

Supplementary material:

Anatomical, phenological and genetic aspects of the host-parasite relationship between *Andrena vaga* (Hymenoptera) and *Stylops ater* (Strepsiptera)

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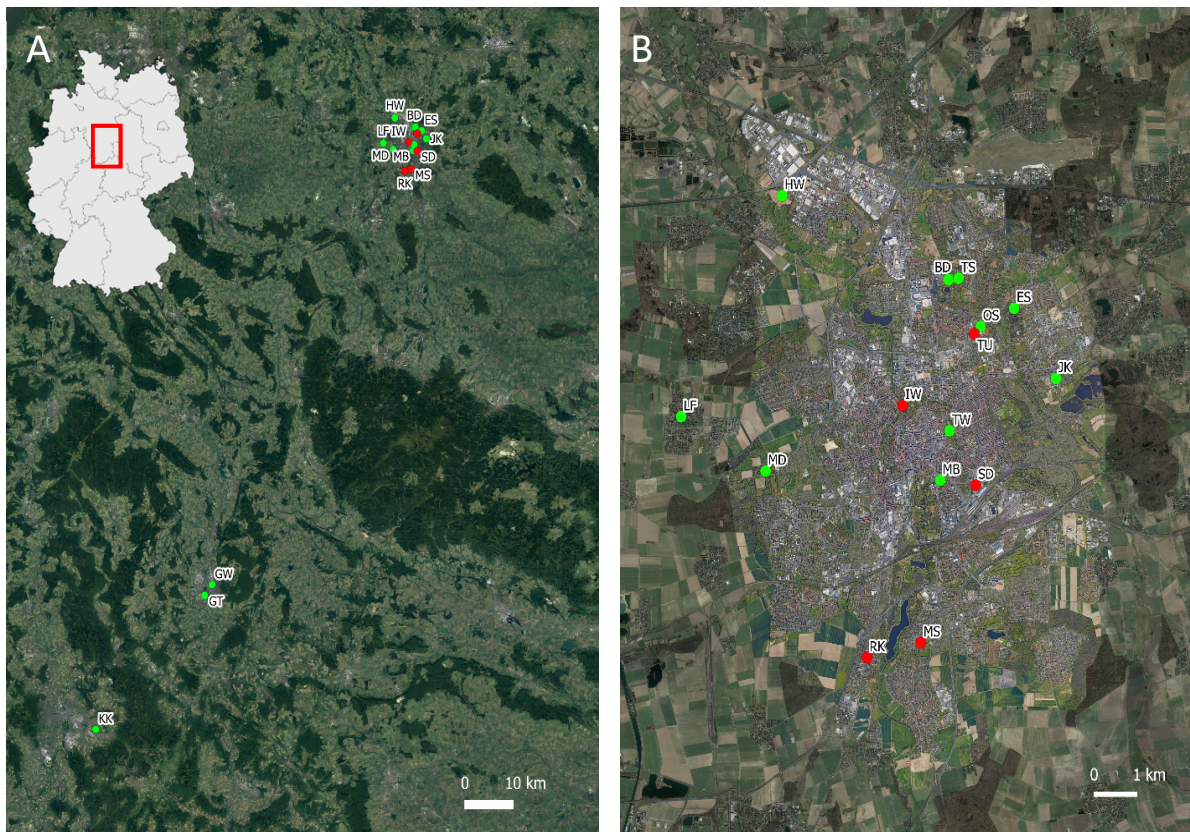


Figure S1: Maps of the study sites. (A) Location in Germany and spatial relation between sites in Braunschweig, Göttingen and Kassel. (B) spatial relation between sites in Braunschweig. Red dots show sites with high sample sizes that were selected for morphometrical analysis. Ground map: Google Maps (2021) Available at: <https://www.google.com/maps> (Accessed: 25th May 2022) Map crated using QGIS 3.4 'Madeira'

Table S1: Name and location of sites for *Andrena vaga* sampling.

| Site code | Site name | Site city | Coordinates (lat, lon) |
|-----------|---------------------------|--------------|------------------------|
| BD | Bahnübergang Dowesee | Braunschweig | 52.295526, 10.531960 |
| ES | Essener Straße | Braunschweig | 52.290064, 10.554394 |
| GT | Göttingen Tonkuhlen | Göttingen | 51.518852, 9.910113 |
| GW | Göttingen Stadtwall | Göttingen | 51.536757, 9.931576 |
| HW | Hafen Weggabelung | Braunschweig | 52.311458, 10.474944 |
| IW | Inselwall-Park | Braunschweig | 52.271593, 10.516237 |
| JK | Julius Kühn-Institut | Braunschweig | 52.276727, 10.568632 |
| KK | Kalkberg Kassel | Kassel | 51.294123, 9.586104 |
| LF | Lamme Friedhof | Braunschweig | 52.269460, 10.440353 |
| MB | Mühlbergweg | Braunschweig | 52.257347, 10.529043 |
| MD | Madamenweg Discgolfanlage | Braunschweig | 52.259126, 10.469246 |
| MS | Melverode Sportplatz | Braunschweig | 52.226508, 10.522466 |
| OS | Ottenroder Straße | Braunschweig | 52.286607, 10.542855 |
| RK | Rüningen Kirche | Braunschweig | 52.223645, 10.504000 |
| SD | Schillstraße Denkmal | Braunschweig | 52.256453, 10.541196 |
| TS | Theisenstraße | Braunschweig | 52.295768, 10.535313 |
| TU | TU Nordcampus | Braunschweig | 52.285188, 10.540780 |
| TW | Theaterwall-Park | Braunschweig | 52.266801, 10.532239 |

Table S2: Dates of *Andrena vaga* sampling.

| Site | Sampling date | Bees collected | Collector(s) |
|------|---------------|----------------|-----------------------------------|
| JK | 23.02.2021 | 26 | Marc Hoffmann |
| BD | 24.02.2021 | 7 | Jana Deierling |
| HW | 24.02.2021 | 4 | Jana Deierling |
| IW | 24.02.2021 | 29 | Marc Hoffmann |
| SD | 24.02.2021 | 47 | Harmen Hendriksma |
| MB | 24.02.2021 | 10 | Harmen Hendriksma |
| TW | 24.02.2021 | 21 | Marc Hoffmann |
| BD | 25.02.2021 | 14 | Jana Deierling |
| LF | 25.02.2021 | 11 | Dennis Leer |
| MB | 25.02.2021 | 7 | Henri Greil and Harmen Hendriksma |
| MD | 25.02.2021 | 25 | Dennis Leer |
| MS | 25.02.2021 | 46 | Henri Greil and Harmen Hendriksma |
| OS | 25.02.2021 | 12 | Dennis Leer and Jana Deierling |
| RK | 25.02.2021 | 55 | Henri Greil and Harmen Hendriksma |
| TS | 25.02.2021 | 20 | Jana Deierling |
| TU | 25.02.2021 | 23 | Dennis Leer and Jana Deierling |
| JK | 02.03.2021 | 3 | Jana Deierling |
| LF | 02.03.2021 | 4 | Marc Hoffmann |
| MD | 02.03.2021 | 14 | Marc Hoffmann |
| BD | 03.03.2021 | 5 | Jana Deierling |
| ES | 03.03.2021 | 38 | Henri Greil |
| GT | 03.03.2021 | 12 | Felix Klaus |
| GW | 03.03.2021 | 3 | Felix Klaus |
| KK | 03.03.2021 | 23 | Ira Waldow |
| MB | 03.03.2021 | 10 | Marc Hoffmann |
| OS | 03.03.2021 | 10 | Marc Hoffmann |
| TS | 03.03.2021 | 9 | Jana Deierling |
| TU | 03.03.2021 | 19 | Marc Hoffmann |
| JK | 12.04.2021 | 14 | Marc Hoffmann |
| BD | 24.04.2021 | 13 | Hanna Gardein |
| IW | 24.04.2021 | 15 | Marc Hoffmann |
| SD | 24.04.2021 | 15 | Marc Hoffmann |
| MB | 24.04.2021 | 14 | Hanna Gardein |
| TS | 24.04.2021 | 11 | Hanna Gardein |
| TW | 24.04.2021 | 14 | Marc Hoffmann |
| RK | 25.04.2021 | 10 | Hanna Gardein |
| ES | 09.05.2021 | 10 | Hanna Gardein |
| TU | 09.05.2021 | 10 | Hanna Gardein |
| MS | 15.05.2021 | 13 | Hanna Gardein |
| MD | 16.05.2021 | 11 | Hanna Gardein |

Table S3: Protocol for DNA extraction from a single *Stylops* sample.

| Step | Action |
|------|---|
| 1 | Crushed tissue (cephalothorax of females, whole body of males) + 410 μ L extraction buffer (Tris 0.01 M, NaCl 0.1 M, EDTA 0.01 M, ddH ₂ O) + 80 μ L SDS (10%) + 20 μ L Proteinase K (20 mg/ml) |
| 2 | incubate shaking at 37°C in thermoshaker overnight |
| 3 | Centrifuge at 19,980 \times g for 5 min. |
| 4 | Supernatant + 180 μ L NaCl (5 M), shake strongly 50x |
| 5 | Centrifuge at 19,980 \times g for 5 min. |
| 6 | Supernatant + 420 μ L Isopropanol (-20°C), shake gently 20x |
| 7 | Centrifuge at 19,980 \times g for 5 min |
| 8 | Discard supernatant, + 250 μ L Ethanol (80%), shake strongly 50x |
| 9 | Centrifuge at 19,980 \times g for 5 min |
| 10 | Repeat step 8-9 |
| 11 | Dry until ethanol is completely evaporated |
| 12 | Dilute in 50 μ L demineralized H ₂ O |

Table S1: Primers used for amplification of *Stylops* genes.

| Amplified gene (region) | Primer name | Primer sequence (5'-3') | Source |
|-------------------------|-------------|-------------------------|----------------------|
| <i>COI</i> | CO122For | TCWACAAATCATAAAATAATTGG | Júzová et al. 2015 |
| <i>COI</i> | CO1669Rev | TCCTCCTCCTAAAGGRTCRAA | Júzová et al. 2015 |
| <i>H3</i> | H3F | ATGGCTCGTACCAAGCAGACVGC | Colgan et al., 1998 |
| <i>H3</i> | H3R | ATATCCTTRGGCATRATRGTGAC | Colgan et al. 1998 |
| <i>18S</i> | 18Sa-F | ATTAAAGTTGTTGCGGTT | Whiting et al., 1997 |
| <i>18S</i> | 18Sb-R | GAGTCTCGTTCGTTATCGGA | Whiting et al., 1997 |

References:

- Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J et al. (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, 46, 419-437. <https://doi.org/10.1071/ZO98048>
- Júzová K, Nakase Y, Straka J (2015) Host specialization and species diversity in the genus *Stylops* (Strepsiptera: Stylopidae), revealed by molecular phylogenetic analysis. *Zoological Journal of the Linnean Society*, 174, 228-243. <https://doi.org/10.1111/zoj.12233>
- Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997) The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic biology*, 46, 1-68. <https://doi.org/10.1093/sysbio/46.1.1>

Table S5: Components for a single PCR reaction. * depending on DNA concentration

| Ingredient | Volume (µL) |
|-------------------------------------|---------------|
| 5x GoTaq buffer (Promega) | 2.5 |
| dNTPs (10 mM) | 0.25 |
| Forward Primer (10 µM) | 0.3 |
| Reverse Primer (10 µM) | 0.3 |
| GoTaq Polymerase (Promega) (5 u/mL) | 0.1 |
| Milli-Q® - H ₂ O | 7.05 - 8.05 * |
| DNA-extract | 1 - 2 * |

Table S6: Used PCR cyclers programs. * depending on DNA concentration

| Gene | Temperature (°C) | Time (min) |
|-----------------|------------------|-------------|
| COI | 94 | 02:00 |
| | 94 | 00:45 |
| | 50 | 00:45 |
| | 72 | 01:00 |
| | 72 | 05:00 |
| | | } 30-35 x * |
| H3 / 18S | 95 | 02:00 |
| | 95 | 01:00 |
| | 52.6 | 01:00 |
| | 72 | 01:00 |
| | 72 | 05:00 |
| | | } 35 x |

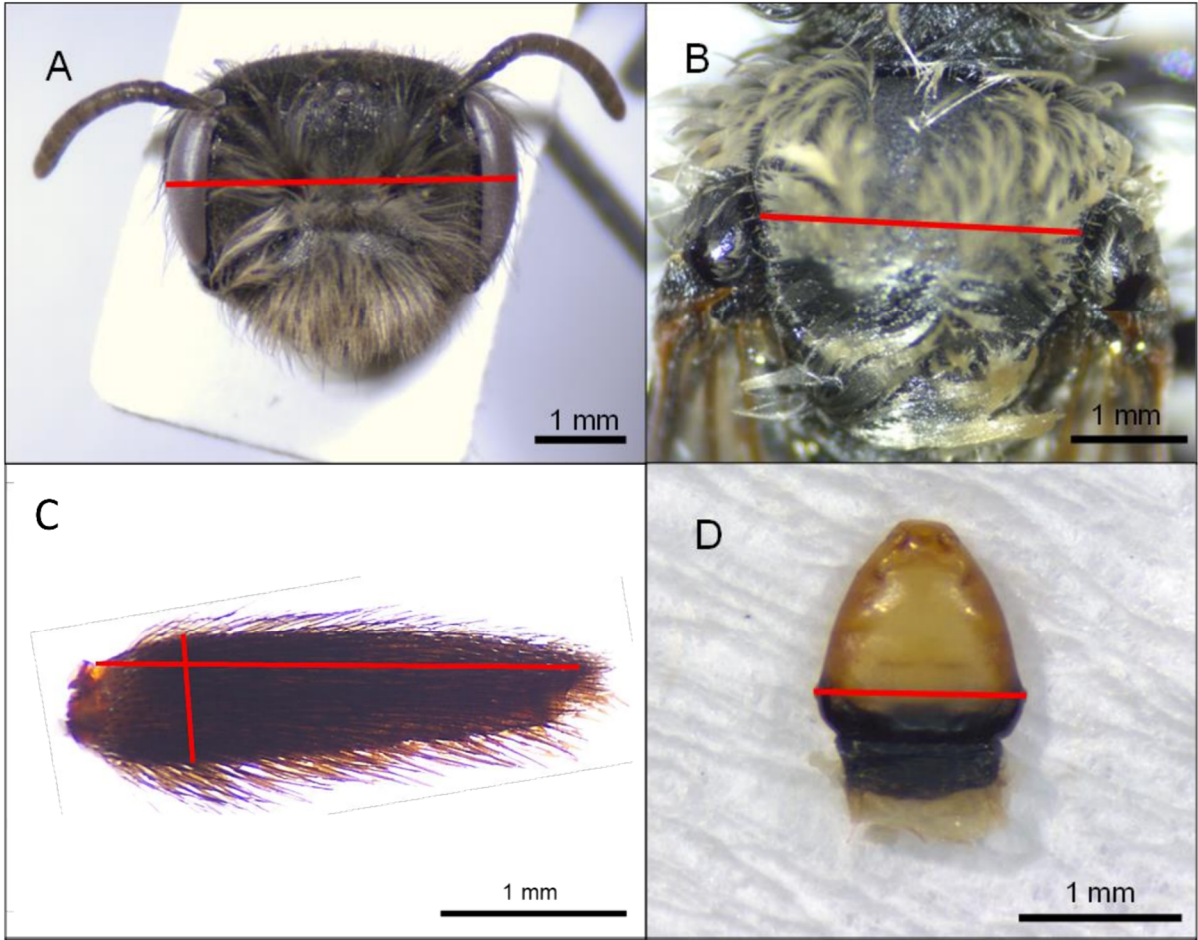


Figure S2: Morphological measurements taken on *A. vaga* and *S. ater*. (A) head width of *Andrena*, (B) intertegular distance of *Andrena*, (C) metabasitarsus length and width of *Andrena*, (D) Cephalothorax width of *Stylops*.



Figure S3: Ovary scores of *A. vaga* females. 1 - no clear egg development, ovaries thread-like; 2 - only small, underdeveloped eggs; 3 - large eggs of at least 1000 μm in the distal part of the ovaries (de - developed egg, lo - lateral oviduct, ov - ovary, ue - underdeveloped egg).

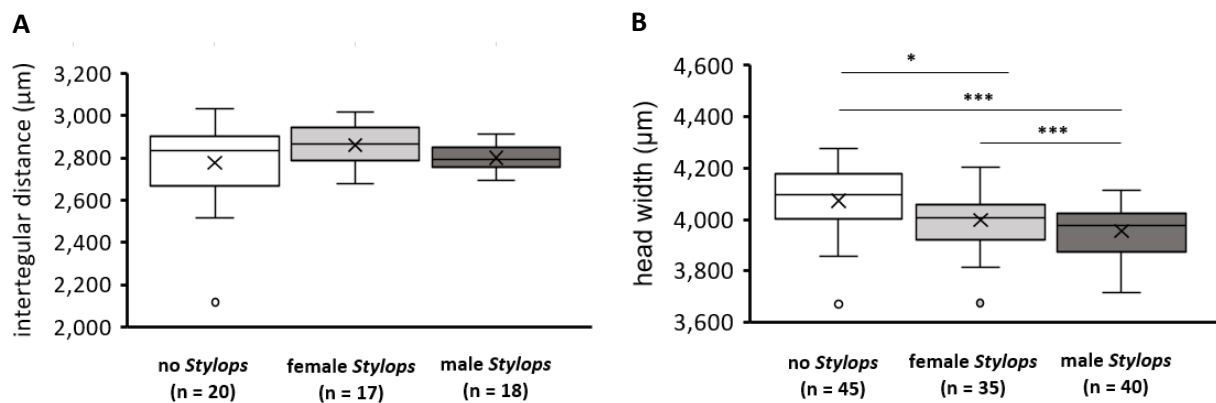


Figure S4: (A) intertegular distance of *A. vaga* females of different stylopization status (sites TU and RK), (B) head width of *A. vaga* females of different stylopization status (alternative model: all sites except MS pooled). Boxplots show minima and maxima (whiskers), medians and first and third quartiles (boxes), means (cross) and outliers (empty circles) (* $p < 0.05$, *** $p < 0.001$).

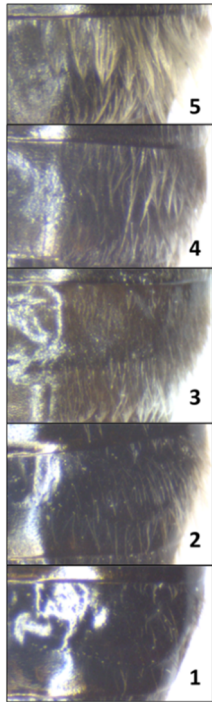
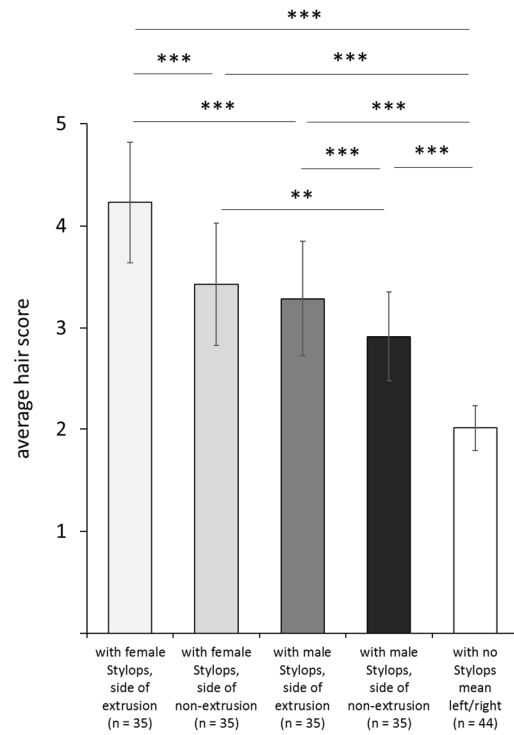
A**B**

Figure S5: Hairiness of tergite 4 in *A. vaga* females of different parasitism groups. (A) Hair scores: 1 - almost no hair, 2 - sparse and short hair, 3 - partly dense and short hair, 4 - dense and partly long hair, 5 - very dense and long hair. (B) Average hair score by different parasitism types and position, error bars show standard deviations (** $p < 0.01$, *** $p < 0.001$).