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Extraordinary drilling capabilities of the tiny parasitoid *Eupelmus messene* Walker (Hymenoptera, Eupelmidae)

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Abstract

In the course of evolution, animals and particularly insects, have developed efficient and complex mechanisms for survival. Biomimetics aims to find applications for these features of organisms (or organs) in industry, agriculture, and medicine. One of these features is the thin, flexible, and mobile insect ovipositor, which is also capable of carrying substances and drilling various substrates, usually of plant origin. Despite the well-studied structure of the ovipositor, the principles of its operation and real possibilities remain poorly understood. In our study, we first discovered an unusual behavioral pattern of oviposition of the female parasitoid *Eupelmus messene* Walker (Hymenoptera: Eupelmidae): she drilled with her ovipositor through the wall of a polystyrene Petri dish and laid her egg outside the dish. Due to the transparency of the plastic, we described the technique of ovipositor movement and studied its structure using scanning electron microscopy. Our research may contribute to developing minimally invasive guided probes and various other instruments.

Keywords

Chalcidoidea, gall, oviposition, ovipositor structure, parasitoid

Introduction

Insects inspire researchers to develop new biomimetic substances for industry and biomedicine (Elvin et al. 2005); to improve mobility, strength, and control systems of robots; to develop micro-electro-mechanical devices (MEMS) for supersensitive sensory systems in medicine, etc. (Gorb 2011); to produce new materials (Scarangella et al. 2020).

One of the examples of structures that may be beneficial for bionics is the ovipositor which in parasitoid wasps is a flexible and thin egg-laying organ used to drill holes in various natural materials, for example, host puparia, plant buds, stems, and galls (Askew 1971; Peters and Abraham 2010; Polidori et al. 2013). Studying the drilling process in nature is challenging due to the dense structure of plant substrates (for ex., fig syconia) (Kundanati and Gundiah 2014). Transparent gel-like substrates on the other hand, allow a detailed investigation of ovipositor insertion and steering (Gouache et al. 2010; Quicke 2014; Cerkvenik et al. 2017; van Meer et al. 2020); however, the mechanisms may differ due to the differences in stiffness of natural and artificial substrates.

Here we present the first observation of drilling and egg laying by the parasitoid wasp *Eupelmus messene* Walker (Hymenoptera: Eupelmidae) in a transparent and solid substrate – wall of a polystyrene Petri dish.

Eupelmus messene, which was recently separated from its synonym *Eupelmus vesicularis* Retzius (Hymenoptera: Eupelmidae) by Fusu (2017), is a 2–3 mm wasp. Among other hosts, it is a parasitoid of the gall wasp *Aulacidea hieracii* Linnaeus (Hymenoptera: Cynipidae). The latter forms a gall on the stems of the hawkweed *Hieracium × robustum* Fr. (Anikin and Nikelshparg 2017). The female of *E. messene* drills the walls of the gall with her ovipositor in search of a gall wasp larva and, upon finding it, lays an egg next to it (Gokhman and Nikelshparg 2021). Study of the drilling the gall is challenging due to its opaque and dense structure, whereas the transparency of the polystyrene made it possible to describe the drilling process and oviposition in detail.

Methods

Insects

We reared 56 females from galls of *H.× robustum* collected in the fields near the city of Saratov, Russia (51°32'00"N, 46°00'00"E) during the years 2017–2023. 18 females were used to observe drilling through plastic in the Petri dishes without galls while 38 were used to observe drilling of their natural host in the Petri dishes with galls. Each female was moved to a separate Petri dish, excluding the first specimen that showed such a behavior. This wasp was transferred to the sterile Petri dish later after drilling 3 perforations. *Eupelmus messene* was kindly identified by Lucian Fusu (Faculty of Biology, Alexandru Ioan Cuza University, Iasi, Romania) and V. E. Gokhman (Botanical Garden, Moscow State University, Moscow, Russia). The voucher specimen of the *E. messene* has been kindly deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes by Gary A. P. Gibson (Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada).

Drilling process visualization

To study the drilling process, we placed *E. messene* females into a polystyrene Petri dish (diameter 90 mm, wall thickness 1 mm, manufactured by MiniMed), kept at a temperature of 23–25 °C and relative humidity of 30–40%, and provided with food (diluted sugar syrup) and water. The control lot drilled galls on *H.* × *robustum*. Photo and video materials were obtained using a Canon S100 camera and a Micromed MC-2-ZOOM stereoscopic microscope. About 1000 video segments were captured (from 1 to 180 minutes each) to analyze *E. messene* behavior. Measurement of ovipositor movements were made from the videos using the software Movavi Video Editor Plus 2021. Fig. 1A–C and Suppl. material 1 contain information about one female whereas the statistical analysis of the movements was made using the video data of all females that drilled the plastic and galls in the period 2017–2023.

Scanning electron microscopy imaging

The insect was studied with the scanning electron microscope (SEM Jeol JSM-6380) after fixation in 70% ethanol, dehydration (rising ethanol series and acetone), critical point drying (Hitachi HCP-2), and gold coating (Giko IB-3).

Results

Out of 18 females that were placed in Petri dishes without host galls, only 5 started drilling the wall of the Petri dish (Fig. 1A). Also, 3 wasps drilled the plastic in the other group of 38 females despite having the host galls inside the Petri dish. Each drilled between one and five holes (mean±SE = 1.75±0.5). The process of drilling each perforation in the polystyrene wall took more than two hours. Periodically the process was interrupted by feeding, drinking, or washing, after which the female found the same place in the Petri dish and continued drilling. In the end, the insect managed to complete the perforation in the plastic wall and lay one egg on the outside of the Petri dish (Fig. 1B, See Suppl. material 1). This process was repeated 4 more times, by the same female. After the female drilled 3 holes, it was transferred to a new Petri dish, where she drilled 2 more times. We recorded the drilling of four perforations out of five (See Suppl. material 1).

Several strictly sequential movement patterns for drilling a polystyrene dish can be distinguished:

1. Pushing movements resembling shaking, with a frequency of 5.7 ± 0.5 Hz and small amplitude, 0.21 ± 0.07 mm;
2. Rotational movements – the insect rotates the ovipositor in both directions up to 360 degrees – such movements were often combined with pushing movements;
3. Ejection movements, jerky movements of the ovipositor towards the insect body with a frequency of 0.8 Hz and an amplitude of 0.58 ± 0.07 mm. To move upwards, *E. messene* straightened its legs and partially retracted the ovipositor inside the body but never completely removed it from the drilled perforation.

4. Cementing step, this is the longest uninterrupted step in oviposition, lasting about 6–7 minutes, resulting in dome-shaped insulation at the entrance of the perforation, consisting of the UV fluorescent biological substance (Fig. 1C).

Drilling of natural substance (a plant gall) differs from drilling the polystyrene substrate. We studied in detail the drilling by 9 *E. messene* females out of 38 at six winter galls on *H.* × *robustum*. The frequency of pushing movements was 4.66 ± 0.34 Hz while the amplitude was 0.24 ± 0.03 mm. Thus, such movements in galls are less frequent than in polystyrene. Rotational movements were also observed, but we never observed ejection movements while the wasp was drilling a gall.

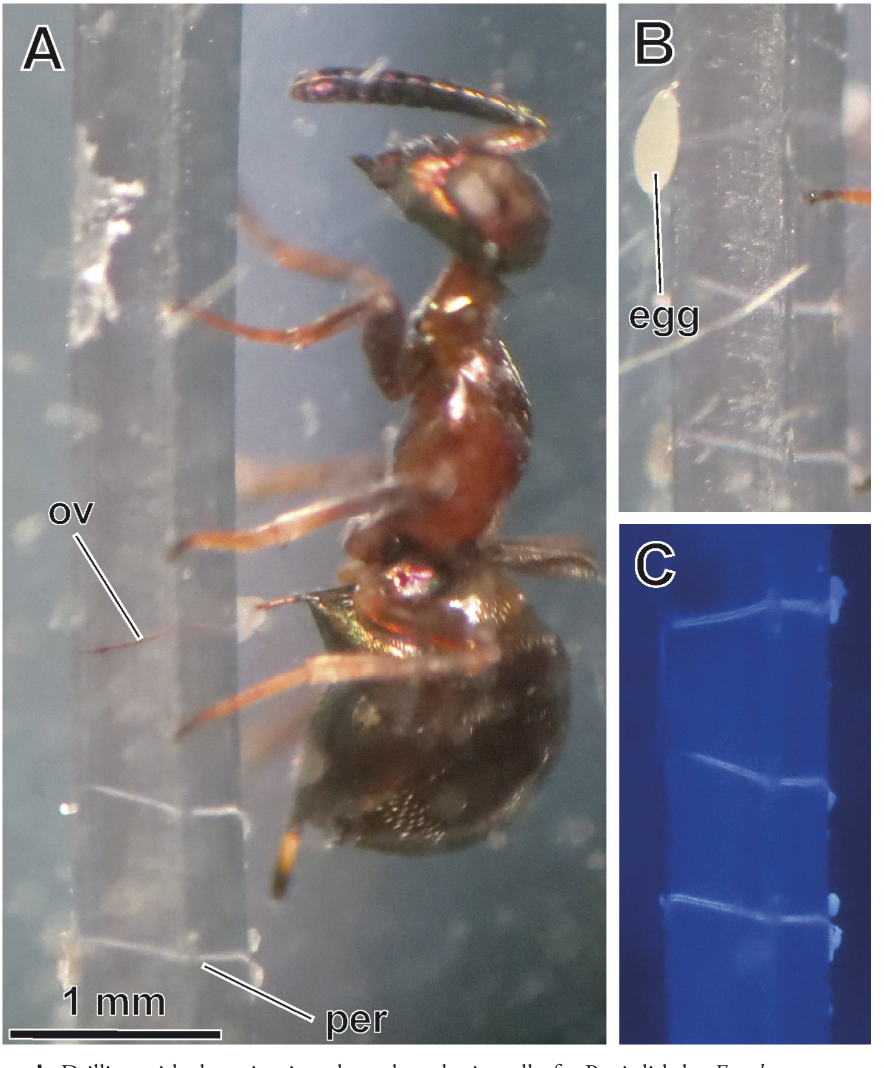

Figure 1. Drilling with the ovipositor through a plastic wall of a Petri dish by *Eupelmus messene* (**A**), a newly laid egg into the external environment (**B**), and UV fluorescent biological substance inside the perforations (**C**). ov – ovipositor, per – perforation, egg – egg.

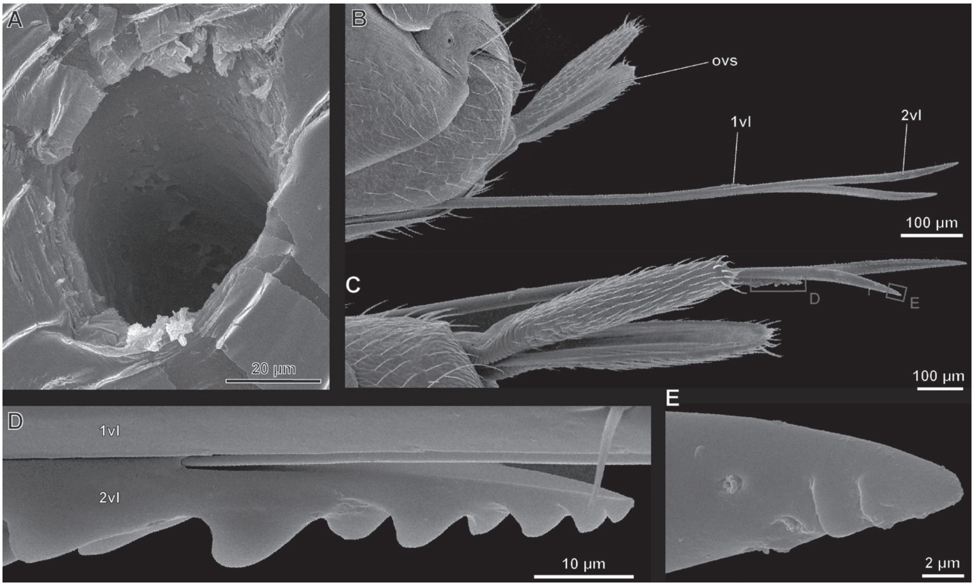

Figure 2. Ovipositor structure of the *Eupelmus messene* and perforation drilled in plastic, SEM **A** perforation in the Petri dish made by *E. messene* **B–E** structure of the *E. messene* ovipositor, lateral view: **B** apex of gaster **C** ovipositor **D** apex of 1st valvifer **E** apex of 2nd valvifer. ovs – ovipositor sheath, 2vl – 2nd valvula, 1vl – 1st valvula.

The structure of the ovipositor and the drilled perforation in the Petri dish was examined using a scanning electron microscope. The diameter of the drilled perforation is about 30 µm (Fig. 2A). As in all apocritans the ovipositor consists of paired ovipositor sheaths (3rd valvulae), paired 2nd valvulae that enclose a pair of 1st valvulae. The 1st and 2nd valvulae form the ovipositor stylets. The exerted part of the stylets in the measured specimen has a total length of 2 mm and diameter of 20 µm (Fig. 2B–E). The apical part of 1st valvula has a sawtooth structure (Fig. 2D). The length of this section is 70 µm, with about 6 teeth in a row; height of each tooth is 1 to 4.5 µm. We assume that with the help of the multiple teeth on the stylet, the *E. messene* not only cuts the perforation but also ejects excess material.

Discussion

We described an interesting case of *E. messene* spontaneous drilling in a polystyrene Petri dish wall for oviposition in the absence of a host stimulus. The reason for such behavior of the parasitoid wasp remains unknown. Since plant gall is opaque, the mechanism of egg-laying behavior has never been described in detail. The unusual behavior of the female allowed us to characterize the drilling patterns. We distinguished four steps of drilling: pushing movements, rotational movements, ejection movements, as well as the cementing step. However, in natural gall, we never observed ejection movements. We suppose that such a type of movement is required to rake out plastic particles, which is unnecessary for more elastic plant gall substrate. Also, we suggest that pushing movements in galls are less frequent than in plastic as gall has other material properties than polystyrene. Importantly, fresh summer galls are suggested to be less solid than winter ones used here. Thus, we assume that the frequency of pushing movements would be decreased even more. The process of drilling the polystyrene and gall tissue may differ due to the difference in stiffness, however, a more detailed study of the gall and polystyrene material properties should be conducted. To mimic the drilling of wasps, Cerkvenik et al. (2017) applied gellan gel. However, we suggest that polystyrene is a more relevant model material to study drilling and oviposition in natural substrates, especially autumn firm galls.

The transparency of the polystyrene allows for describing the features of drilling in a solid substrate invisible in natural materials. Studying in details the drilling behavior of parasitic mycrohymenopterans can be useful in medicine for the creation of minimally invasive guided probes in neurosurgery (Frasson et al. 2010; Ramadi et al. 2019), the development of orthopedic surgical instruments (Nakajima and Schwarz 2014), needle biopsies using functionally graded tools (Kundanati and Gundiah 2014). Due to the flexibility, potential hardness, and effective types of movement, ovipositor structures can be used to develop methods of vertical and directional drilling, whereas ultra-low mass and energy efficiency make it possible to use ovipositor features for studying space structures and celestial bodies, extraterrestrial drilling or sampling (Gouache et al. 2010). Some eupelmid species (such as *Eupelmus vuilleti* Craw (Hymenoptera: Eupelmidae) and *Eupelmus microzonus* Förster (Hymenoptera: Eupelmidae)) provide a protective web-like coating for their eggs (Leveque et al. 1993; Gokhman and Nikelshparg 2021), but *E. messene* has not previously shown any ability to protect its offspring. Due to the transparency of the plastic Petri dish, we found that after laying the egg, the female carefully cements the drilled perforation with a yet unknown biological substance. We suggest that this substance plays a role in isolation from temperature fluctuations, water, microorganisms, etc. It should be noted that it is practically impossible to detect such perforation cementing under natural conditions.

Conclusion

We revealed the phenomenon of *E. messene* drilling the plastic Petri dish using three types of ovipositor movements: pushing, rotational, and ejection. In the natural gall, the ejection movements were absent, and the pushing movements were less frequent in the gall than in plastic. Additionally, we provided the first evidence that *E. messene* isolates the perforation after the oviposition. The structure of the ovipositor was described with SEM. The proposed analysis may contribute to developing minimally invasive guided probes for medicine or methods of vertical and directional drilling of rocks.

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References

Anikin VV, Nikelshparg MI (2017) Details of parasitism by *Eupelmus* sp. (Hymenoptera: Eupelmidae) on *Aulacidea hieracii* (Hymenoptera: Cynipidae), a gall-maker of the hawkweed, *Hieracium virosum*. Entomological and parasitological studies in the Volga Region 14: 67–71. [In Russian]

Askew RR (1971) Parasitic insects. Heinemann Educational Bks., London. [ISBN 978-0435620455]

Cerkvenik U, van de Straat B, Gussekloo SWS, van Leeuwen JL (2017) Mechanisms of ovipositor insertion and steering of a parasitic wasp. Proceedings of the National Academy of Sciences of the United States of America 114: E7822–E7831. https://doi.org/10.1073/pnas.1706162114

Elvin CM, Carr AG, Huson MG, Maxwell JM, Pearson RD, Vuocolo T, Liyou NE, Wong DCC, Merritt DJ, Dixon NE (2005) Synthesis and properties of crosslinked recombinant pro-resilin. Nature 437: 999–1002. https://doi.org/10.1038/nature04085

Frasson L, Ko SY, Turner A, Parittotokkaporn T, Vincent JF, Rodriguez y Baena F (2010) STING: A soft-tissue intervention and neurosurgical guide to access deep brain lesions through curved trajectories. Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine 224(6): 775–788. https://doi.org/10.1243/09544119JEIM663

Fusu L (2017) An integrative taxonomic study of European *Eupelmus* (*Macroneura*) (Hymenoptera: Chalcidoidea: Eupelmidae), with a molecular and cytogenetic analysis of *Eupelmus* (*Macroneura*) *vesicularis*: several species hiding under one name for 240 years. Zoological Journal of the Linnean Society 181(3): 519–603. https://doi.org/10.1093/zoolinnean/zlw021

Gokhman VE, Nikelshparg MI (2021) *Eupelmus messene* Walker, 1839 and *E. microzonus* Förster, 1860 as parasitoids of *Aulacidea hieracii* (Bouché, 1834) (Hymenoptera, Eupelmidae, Cynipidae). Journal of Hymenoptera Research 84: 87–102. https://doi.org/10.3897/jhr.84.68556

Gorb SN (2011) Insect-Inspired Technologies: Insects as a Source for Biomimetics. In: Vilcinskas A (Ed.) Insect Biotechnology. Biologically-Inspired Systems, vol 2. Springer, Dordrecht, 241–264. https://doi.org/10.1007/978-90-481-9641-8_13

Gouache T, Gao Y, Gourinat Y, Coste P (2010) Wood Wasp Inspired Planetary and Earth Drill. In: Mukherjee A (Ed.) Biomimetics Learning from Nature, InTech Open, 467–486. https://doi.org/10.5772/8775

Kundanati L, Gundiah N (2014) Biomechanics of substrate boring by fig wasps. Journal of Experimental Biology 217(11): 1946–1954. https://doi.org/10.1242/jeb.098228

Leveque L, Monge J-P, Rojas-Rousse D, Van Alebeek F, Huignard J (1993) Analysis of multiparasitism by *Eupelmus vuilleti* (Craw) (Eupelmidae) and *Dinarmus basalis* (Rond) (Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic) (Coleoptera Bruchidae). Oecologia 94: 272–277. https://doi.org/10.1007/BF00341327

Nakajima K, Schwarz O (2014) How to use the ovipositor drilling mechanism of Hymenoptera for developing a surgical instrument in biomimetic design. International Journal of Design & Nature and Ecodynamics 9(3): 177–189. https://doi.org/10.2495/DNE-V9-N3-177-189

Peters RS, Abraham R (2010) The food web of parasitoid wasps and their non-phytophagous fly hosts in birds' nests (Hymenoptera: Chalcidoidea, and Diptera: Cyclorrhapha). Journal of Natural History 44: 9–10, 625–638. https://doi.org/10.1080/00222930903437317

Polidori C, García AJ, Nieves-Aldrey JL (2013) Breaking up the wall: Metal-enrichment in ovipositors, but not in mandibles, co-varies with substrate hardness in gall-wasps and their associates. PLoS ONE 8: e70529. https://doi.org/10.1371/journal.pone.0070529

Quicke DLJ (2014) The Ovipositor and Ovipositor Sheaths. In: Quicke DLJ (Ed.) The Braconid and Ichneumonid Parasitoid Wasps. John Wiley & Sons, Ltd, Chichester, UK, 35–55. [ISBN: 978-1-118-90705-4] https://doi.org/10.1002/9781118907085.ch3

Ramadi KB, Cima MJ (2019) Materials and devices for micro-invasive neural interfacing. MRS Advances 4: 2805–2816. https://doi.org/10.1557/adv.2019.424

Scarangella A, Soldan V, Mitov M (2020) Biomimetic design of iridescent insect cuticles with tailored, self-organized cholesteric patterns. Nature Communications 11: 4108. https://doi.org/10.1038/s41467-020-17884-0

van Meer NMME, Cerkvenik U, Schlepütz CM, van Leeuwen JL, Gussekloo SWS (2020) The ovipositor actuation mechanism of a parasitic wasp and its functional implications. Journal of Anatomy 237(4): 689–703. https://doi.org/10.1111/joa.13216

Supplementary material 1

Eupelmus messene drilling the wall of the polystyrene Petri dish

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Data type: mp4

Explanation note: A detailed captioned video recording of *Eupelmus messene* drilling the wall of the polystyrene Petri dish. ×10 and ×1.5 – speed is increased by 10 and 1.5 times, respectively.

Link: https://doi.org/10.3897/jhr.96.107786.suppl1