicindelidae, Scarabaeidae, Silphidae), Heteroptera (Cydnidae, Reduviidae), Hymenoptera (Apidae, Bradynobaenidae, Mutillidae, Crabronidae, Formicidae, Rhopalosomatidae) and Lepidoptera (Nymphalidae) (Hinton 1945, 1955; Downey 1966; Nelson 1979;

Schal et al. 1982; Tremevan 1985; Tschuch and Brothers 2000; Drosopoulos and Claridge 2005; Kočárek 2009; Olofsson et al. 2012; Dolle et al. 2018).

Sound production for intra- or interspecific communication is rare in bees. It has been observed in the males of several solitary species during courtship and mating (Torchio 1990; Weislo and Buchmann 1995; Toro and Riveros 1998; Conrad et al. 2010) and in several social species of corbiculate Apidae for queen-queen, queen-worker and worker-worker communication, defense or recruitment to food sources (Kirchner and Röschard 1999; Hrnčir et al. 2005). In most cases, these sounds are produced by thoracic or more rarely wing vibrations rather than specialized structures (Tschuch and Brothers 2000). An exception is *Meganomia binghami* (Cockerell) (Melittidae), in which the males produce loud rasping sounds during mating with stridulatory areas on the sterna (Rozen 1977).

Given this rather silent nature of bees, it turned out as a surprise when we recently realized that the postdefecating larvae (“prepupae”) of the stem-nesting osmiine bee *Hoplitis (Alcidamea) tridentata* (Dufour and Perris) (Megachilidae) produce two different types of sounds upon disturbance, one of which is very quiet and difficult to hear by bare ear (“tapping sounds”), whereas the other is loud and well audible (“stroking sounds”). A literature survey revealed that the stroking sounds were already briefly mentioned by Enslin (1925), Malyshev (1937) and Westrich (1989), who describe them as cracking, humming or barking and assume that the prepupae produce them by rapidly contracting their body inside the cocoon.

In this study, we i) describe the prepupal disturbance sounds of *Hoplitis tridentata*, ii) identify the structures that produce them, iii) present the results of experiments on prepupal sound production, iv) examine the prepupae of other osmiine bee species for the presence of sound producing structures similar to those of *H. tridentata* and v) discuss possible functions of the disturbance sounds.

Methods

Bee species

Hoplitis tridentata is a 10–12 mm long Palearctic osmiine bee (Megachilidae, Osmiini), which has a vast distribution ranging from Europe and northern Africa eastwards to Western Siberia and Central Asia (Müller 2020). In Central Europe, the species' flight period lasts from the end of May to mid August (Swiss Bee Team 2000). The females nest in burrows excavated in dead pithy stems (e.g. of *Artemisia*, *Cirsium*, *Cynara*, *Ferula*, *Onopordum*, *Rubus*, *Verbascum*; Figs 1, 2) or – more rarely – in hollow stems (e.g. of *Dipsacus*, *Phragmites*) (Enslin 1925; Malyshev 1937; Westrich 2018; Müller 2020). They enter the stem either through a hole bitten laterally through the stem wall (Fig. 1) or at its broken tip (Fig. 2). The nests usually contain 6–12 and occasionally up to 34 brood cells, which are arranged in a linear series within the maximally 36 cm long nesting burrow (Enslin 1925; Malyshev 1937). Each brood cell is provisioned

with pollen of Fabaceae (e.g. *Lathyrus*, *Lotus*, *Onobrychis*, *Vicia*), sometimes admixed with pollen of *Echium* (Boraginaceae), before a single egg is laid onto the provision and the cell is closed with a wall of masticated green leaves (Westrich 2018; Müller 2020). The egg hatches after a few days and the larva devours the food provision within less than a month, before it spins a cocoon and overwinters as prepupa inside the cocoon (Malyshev 1937; Fig. 3). Pupation and metamorphosis to the adult stage take place in early summer of the following year. In Central Europe, the prepupal stage lasts roughly ten months, i.e. from August to May.

The nests of *Hoplitis tridentata* are attacked by numerous brood parasites (Dufour and Perris 1840; Giraud 1866; Laboulbène 1877; Enslin 1925; Bouček 1952; Le Goff 1997; Baur and Amiet 2000; Schwarz 2007; Westrich 2018, 2020), which act either as cleptoparasites that devour the larval food provisions or as parasitoids that feed on the larvae. The species' known cleptoparasites include the megachilid bees *Stelis minuta* Lepeletier and Audinet-Serville and *S. ornatula* (Klug), the sapygid wasp *Sapyga quinquepunctata* (Fabricius), the gasteruptionid wasp *Gasteruption thomsoni* (Schletterer), the meloid beetle *Zonitis immaculata* (Olivier) and the sarcophagid fly *Miltogramma murinum* Meigen. Known parasitoids are the ichneumonid wasps *Hoplocryptus bellosus* (Curtis) and *H. signatorius* (Gravenhorst), the leucospidid wasps *Leucospis biguetina* Jurine and *L. dorsigera* Fabricius, the chalcidid wasp *Neochalcis osmicida* (Saunders) and the tachinid fly *Triathria setipennis* (Fallén).

Origin of bee material

To obtain nests of *Hoplitis tridentata*, trap nests were positioned in suitable habitats in northern Switzerland (Glattfelden/Zurich) and in southern Switzerland (Kalpetran/Valais) in spring 2020. Each trap nest consisted of a bundle of five dry *Rubus* stems of 50 cm length (Fig. 4). In total, 35 sealed nests were collected in late summer 2020, of which 12 were opened for audio and video recording, examination of larval morphology and experiments on prepupal sound production; the remaining nests – although partly used for other experiments – remained intact and were returned to the field at the end of the study.

Sound recording and analysis

Acoustic recordings were performed with a Batlogger M (Elekon AG, Luzern, Switzerland). This device records wav-files with a sampling rate of 312.5 kHz with 16 bit sampling depth to a SD-memory card. Its microphone shows a relatively flat frequency response (± 5 dB) from the low audio range up to 150 kHz. Recordings of 10.5 s duration were triggered manually at a distance of 2–20 cm between microphone and the nest stem.

The wav-files were analysed with the software Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019). To calculate temporally well-resolved spectrograms, we used a Blackman window of size 64, overlapping by 90.6%, and zero-buffered the windows for a FFT size of 1024 points. In Raven Pro we designed a band-limited

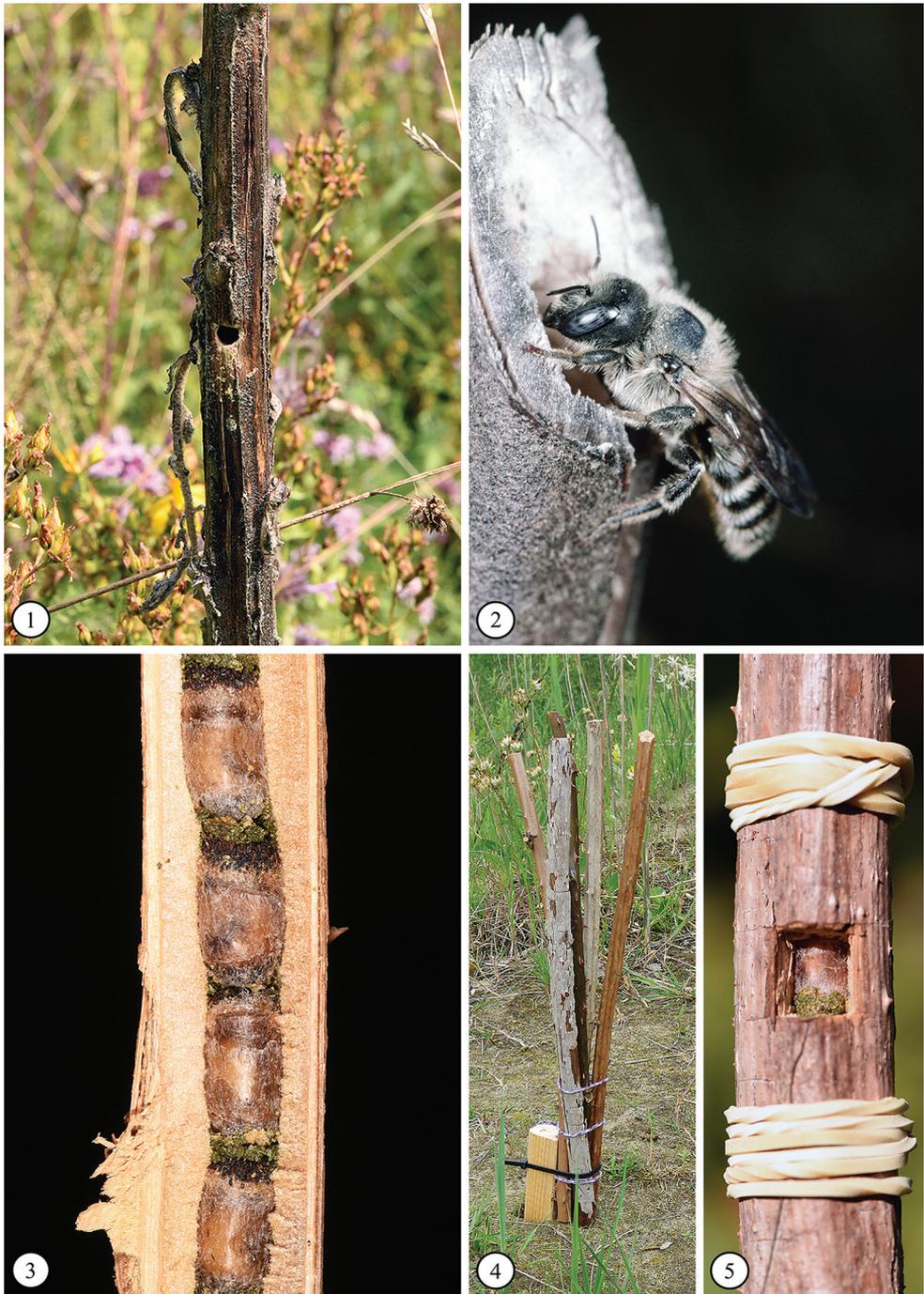
energy detector that was run over 52 high quality recordings of 10.5 s duration each. The detector was iteratively optimized to identify signals of 0.15–4.0 ms duration with a minimum temporal separation of 0.46 ms, searching in the frequency band of 0.1–150 kHz with a SNR threshold of 12 dB and a minimum occupancy of 70%. Spectral and temporal parameters were measured automatically within the detection window. To achieve robust signal measurements from the spectrograms, we used parameters based on temporal and spectral cumulative energy distributions (5%, 95% and differences thereof), which Raven Pro automatically calculates.

Experiments on prepupal sound production

Intended as a first step towards a better understanding of the possible function of the prepupal sounds of *Hoplitis tridentata*, we performed ten experiments (Table 1). For these experiments, we used four types of nests: i) eight trap nest bundles still positioned at their original site on an area of about 40 m² containing one (n = 1), two (n = 3), three (n = 3) and five (n = 1) sealed nests; ii) ten sealed nests with an unknown number of brood cells; iii) four sealed nests (with 2, 4, 9 and 10 prepupae, respectively), which were longitudinally split into two halves, closed with rubber band and opened again for the experiments; and iv) five sealed nests (with 4, 5, 7, 10 and 12 prepupae, respectively), which were longitudinally split into two halves, provided with a single rectangular window exactly corresponding to the position of a cell and closed with rubber band, resulting in one exposed prepupa within its cocoon (Fig. 5) and 3–11 prepupae well protected inside the stem; due to the semitransparent wall of the cocoon, the behaviour of the exposed prepupa inside its cocoon could easily be observed under

Table 1. Description of experiments on prepupal sound production.

Experimental question	Experiment number	Experimental procedure	Nest type
Do the prepupae spontaneously produce sounds?	1	Eight nest bundles were auscultated for 15 min during sunny weather from a short distance.	i
	2	Ten nests were auscultated together during one hour from a short distance.	ii
Which disturbances cause the prepupae to produce sounds?	3	Ten nests were individually subjected to a strong movement by turning the stems five times from a vertical to a horizontal position and back.	ii
	4	Four nests were individually subjected to a strong increase in temperature to 40 °C by irradiating them with a 150 watts infrared heat lamp (Beurer IL21) for 90 sec from a distance of 20 cm.	iii
	5	Ten nests were individually subjected to vibration by holding a vibrating small tuning fork to the stem wall.	ii
	6	Exposed prepupae in five nests were individually subjected to light by illuminating them with a torch for two minutes from a distance of 2 cm.	iv
Is the production of stroking and tapping sounds by a single prepupa linked?	7	In five nests, sound production by the exposed prepupa was recorded after it was stimulated to produce stroking sounds by carefully denting its cocoon wall with a stick.	iv
	8	In ten nests, the duration of the tapping sounds was recorded after the nests were individually turned five times from a vertical to a horizontal position and back.	ii
Do the sounds produced by a prepupa trigger sound production by other prepupae within the same nest?	9	In five well-fixed nests, the exposed prepupa was stimulated to produce stroking sounds by carefully denting its cocoon wall with a stick, before the nests were auscultated for stroking and tapping sounds of other prepupae.	iv
	10	In five nests the exposed prepupa was stimulated to produce tapping sounds by illuminating it with a torch, before the nests were auscultated for stroking and tapping sounds of other prepupae.	iv



Figures 1–5. *Hoplitis tridentata* **1** nest entrance in a dead stem of *Verbascum* **2** female entering her nest at the broken tip of a dead stem of *Verbascum* (photo A. Krebs) **3** linear series of brood cells within a dead stem of *Rubus* each containing a prepupa inside the cocoon **4** trapnest bundle consisting of five 50 cm long stems of *Rubus* **5** experimental nest stem with exposed brood cell.

good light conditions. In all experiments, the stroking sounds were registered by ear, whereas the tapping sounds were perceived with the aid of a bat detector (SSF Bat2, microelectronic Volkmann) set to 30 kHz and held at short distance from the stem. It proved to be impossible to differentiate between the sounds of different prepupae through the stem wall, i.e. to judge whether successive sounds were produced by one or more individuals; to address this inaccuracy, we apply the term stroking or tapping sound “event”, which is defined as sound production by at least one prepupa. The ambient temperature for the field and the lab experiments was 23–25 °C. Experiments 1 and 8 were run once, experiments 2–5 twice and experiments 6, 7, 9 and 10 three times. The results of the different runs were pooled for each experiment.

Sound producing structures in other osmiine bees

To address the question whether the prepupae of other osmiine bees are equipped with similar sound producing structures like *Hoplitis tridentata*, we examined the prepupae of the following eleven Central European species belonging to four genera and ten subgenera obtained from nests collected by the first author in 2020 and by P. Bogusch in the frame of studies on reed gall inhabiting aculeate Hymenoptera (Bogusch et al. 2015): *Chelostoma* (*Chelostoma*) *florisomne* (Linné), *C.* (*Gyrodromella*) *rapunculi* (Lepeletier), *Heriades* (*Heriades*) *truncorum* (Linné), *Hoplitis* (*Alcidamea*) *leucomelana* (Kirby), *H.* (*Hoplitis*) *adunca* (Panzer), *O.* (*Helicosmia*) *caerulescens* (Linné), *O.* (*Hoplosmia*) *spinulosa* (Kirby), *O.* (*Melanosmia*) *uncinata* Gerstäcker, *O.* (*Metallinella*) *brevicornis* (Fabricius), *O.* (*Osmia*) *bicornis* (Linné) and *O.* (*Osmia*) *cornuta* (Latreille). Furthermore, we reviewed the literature on osmiine bee larvae for possible indications that the prepupae possess sound producing structures, such as: i) clypeus projecting over the antennae in lateral view, ii) colour of the clypeus differing from that of the surrounding cuticle, iii) presence of a projecting horseshoe-shaped ridge around the anus, and iv) colour of the area around the anus differing from that of the surrounding cuticle. In total, we checked larval descriptions for 43 species belonging to eight genera and 19 subgenera.

Results

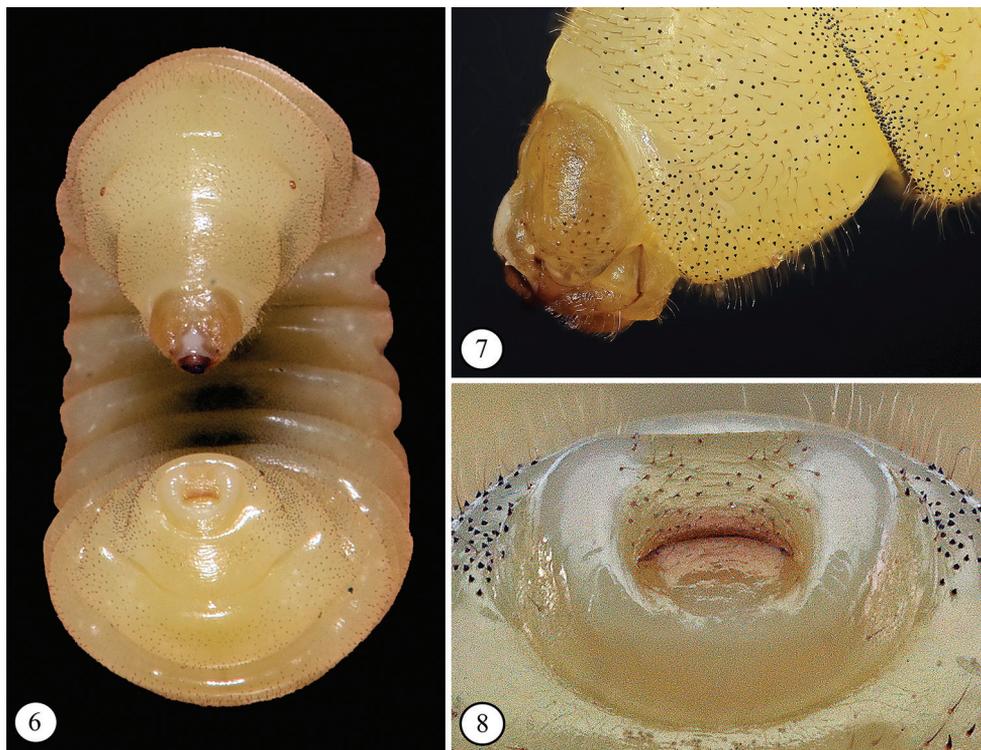
Prepupal sounds

The prepupae of *Hoplitis tridentata* produce two types of sounds, which considerably differ in their intensity. In unopened nest stems and under complete silence, the “tapping sounds” are audible by bare ear only within 10–20 cm (Suppl. material 1), whereas the “stroking sounds” can be heard up to a distance of 2–3 m (Suppl. material 2). The different loudness is also reflected by the strength of the vibrations that can be felt while touching the nest stem: the tapping sounds cannot be felt with the fingertips, whereas the stroking sounds are clearly perceptible.

The prepupae produce the sounds with two projecting body callosities, which are localized on the clypeus and around the anus (Figs 6–8). The clypeal callosity is of roughly triangular shape, while the anal callosity has the form of a horseshoe-shaped ridge, which surrounds the anus. The two callosities are distinctly harder than the surrounding cuticle and stand out by their (snow-)white coloration; in rare cases, the clypeal callosity is completely and the anal callosity partly brownish pigmented.

In larvae that have either started to spin their cocoon or just have finished cocoon construction, the callosities have not yet reached their final functional state: compared with the final state, the clypeal callosity is distinctly softer albeit already white and projecting, while the anal callosity is distinctly softer, still of the same colour as the surrounding cuticle and less projecting. Thus, the callosities seem to reach their functional state only after the prepupae have finalized the cocoon.

The prepupae produce the tapping sounds by knocking the clypeal callosity against the cocoon wall (Fig. 9; Suppl. material 5); each contact with the cocoon wall results in one tapping sound. The tapping sounds are usually produced in long uninterrupted series of one to four knocks per second. To produce the stroking sounds, the prepupae



Figures 6–8. *Hoplitis tridentata* **6** prepupa with a triangularly shaped white callosity on the clypeus and a horseshoe-shaped white callosity around the anus, which both are used for sound production **7** clypeal callosity in profile distinctly projecting over the antennae (photo J. Sommerhalder) **8** anal callosity (photo J. Sommerhalder).

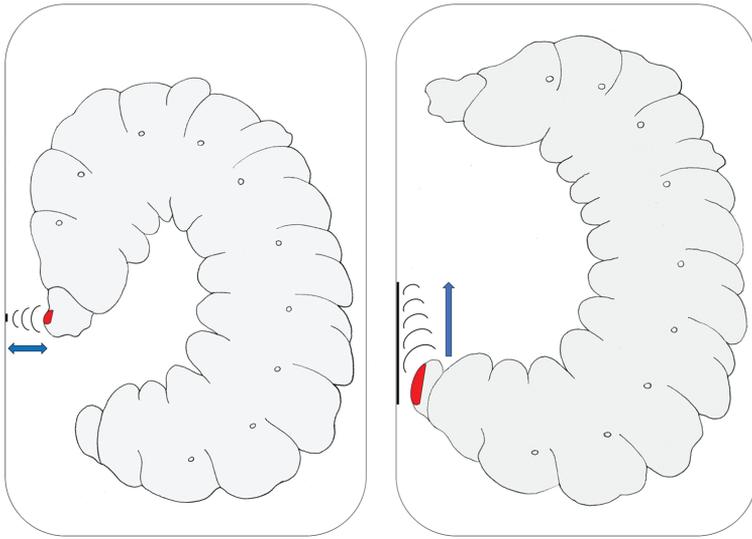


Figure 9. Sound production in prepupae of *Hoplitis tridentata*. The tapping sounds (left) are produced by knocking the clypeal callosity against the cocoon wall and the stroking sounds (right) by rapidly moving the anal callosity over the cocoon wall.

stretch their body and bring the abdominal tip in contact with the cocoon, before the anal callosity is rapidly pulled forward over the cocoon wall (Fig. 9; Suppl. material 6); each pull results in one stroking sound. The stroking sounds are produced one to maximally five times in quick succession and never in long series as the tapping sounds. After stroking, the prepupa invariably starts producing tapping sounds (see Experiments section). Although not substantiated by data, the number of tapping sounds per second and the number of consecutive stroking sounds seem to increase with increasing intensity of the disturbance.

Except for small wart-like protuberances, the inner cocoon wall of *Hoplitis tridentata* lacks special projections such as ridges or teeth, which might help in producing or amplifying the sounds when the prepupae move their anal callosity over the cocoon wall. The wart-like protuberances are unlikely to participate in sound production as their density considerably varies between the cocoons of different individuals, as they are not confined to defined zones of the inner cocoon surface and as similar protuberances also occur in the cocoons of other osmiine bee species (A. Müller, unpublished data). Nevertheless, the cocoon wall might play an important role in sound production going well beyond its function as a mere abutment for the sound producing structures. In fact, even a slight touch of the cocoon wall by the experimenter leads to a crackling sound readily audible both to the unaided ear and by a bat detector set to the ultrasonic range. It would be worthwhile to compare the physical and morphological properties of the cocoon of *H. tridentata* with those of related *H. (Alcidamea)* species, which do not produce sounds. Such a comparison, however, is beyond the scope of this study.

Table 2. Basic temporal and spectral characteristics of the prepupal sounds of *Hoplitis tridentata*. Mean values are given with standard deviation in brackets. In total, 2592 tapping sounds and 148 stroking sounds were measured.

Sound characteristics	Tapping sounds	Stroking sounds
Duration	0.63 (± 0.48) ms	40.6 (± 16.0) ms
Number of syllables	1	15.3 (± 7.2)
Interval between syllables	–	2.8 (± 0.64) ms
Duration of syllable	0.63 (± 0.48) ms	0.48 (± 0.33) ms
Duration of syllable (90% energy sum)	0.46 (± 0.30) kHz	0.38 (± 0.24) kHz
Lower frequency (5% energy sum)	11.91 (± 4.60) kHz	12.07 (± 3.70) kHz
Peak frequency (peak energy)	23.94 (± 9.56) kHz	24.44 (± 8.13) kHz
Upper frequency (95% energy sum)	54.31 (± 13.16) kHz	56.22 (± 11.70) kHz
Bandwidth of syllable (90% energy sum)	42.39 (± 12.24) kHz	44.15 (± 11.64) kHz

Sound characteristics

The tapping sounds are extremely brief lasting less than 1 ms and consist of a single syllable (Table 2, Fig. 10). In contrast, the stroking sounds are considerably longer with a mean duration of 40 ms and consist of an average of 15 syllables (Fig. 11). The single syllables of a stroking sound are separated by a temporal spacing of approximately 2.8 ms and can only be perceived by the human ear after a strong time expansion of $10 \times$ (Suppl. material 3). The composition of the stroking sounds from single syllables suggests that the anal callosity is jerkily rather than evenly moved over the cocoon wall.

The tapping sounds and the single syllables of the stroking sounds show very similar temporal and spectral characteristics (Table 2). Both are very brief and explosive

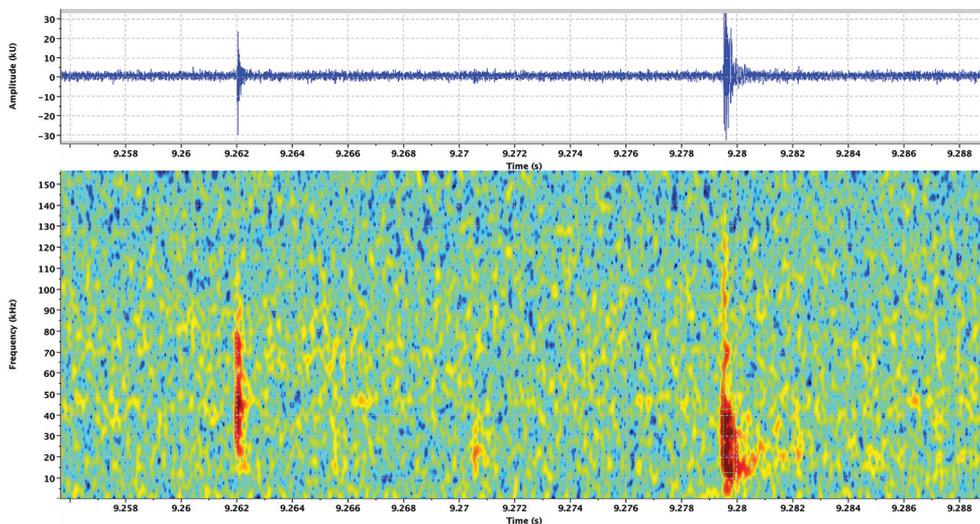


Figure 10. Amplitude plot (above) and spectrogram (below) of two prepupal tapping sounds of *Hoplitis tridentata* originating from two individuals inhabiting the same nest; the sounds were recorded with a batlogger in a distance of 20 cm from the opened nest stem.

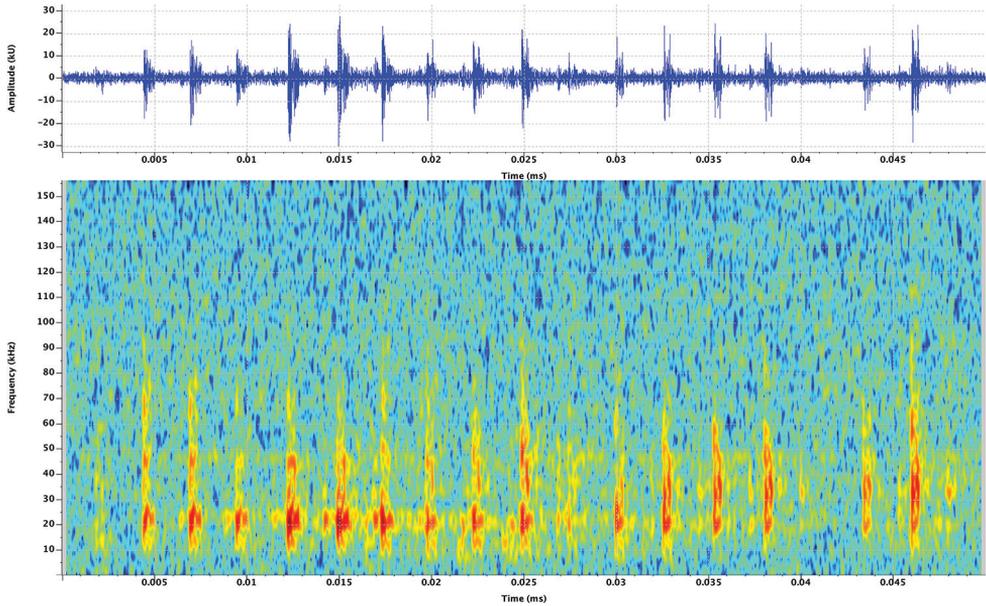


Figure 11. Amplitude plot (above) and spectrogram (below) of a prepupal stroking sound of *Hoplitis tridentata* of 46 ms duration containing 15 syllables; the sounds were recorded with a batlogger in a distance of 20 cm from the opened nest stem.

containing very few non-sinusoidal waves resulting in very broad frequency spectra, which regularly reach into the ultrasonic range, occasionally even above 100 kHz.

Experiments on prepupal sound production

Spontaneous prepupal sounds were absent (experiment 1) or very rare with 0.4 stroking and 0.15 tapping sound events per stem and hour (experiment 2). Movement of the stem and increase in temperature (experiments 3 and 4) stimulated prepupal sounds in every trial ($n = 20$ and $n = 8$, respectively), all of which were both stroking and tapping sound events. Vibration of the stem (experiment 5) stimulated prepupal sounds in 95% of the trials ($n = 20$), of which 32% were both stroking and tapping sound events and 68% only tapping sound events. Exposure to light (experiment 6) stimulated prepupal sounds in 87% of all trials ($n = 15$), of which 8% were both stroking and tapping sound events and 92% only tapping sound events. Stimulated stroking sounds (experiment 7) were followed by tapping sounds by the same prepupa in every trial ($n = 15$). Tapping sound events after the end of a disturbance (experiment 8) lasted on average 21.1 min and ranged from 8.5 min to 36.3 min ($n = 10$). Stimulated stroking sounds (experiment 9) triggered sound production by other prepupae within the nest in 93% of the trials ($n = 15$), of which 71% were both stroking and tapping sound events, 22% only tapping sound events and 7% only stroking sound events. Stimulated tapping sounds (experiment 10) never triggered sound production

by other prepupae within the nest ($n = 15$). In summary, the experiments revealed that i) the prepupae do not or only exceptionally produce sounds spontaneously, ii) the disturbances stimulating sound production are rather unspecific encompassing stem movement and vibration, increase in temperature, exposure to light and denting of the cocoon wall, iii) the tapping sounds and the stroking sounds appear to represent two levels of escalation with the former being produced after a weak disturbance but the latter only after a strong disturbance, iv) the stroking sounds are followed by extended periods of tapping sounds after the disturbance has ended, whereas the stroking sounds stop within 10–15 sec to maximally 30 sec after the end of the disturbance, and v) the stroking sounds trigger sound production by other prepupae of the same nest, which is not the case for the tapping sounds.

Sound producing structures in other osmiine bees

The prepupae of eleven Central European osmiine bee species belonging to four genera and ten subgenera (see Methods section) all lacked clypeal and anal callosities. The descriptions of osmiine bee prepupae in the literature either did not suggest the presence of sound producing structures or proved to be insufficient for a proper assessment (Suppl. material 7). However, a few Nearctic *Hoplitis* (*Alcidamea*) species, such as *H. biscutellae* (Cockerell), *H. hypocrita* (Cockerell) and probably also *H. fulgida* (Cresson), *H. producta* (Cresson) and *H. uvulalis* (Cockerell), were recorded by Rozen and Praz (2016) to possess a “projecting unpigmented ridge ringing anus except for ventral one-quarter”, which corresponds well to the anal callosity of *H. tridentata* and might suggest the presence of sound producing structures also in these species. This suggestion also holds for the Palaearctic species *H. (Alcidamea) acuticornis* (Dufour and Perris), whose prepupae were observed by Enslin (1925) to strongly move upon disturbance exactly as *H. tridentata* does when producing stroking sounds. Interestingly, these six *H. (Alcidamea)* species do not only belong to the same subgenus as *H. tridentata* but also nest in plant stems, either obligatorily, preferentially or regularly (Graenicher 1905; Comstock 1924; Enslin 1925; Hicks 1926, 1934; Rau 1928; Linsley and MacSwain 1943; Michener 1947; Fischer 1955; Hurd and Michener 1955; Medler 1961; Parker and Bohart 1966, 1968; Parker 1975; Clement and Rust 1976; Rust 1980; Tepedino and Parker 1984; Frohlich et al. 1988). In summary, sound producing structures similar to those of *H. tridentata* appear to be absent in most osmiine bee taxa with the possible exception of a few related stem-nesting *H. (Alcidamea)* species in both the Palaearctic and the Nearctic region.

Discussion

The finding that the prepupae of *Hoplitis tridentata* produce well audible sounds is the first record of sound production in larvae of bees and – to the best of our knowledge – also of Hymenoptera. These prepupal sounds are almost exclusively produced after

disturbance, which qualifies them as typical disturbance sounds (Drosopoulos and Claridge 2005). Although disturbance sounds are widespread in insects (see Introduction section), their function has been examined only in a few species, where they were unambiguously found to deter antagonists (Bauer 1976; Smith and Langley 1978; Masters 1979; Lewis and Cane 1990; Olofsson et al. 2012). It is most likely that the prepupal sounds of *H. tridentata* also serve an anti-antagonist function, which would be highly adaptive as the prepupae spend many months within exposed and rather thin-walled plant stems, where they are substantially more susceptible to predators and parasites than the offspring of bee species developing in other substrates, for example in the ground or in dead wood.

The spectrum of potential antagonists affecting *Hoplitis tridentata* in the prepupal stage encompasses two main groups, i.e. vertebrate predators such as birds, which peck open the stems and devour the prepupae, and insect brood parasites such as parasitoid wasps, which insert the eggs through the stem wall and whose larvae feed on the prepupal bodies. These two groups of antagonists perceive vibrational signals differently, i.e. the predators mainly as air-borne sounds and the parasites probably exclusively as substrate-borne vibrations. As the sounds produced by the prepupae of *H. tridentata* are both air-borne and substrate-borne, the quality of the prepupal sounds does not allow us to decide whether the sounds have evolved against vertebrate predators or insect brood parasites. Similarly, the disturbances that stimulated prepupal sound production in the experiments hardly allow any conclusions on the natural triggers of the sounds and suggest that the prepupae react rather unspecifically to any disturbance whether naturally occurring or not. As discussed below, we nevertheless hypothesize that the intended recipients of the prepupal sounds are insect brood parasites, that the sounds act as substrate-borne vibrations, and that the sounds are triggered by the presence of parasites on the nest stem.

Among the three potential mechanisms which might underlie the deterrent effect of disturbance sounds (see Introduction section), acoustic aposematism is highly unlikely to act in *Hoplitis tridentata* because the prepupae are unlikely to be toxic or in any other way dangerous for vertebrate predators and insect brood parasites. It appears also to be improbable that the disturbance sounds have evolved to startle a predator or parasite because the prepupae cannot take advantage of the attacker's short-term confusion for escape as they are enclosed within their cocoons; furthermore, startling vertebrate predators by sound seems to be counteradaptive as the predators might learn to use the sounds to localize nests after they have found that the prepupae are harmless. Instead, we hypothesize that sound production in *H. tridentata* has evolved to render it difficult for parasitoid wasps with a peculiar host-searching strategy to precisely localize the prepupae within the plant stem.

Parasitoid wasps usually localize hidden hosts by scent or vibrations caused by host movement and feeding (Xiaoyi and Zhongqi 2008). However, numerous ichneumonid wasps of several subfamilies as well as orussid wasps employ a special form of echolocation to localize deeply concealed and often immobile larvae, prepupae and pupae of their hosts – a host-searching strategy known as vibrational sounding (Henaut

and Guerdoux 1982; Wäckers et al. 1998; Broad and Quicke 2000; Vilhelmsen et al. 2001; Otten et al. 2002; Fischer et al. 2003; Laurenne et al. 2009). During vibrational sounding, the wasps transmit vibrations through potential host substrate by drumming their modified antennal tips onto the substrate surface and gain information on host occurrence and host position based on the reflected signals, which are perceived by the subgenual organs in the tibiae of all legs (Otten et al. 2002). We suggest that the substrate-born vibrations induced by sound producing prepupae of *H. tridentata* might interfere with the perception of the reflected signals by the echolocating wasps. As the wasps applying vibrational sounding probably have to perceive very small differences in the arrival times of the reflected signals between fore, middle and hind legs to identify the exact position of the host inside the substrate relative to their body (Otten et al. 2002), the vibrations produced by the prepupae of *H. tridentata* might be highly effective in impairing the process of host localization by echolocation.

There are indeed two ichneumonid species of the genus *Hoplocryptus* (Cryptinae) among the known wasp parasitoids of *Hoplitis tridentata* (see Bee species section), which possess strongly modified antennal tips in the females (Laurenne et al. 2009), clearly indicating that these antagonists of *H. tridentata* apply vibrational sounding for host location. Two properties of the sounds produced by the prepupae of *H. tridentata* also support the hypothesis that the prepupal sounds serve to impair host finding by echolocating wasps. First, the tapping sounds were found to be continued for a surprisingly long period of up to more than 30 min after the disturbance has ended; parasitoid wasps often take prolonged walks over the substrate before localizing concealed hosts; thus, prolonged tapping by the prepupae may be a strategy to disturb the wasp's host localization during its surface exploration. Second, the sounds produced by the prepupae after a strong disturbance were found to trigger sound production by other prepupae inhabiting the same nest resulting in a simultaneous albeit unsynchronized percussion by several individuals; such a "chorus" of prepupae tapping from different positions within the nest stem (Suppl. material 4) is expected to reinforce the disturbance effect against echolocating wasps and to expand it along the whole stem, thereby acoustically concealing the exact position of the prepupae within the nest. A similar chorusing behaviour is known from the larvae of the Palaearctic cerambycid beetle *Icosium tomentosum* Lucas, which often develop in groups between wood and the bark of thin dry branches of Cupressaceae and produce disturbance sounds by scraping their strongly sclerotized mandibles against the inner bark surface (Kočárek 2009); the sound produced by the beetle larva of *I. tomentosum* consists of long series of up to eight very short pulses per second, lasts up to three minutes beyond the end of the disturbance and induces sound production by other larvae occupying the same or a nearby branch resulting in a chorus of several simultaneously scraping larvae. The striking similarities between *H. tridentata* and *I. tomentosum* not only with respect to the chorusing behaviour but also with respect to development place, aggregated occurrence of larvae and sound quality suggest that the disturbance sounds of these two unrelated taxa probably have evolved against the very same antagonists. Indeed, the chorusing behaviour of *I. tomentosum* is hypothesized by Kočárek (2009) to be an adaptation to reduce the success of parasitoid wasps and predators in locating their hosts.

The prepupae of *Hoplitis tridentata* produce two types of sounds, which differ in several characteristics. The tapping sounds, which are produced by knocking the clypeal callosity against the cocoon wall, are quiet and require little energy; they are readily induced by a weak disturbance and continued long beyond the end of the disturbance, and they do not trigger sound production by other prepupae. In contrast, the stroking sounds, which are produced by moving the anal callosity over the cocoon wall, are loud and require much energy; they are induced only after a strong disturbance and stop shortly after the disturbance has ended, and they trigger sound production by other prepupae. These different characteristics suggest that the two types of sounds have different functions, which however are most probably linked and work in combination. We envisage the following scenario for the two sounds to work together: when one prepupa perceives the presence of a parasitoid wasp near its cell due for example to vibrations caused by the drumming wasp antennae or by the insertion of the wasp ovipositor, it starts to stroke; the vibrations elicited by this stroking spread through the stem and alert other prepupae, which immediately begin to stroke and/or tap, eventually resulting in a continuing percussion by numerous prepupae along the whole nest stem. Under this scenario, the main function of the tapping sounds is to impair host location by echolocating parasitoid wasps, whereas the main function of the stroking sounds is to induce sound production by alerting other nest inhabitants. As the nest inhabitants are siblings, the simultaneous percussion by the prepupae of *H. tridentata* can be regarded as an extraordinary form of collaboration, which contributes to the inclusive fitness of all individuals within the nest.

Conclusions

The production of sounds by the larvae of bees as reported in this study for the stem-nesting osmiine bee *Hoplitis tridentata* is a new facet in the fascinating biology of solitary bees as is the suspected collaboration against antagonists between siblings inhabiting the same nest. While there is little doubt that the prepupal sounds of *H. tridentata* serve an anti-antagonist function, the assumption that they have evolved to disturb host location by echolocating wasp parasitoids is for now speculative and has to be tested experimentally.

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Supplementary material 1

Tapping sounds of a single prepupa of *Hoplitis tridentata*

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sounds were recorded with a batlogger in a distance of 2 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl1>

Supplementary material 2

Four stroking sounds followed by tapping sounds of a single prepupa of *Hoplitis tridentata*

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sounds were recorded with a batlogger in a distance of 2 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl2>

Supplementary material 3

One stroking sound of a single prepupa of *Hoplitis tridentata* slowed down ten times

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sound was recorded with a batlogger in a distance of 20 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl3>

Supplementary material 4

Simultaneous percussion by several tapping prepupae of *Hoplitis tridentata* inhabiting the same nest

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sounds were recorded with a batlogger in a distance of 20 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl4>

Supplementary material 5

Two prepupae of *Hoplitis tridentata* producing tapping sounds

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: Two prepupae of *Hoplitis tridentata* producing tapping sounds by continuously knocking the clypeal callosity against the cocoon wall; the right brood cell contains a cocoon of *Sapyga quinquepunctata*.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl5>

Supplementary material 6

Prepupa of *Hoplitis tridentata* producing stroking sounds

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: Prepupa of *Hoplitis tridentata* (left brood cell) producing stroking sounds by rapidly moving the anal callosity five times in quick succession over the cocoon wall; note that the prepupa in the middle brood cell is tapping; the right brood cell contains a cocoon of *Sapyga quinquepunctata*.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl6>

Supplementary material 7

Literature review on potential sound producing structures in the prepupae of osmiine bee species other than *Hoplitis tridentata*

Authors: Andreas Müller

Data type: species data

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