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A Worker-Like Female of *Myrmica sabuleti* Meinert, 1861 (Hymenoptera: Formicidae: Myrmicinae) in a Pitfall Trap with Five Mermithids (Nematoda: Mermithidae) Protruding from the Gaster

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Abstract

A worker-like female of *Myrmica sabuleti* Meinert, 1861, pitfall-trapped near Jena, Germany, in late summer 2016, was infested by five postparasitic juvenile mermithids. They poked out of the ant´s gaster as a trail of seven filaments of various lengths. Apart from its swollen gaster, the ant differed from conspecifics in several morphometric parameters. Using both morphological and molecular techniques, the parasite family Mermithidae was confirmed. Our stray find raises multiple questions concerning the genus and species identity of the parasite, its biology, and the infestation rate of the host ant population. More mermithid awareness by the various researchers working with *Myrmica* will help, but directed fieldwork, experimental life-history research, and molecular studies are needed to emancipate progress in ant-mermithid research from serendipity.

Introduction

Nematodes of 10 families are known as ant parasites worldwide, with Mermithidae as the largest and most conspicuous one (summarized by Poinar, 1975; Poinar, 2012). They occasionally can be found either by dissecting the gaster of a host ant or because they attract attention when leaving their host. Sometimes, mermithids considerably change the morphology of the (female or male) hosts compared with unparasitized conspecifics, forming so-called parasitogenic phenotypes (e.g. Kloft, 1949; Czechowski et al., 2007a; Csősz & Majoros, 2009; Csősz, 2012; Poinar, 2012; de Bekker et al., 2018). Wheeler (1928) distinguished three discrete aberrant forms of female infested ants, mermithergates (resembling workers), gynaecoid mermithergates (intermorphs), and

mermithogynes (resembling gynes). Csősz and Majoros (2009) hypothesized that all infested adult females originate from gyne-destined larvae even if resembling workers.

Mermithids in ants have been noticed for a long time, with the first report dating back to 1747 (Poinar, 2012). Nevertheless, knowledge is still scarce, and the number of reliably documented instances of mermithids in ants was estimated at just "> 50" (Lachaud & Perez-Lachaud, 2015). Particularly, the life history of most ant mermithids has remained mysterious, and even for the best analyzed systems, crucial questions remain open (e.g., Kaiser, 1986; Poinar et al., 2007). All resolved instances have in common that the parasite enters its host as preparasitic juvenile, grows in the gaster, and leaves the gaster as postparasitic juvenile, causing its host's death (Poinar, 2012). Most frequently, a single mermithid per ant occurs, densely coiled up within the gaster

and invisible from outside; the highest number of individuals in a single ant was nine (Poinar et al., 2007).

Several species of the myrmicine genus *Myrmica* have been reported as hosts of mermithids: *M. gallienii* Bondroit, 1920, *M. rubra* (Linnaeus, 1758), *M. ruginodis* Nylander, 1846, *M. rugulosa* Nylander, 1846, *M. sabuleti* Meinert, 1861, *M. scabrinodis* Nylander, 1846, and *M. schencki* Viereck, 1903 (Czechowski et al., 2007a; b and references therein). Interpreting these host records, of which several date back to the first half of the 20th century, needs be done with a grain of salt, though, because the morphological changes of ants parasitized by mermithids often make species identification difficult (and have even resulted in erroneous species descriptions; Csősz, 2012; Borowiec & Salata, 2015) and because the species-level identification of *Myrmica* has remained difficult, even when not parasitized (Radchenko & Elmes, 2010; Seifert, 2018).

Here, we describe a case of gregarious parasitism with five mermithids protruding from the gaster of a pitfall-trapped worker-like *Myrmica* ant. Based on morphometric and molecular-genetic analyses of the host and the parasite, respectively, we discuss questions about parasite taxonomy, parasite biology, and the host's infestation rate. Like in many other instances of mermithids in ants, many questions remain open – more directed research efforts from various directions will be needed to solve the many exciting questions in this field.

Material and Methods

Sampling

As part of a 2012-2017 monitoring project for studying arthropod communities by the Institute of Ecology and Evolution (Friedrich-Schiller-University of Jena), a pitfall-trapping campaign was conducted in the Leutra valley near Jena/Thuringia, Germany; for details, see Köhler (2018) and Autorenkollektiv (2019). Briefly, a total of 72 pitfall traps were exposed in groups of four traps (at the corners of 1.4 × 1.4 m squares) along three transects from mid April to mid October. The traps used were hard plastic tubes with an opening diameter of 4.7 cm and a depth of 9 cm; the trapping liquid was a 1,2-propandiol:water = 3:1 (v/v) mixture plus a dash of liquid soap to reduce surface tension and a dash of quinine sulfate powder to repel small mammals. The infested *Myrmica* specimen was trapped on a xerothermous, south-exposed (22° inclination) cherry meadow orchard with *Bromus*-rich grassland, partly surrounded by a sparse pine forest (50° 52' 15'' N / 11° 33' 48'' E, 240 m above sea level) during trap exposure 19.IX.-05.X.2016. This ant specimen was the only ant of the monitoring project for which parasite infestation was detected; upon sorting, it was transferred to 70% ethanol. The parasitized ant host and one parasite individual were deposited in the ant and nematode collection, respectively, of Senckenberg Museum für Naturkunde Görlitz, Germany.

Measurements and photographs

The externally visible mermithid filaments were measured with a Zeiss stereomicroscope (SM XX) with measuring ocular using 12.5× and 50× magnification. Photographs were taken using a Zeiss stereomicroscope (Stemi 305) with integrated HD IP Wi-Fi camera and transmitting light (Institute of Ecology and Evolution, University of Jena). Later, the ant was dissected in tap water in a wax bowl under a Wild M5 dissecting microscope at 25× magnification using two watchmaker forceps Dumont No. 5. After dissection, the entire mermithids were measured and photographs taken under 50× magnification. During further handling, one worm got lost.

Identifications

Host species identification was done by (a) using the characters in the key of Seifert (2018) to exclude less similar species of the *Myrmica scabrinodis* and *M. sabuleti* species complexes, (b) running the infected specimen as wild-card in a two-class linear discriminant analysis (LDA) comparing nine *M. bibikoffi* Kutter, 1963 workers from three sites in Germany and Switzerland and 77 *M. sabuleti* workers from 34 sites in the whole Westpalaearctic range, and (c) running the same individuals in a principal component analysis (PCA).

The stereomicroscopic investigation methods, the removal of allometric variance (RAV), and the set of 19 investigated characters used in LDA and PCA are described in Seifert et al. (2014). LDA and PCA were run using the SPSS 16.0 software package (IBM, USA).

For morphological identification of the nematodes, two of the five individuals were processed into glycerin (Poinar, 1975) and studied with a Nikon SMZ-10 R stereoscopic microscope and a Nikon Opti-phot compound microscope at magnifications of up to 800× (G. Poinar, Corvallis/Oregon, USA).

For molecular identification, one nematode was analyzed via the 18S ribosomal DNA (18SrDNA) and the cytochrome c oxidase I (COI) marker. Nematode DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Germany) following the instructions of the manufacturer. 18S PCR was performed in 1× QuantiTect Probe PCR mastermix containing 0.2 µM NF1 primer, 0.2 µM 18Sr2b primer (both Porazinska et al., 2009), and 2 µl DNA extract in 10 µl total volume. COI PCR reactions contained 1× MyTaq buffer (Bioline, UK), 0.2 µM NEM_COI_F primer, 0.2 µM NEM_COI_R primer (Malysheva et al., 2016), 0.25 U MyTaq (Bioline), and 1 µl DNA extract in 10 µl total volume.

The thermal profile for 18S was 10 min initial denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 58 °C, 1 min at 72 °C, and a final elongation of 10 min at 72 °C; the profile for COI was as in Malysheva et al. (2016). Presence of PCR product was verified by gel electrophoresis, and 1 µl amplicon was cloned with the CloneJET PCR Cloning Kit (Thermo) following the instructions of the manufacturer. Insert size was determined by PCR with the vector primers provided with the kit. Plasmid DNA of three colonies with

correct insert size was extracted by alkaline lysis (Sambrook et al., 2001) and Sanger sequenced using the forward vector primer. The obtained sequence was analyzed with the Basic Local Alignment Search Tool (BLAST), which searches for regions of similarity between sequences of a query sequence and the NCBI GenBank database. Because for COI, default settings did not yield any plausible result, "optimize for more dissimilar sequences (discontiguous megablast)" was chosen.

Results

Host ant

There are six *Myrmica* species known from Central Europe (Seifert, 2018) to which the nematode host might possibly belong: *M. sabuleti*, *M. bibikoffi*, *M. scabrinodis*, *M. lonae* Finzi, 1926, *M. vandeli* Bondroit, 1920, and *M. curvithorax* Bondroit, 1920. The latter four species can be clearly excluded by simple eye inspection based on structure of basal scape and head sculpture as described in Seifert (2018). Regarding the former two species, normal workers of *Myrmica sabuleti* without teratological or parasite-induced changes of morphology are easily separable from its temporary social parasite *Myrmica bibikoffi* by different RAV-corrected body ratios. These discriminators are, according to an LDA with stepwise character reduction, a smaller postpetiole width index PpW/CS_{1050}, a smaller postpetiolar hair length index $PpHL/CS_{1050}$, a lower postocular distance index $PoOc/CS_{1050}$, a larger frontal carinae index FL/FR_{1050}, and a shorter spine length index SP/CS_{1050}. However, nematode infection is known in *Myrmica* to result in more massive petiole and postpetiole, a smaller frontal carinae index, and more diverging propodeal spines (Czechowski et al., 2007a; Csősz & Majoros, 2009). This could make the identification of the host specimen problematic.
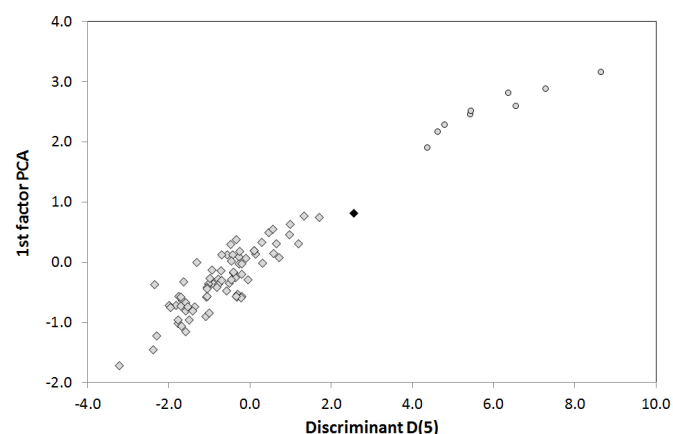
Considering the five diagnostic characters mentioned above and running the Leutra host specimen as wild card in a two-class LDA comparing *M. bibikoffi* and *M. sabuleti*, the host was allocated to *Myrmica sabuleti* with p=0.611. Furthermore, the infested specimen was clearly allocated to *M. sabuleti* in a PCA using these five characters, but it was the most *M. bibikoffi*-like specimen within the *M. sabuleti* cluster (Fig 1) due to its increased width and height of waist segments and the lower frontal carinae index (Table 1). The just low likelihood of belonging to *M. bibikoffi* is also supported by the rarity of this species in general and the fact that it has never been found in the Leutra valley, which has been intensively studied myrmecologically since the 1970s (Seifert, 1982).

Table 1. Morphometric data (average ± standard deviations, minimum and maximum values in square brackets) of workers of *Myrmica bibikoffi*, of the nematode-infested *M. sabuleti* worker from Leutra, and of normal *M. sabuleti* workers from its whole Palaearctic range. The data are corrected in the allometric space. The data for PpHL, MetSp, and MetL are based on a reduced sample of only 77 specimens. For definitions of the morphometric characters, see Seifert et al. (2014).

	bibikoffi (n=9)	*sabuleti* *Leutra* (n=1)	*sabuleti* (n=313)
CS [μm]	1154 ± 76 [1062,1257]	1165	1158 ± 65 [942,1322]
CL/CW (1150)	1.042 ± 0.020 [0.996,1.064]	1.055	1.031 ± 0.015 [0.990,1.074]
SL/CS (1150)	0.810 ± 0.013 [0.795,0.833]	0.848	0.802 ± 0.017 [0.761,0.851]
SW/SL (1150)	0.179 ± 0.008 [0.137,0.179]	0.173	0.199 ± 0.017 [0.159,0.245]
PoOc/CL (1150)	0.444 ± 0.004 [0.440,0.450]	0.424	0.432 ± 0.009 [0.408,0.456]
EYE/CS (1150)	0.194 ± 0.007 [0.183,0.202]	0.200	0.195 ± 0.006 [0.181,0.220]
FL/CS (1150)	0.459 ± 0.013 [0.443,0.484]	0.464	0.457 ± 0.012 [0.421,0.489]
FR/CS (1150)	0.327 ± 0.017 [0.312,0.357]	0.325	0.300 ± 0.014 [0.262,0.356]
FL/FR (1150)	1.405 ± 0.040 [1.333,1.457]	1.423	1.530 ± 0.076 [1.306,1.739]
PeW/CS (1150)	0.321 ± 0.025 [0.300,0.370]	0.321	0.280 ± 0.011 [0.253,0.318]
PpW/CS (1150)	0.461 ± 0.031 [0.430,0.520]	0.482	0.399 ± 0.016 [0.351,0.453]
PeH/CS (1150)	0.364 ± 0.015 [0.345,0.391]	0.365	0.335 ± 0.012 [0.307,0.373]
PeL/CS (1150)	0.499 ± 0.024 [0.465,0.528]	0.533	0.479 ± 0.014 [0.441,0.523]
SP/CS (1150)	0.386 ± 0.021 [0.345,0.419]	0.445	0.398 ± 0.023 [0.303,0.455]
PpHL/CS (1150)	0.211 ± 0.010 [0.198,0.227]	0.164	0.168 ± 0.013 [0.115,0.197]
MetL/CS (1150)	0.239 ± 0.016 [0.214,0.259]	0.245	0.228 ± 0.010 [0.194,0.256]
MetSp/CS (1150)	0.207 ± 0.029 [0.147,0.243]	0.187	0.180 ± 0.018 [0.147,0.250]

Fig 1. Bivariate plot of the score of the 1st factor of a principal component analysis (PCA) and of the score of a linear discriminant analysis (LDA) of the nematode-infested specimen (black rhomb), of nine *Myrmica bibikoffi* workers (grey dots) and of 77 *M. sabuleti* workers (grey rhombs) from the whole W-Palaearctic range. The infested specimen was run in the LDA as wild-card, that is, without imposing a hypothesis. The set of originally 19 characters was reduced to the five most diagnostic ones in both analyses.

The infested female *Myrmica* ant was a mermithergate according to the categorisation by Wheeler (1928) and had no ocelli. It possessed a slightly thickened and longer gaster (~2.5 mm) compared with unparasitized workers from the same trap (~2.0 mm).

Mermithid parasites

At the *Myrmica* worker's back end, seven filaments of various lengths protruded from the gaster, of the same yellow-brown color as the ant itself, presumably because of storage in the same trap liquid (Fig 2a). The nematodes had penetrated the intersegmental membranes between gastral segments three and four (= abdominal segments six and seven), rupturing both tergites and sternites distinctly. The worm parts were more or less straight and rod-like, and of two worms, both anterior and posterior ends were visible outside the ant's gaster, whereas of three worms, only one end (one front and two rear ends) were visible before dissection. The coiled-up parts of the worms were partially visible through the cuticle up to the second gaster segment (Fig 2b). Three filaments were rising more ventrally and rather short (0.9/1.2/2.0 mm), whereas four filaments were rising more dorsally and much longer (3.5/4.8/5.1/6.5 mm), with the longest exceeding the ant size (~5.5 mm) (Fig 2a). Their diameters were between 0.13-0.19 mm at the base and 0.11-0.15 mm at the ends, narrowing only slightly.

After removal of the tergites and sternites, the parts of the worms inside the ant were found coiled and had remained pale, contrasting the yellow-brown parts exposed to the pitfall fluid (Fig 2c). A total of five mermithids could be confirmed, with the largest reaching 12.0 mm total length (Fig 2d). Only small remains of the ant's internal organs were discernible and not clearly identifiable.

Morphologically, the nematodes were identified as postparasitic juvenile mermithids (family Mermithidae). The genus can only be determined in adults (G. Poinar, unpubl., using the key in Poinar, 1975).

This morphological identification was corroborated by sequencing 363 basepairs of the 18Sr DNA (GenBank Accession Number MH793873) and 563 basepairs of COI (GenBank Accession Number MK256737). The 18S BLAST search yielded several hits with 100% query cover, 99% identity match, and e=0 to the genera *Agamermis* and *Hexamermis*; the COI BLAST search yielded hits with 98% query cover, 73% identity match, and e=10⁻⁸¹ to the same genera.
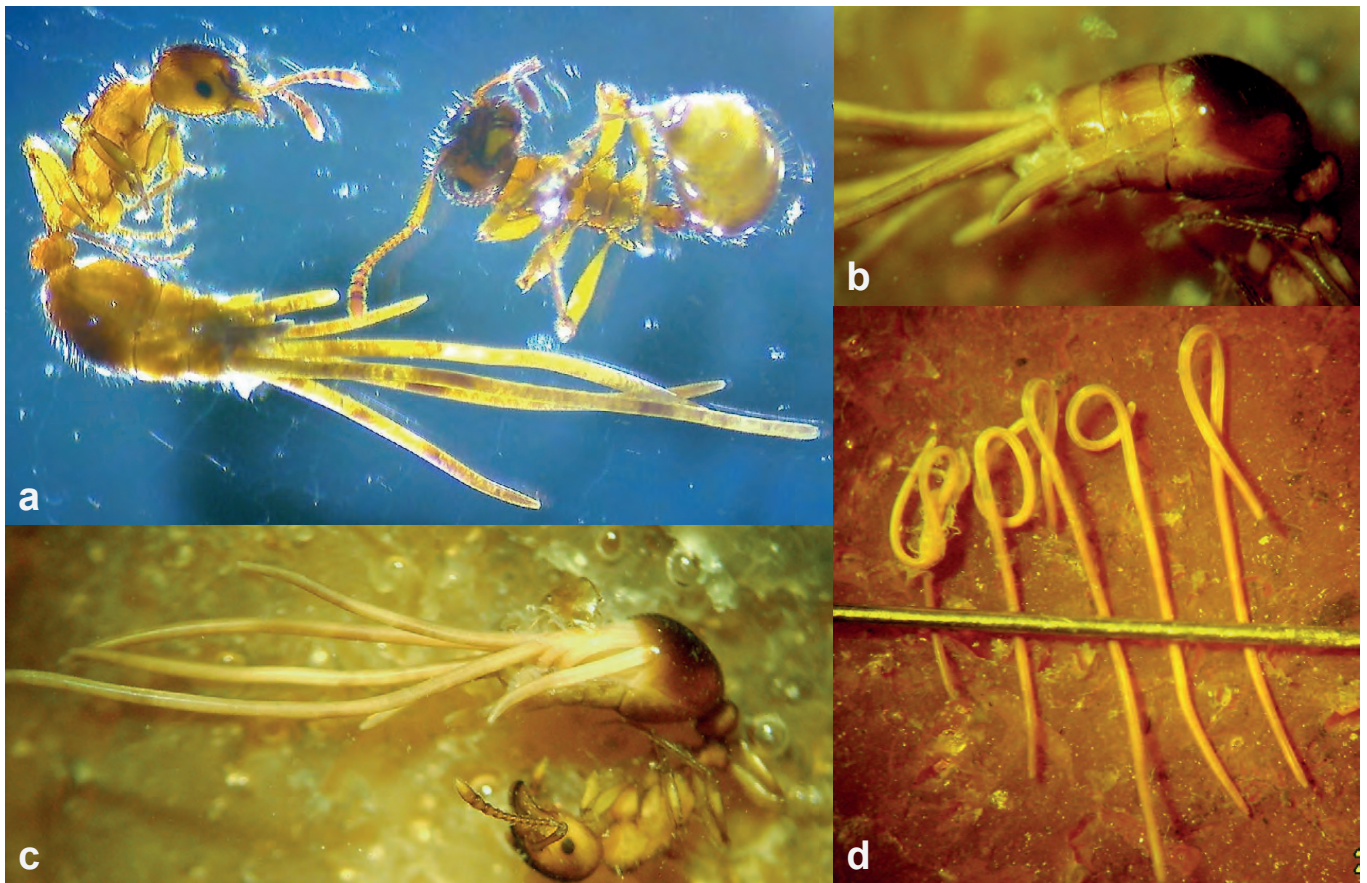

Fig 2. The infested *Myrmica* female and its parasites. (a) Two workers of *Myrmica sabuleti*, on the left infested by mermithids (total ant body length ~5.5 mm, gaster length ~2.5 mm, length of visible part of longest mermithid filament ~6.5 mm, diameters at base 0.13-0.19 mm and at the ends 0.11-0.15 mm), on the right an uninfested conspecific. (b) Through the *Myrmica* gaster, parts of the mermithids are visible. (c) The dissected *Myrmica* gaster shows the compact juvenile mermithids. (d) The five juvenile mermithids of various lengths found in the infested *Myrmica* (length of longest mermithid 12.0 mm). (Photograph (a) by A. Ebeling & I. Wolf; photographs (b-d) by A. Buschinger).

Discussion

Our record of *Myrmica sabuleti*, identified using morphometrics, as host of five postparasitic mermithids, identified using morphology and molecular genetics, was a stray find during a pitfall-trapping campaign with research aims other than ant mermithid infection. Multiple questions about parasite taxonomy, parasite biology, and the infestation rate of the host population can now be addressed, albeit mostly without definitively answering them.

What is the genus and species identity of the parasite?

The morphological identification of postparasitic juveniles, at which stage mermithids leave their ant hosts, is not possible to species nor to genus (Poinar, 1975). Mermithid species from ants have thus often remained unidentified on morphological grounds (e.g., McInnes & Tschinkel, 1996; Csősz & Majoros, 2009; O'Grady & Breen, 2011; Laciny et al., 2017), and the genus and species identity of Mermithidae in *Myrmica* has never been resolved so far (Poinar, 2012).

DNA-sequence-based identification can be a valuable tool (Poinar et al., 2007). The sequences we established are similar to ones from *Agamermis* and *Hexamermis*, which build a monophylum within the Mermithidae (Park et al., 2011; Kubo et al., 2016). Both *Agamermis* and *Hexamermis* have been reported from a broad range of insect hosts (e.g., Achinelly & Camino, 2008; Stubbins et al., 2016; and references therein) but, as far as we know, not from ants (Poinar, 2012 and Web of Science queries on 20 November 2018). However, despite the high similarity to *Agamermis* and *Hexamermis*, uncertainty remains, in that of the safely identified recent mermithid genera found in ants (Poinar, 2012), two (*Allomermis*, *Pheromermis*) are represented in GenBank (Nucleotide query of 21 November 2018) but not the other four (*Agamomermis*, *Camponotimermis*, *Comanimermis*, *Meximermis*).

This uncertainty is typical of current mermithid research – a lack of sequence data from safely identified specimens of the mermithid species at question and thus a lack of comparison has often impeded taxonomic resolution even when molecular analyses were undertaken (hornets: Villemant et al., 2015; ants: Laciny, 2017; moths: Kumar et al., 2018; bumble bees: Tripodi & Strange, 2018). A comprehensive reference DNA sequence database of the mermithid species identified so far in ants would allow improved taxonomic resolution for postparasitic individuals and thus access to the knowledge established for those parasites.

At which developmental stage of the ant did the parasite infest it?

The female *M. sabuleti* presented here showed similar differences from nonparasitized conspecifics to those described by Csősz and Majoros (2009) for mermithogenic *Myrmica gallienii* and to those described by Czechowski et al. (2007a) for mermithogenic *M. sabuleti*. These differences (i.e., not the enlarged gaster) allow us to conclude that the parasites entered the ant before the ant was adult, in line with findings that mermithids that enter ant larvae can be carried over to the adult stage (Wheeler, 1901). Likely, the ovipositing mermithid was close to the ant nest, thus resulting in multiple infections – *Hexamermis* adults, for example, produce eggs in the soil, and from these eggs hatch pre-infective stages that search the surroundings for hosts (Poinar & Gyrisco, 1962).

What is the relevance of the high number of five parasites in the same host?

In most ants parasitized, a single mermithid is found. The highest available numbers of mermithids per ant individual are four in *Myrmica* (Csősz & Majoros, 2009), five in *Solenopsis* and *Lasius* (McInnes & Tschinkel, 1996; O'Grady & Breen, 2011), and nine in *Solenopsis* (Poinar et al., 2007). In all these instances, large numbers of parasitized ants were screened and the higher the number of individuals per ant, the rarer it was detected. Our find is among the highest numbers of mermithid per ant host recorded, but just a single infected worker was found. This combination is improbable (but obviously not impossible) if the frequency distribution of multiple infections (2, 3, 4, etc. nematodes per ant) in the *M. sabuleti* population Leutra is the same as in those other studies. Series of infected *M. sabuleti* from the population analyzed here will be needed to interpret our current find of five parasites as typical or atypical of this particular relationship.

Did the parasite start emerging from the host before or after the host was pitfall-trapped?

Our find of a pitfall-trapped host with parasites protruding from its gaster could mean that the parasites started emerging before the ant got caught (pre-trapping) or just after contact with the trapping liquid (post-trapping). Pre-trapping emergence may be possible, in that the process of emerging from the ant was reported to take, for example, one hour (after which the ant hosts lived for a further hour; O'Grady & Breen, 2011). In support of pre-trapping emergence may be seen that the worms were shaped rod-like and did not, as normally the case, form a tangle – the rod-like posture may have resulted from the ant walking some distance with the worms trailing behind. Alternatively, the rod shape may have resulted from death in the trapping liquid. This aspect could be tested by killing living mermithids in diluted 1,2-propandiol. Walking with such a voluminous trail appears difficult, but before contact with the liquid, the worms may have been much thinner – mermithids were reported to gain volume once in contact with water by imbibing it (Poinar et al., 2007). Not in line with pre-trapping emergence is the report by Kaiser (1986) that the ants remain still during the parasites' emergence and only after completed emergence run around hastily before they die.

Post-trapping emergence would be in line with reports for various ant mermithids that contact of the host with water

was necessary to trigger emergence of the parasite (Crawley & Baylis, 1921; Kaiser, 1986; Maeyama et al., 1994; Poinar et al., 2007; O'Grady & Breen, 2011). From many parasitized grasshoppers pitfall-trapped in the same liquid used here, not a single emergence of the mermithids was observed (G. Köhler, unpubl.), but this may not be in conflict with post-trapping emergence in this ant case because the mermithids of the two hosts may have different emergence triggers. Experiments with living, infected *M. sabuleti* from the population analyzed here will be needed to solve this question.

Did the parasite trigger water-seeking behavior in the host?

Whenever the parasites started emerging from the host, the ant may have been trapped because it fell into the pitfall trap by chance or because the parasite triggered its host to seek a moist environment. Such behavioral manipulation has been postulated for several mermithids in ants (Kaiser, 1986; Mayeama et al., 1994; Poinar et al., 2007). A potential adaptive value of seeking a moist environment appears somewhat problematic in a dry habitat during a dry period (total rainfall measured by a climate station 6 km from the habitat was 16.1 mm during the trapping period 19.IX.-05.X.2016; Jena University of Applied Science, unpubl.) but cannot be refuted entirely. Avoiding exposure to the dry conditions above surface may actually be more adaptive for the parasites. Experimental evidence is needed.

What is the infestation rate of the host population?

The literature record holds both high infestation rates in some ant nests of selected host populations (McInnes & Tschinkel, 1996; Czechowski et al., 2007b; Csősz & Majoros, 2009) and difficulties in finding mermithids in ant populations infested in a previous year (reviewed by O'Grady & Breen, 2011). At the population level, this means clumped spatial distribution with temporal variation. Our find of a single parasitized worker out of thousands *Myrmica* workers trapped in the habitat analyzed mid April to mid October 2016 (G. Köhler, unpubl.) may thus mean that the *M. sabuleti* population has a low infestation rate. Alternatively, it might have a high infestation rate but no trap may have been close to a strongly infested nest or infested ants may be influenced by their parasites to rather stay in their nests during dry periods (see previous section). To characterize the infestation rate of the analyzed population, but also those of other *Myrmica* populations, dedicated screening of many living individuals from many nests will be needed, ideally from multiple years, as done recently in a single-year survey of thousands of bumble bees at the continent scale (Tripodi & Strange, 2018) and in a multiple-year survey of tens of thousands of hornets at the district scale (Villemant et al., 2015). If combined with genetic approaches, such studies would also help to shed some light on the entirely understudied population genetics of nematode parasites in general (Cole & Viney, 2018).

Conclusion

While a lot of research effort has been directed at many of the parasitic organisms infesting *Myrmica* species (Witek et al., 2014), data on mermithids are scarce. As *Myrmica* ants are important study systems for various disciplines (e.g., Radchenko & Elmes, 2010; Seifert, 2018), including the study of lycaenid butterflies and socially parasitic ants (e.g., Barbero et al., 2012; Tartally et al., 2019), awareness of this phenomenon by the various researchers working with *Myrmica* may bring to light further instances of mermithid infections, possibly with living individuals. However, additionally to mermithid awareness when analyzing other aspects of *Myrmica* ants, directed and dedicated efforts in fieldwork, experimental life-history research, and molecular-genetic analyses will be needed to reduce the dependence of mermithid research in *Myrmica* and ants generally on chance.

Authors' contributions

GK noticed the host ant when sorting pitfall-trapped insects. GK and AB measured and photodocumented the nematodes. AB dissected the host ant. BS analyzed the host ant and other *Myrmica* ants using morphometrics and ran statistics on the data. FMS, WA, and BCS-S designed the molecular analyses of the nematodes and interpreted the data. All authors wrote the paper.

References

Achinelly, M.F. & Camino, N.B. (2008). *Hexamermis paranaense* new species (Nematoda, Mermithidae): a parasite of *Diloboderus abderus* (Coleoptera, Scarabaeidae) in Argentina. Iheringia Serie Zoologia, 98: 460-463.

Autorenkollektiv (2019). Schlussbericht zum Forschungsprojekt (FE: 02.0234/2003/LRB): Entwicklung und Wiederbesiedlung von Lebensräumen von Flora und Fauna nach Rückbau einer vorhandenen Autobahn am Beispiel der A 4 (Wirksamkeitsuntersuchungen). Friedrich-Schiller-Universität Jena, Institut für Ökologie und Evolution, unpubl. Schlussbericht i. A. Bundesanstalt für Straßenwesen (BASt), 262 pp.

Barbero, F., Patricelli, D., Witek, M., Balletto, E., Casacci, L.P., Sala, M. & Bonelli, S. (2012). *Myrmica* ants and their butterfly parasites with special focus on the acoustic communication. Psyche, 2012: article 725237. doi: 10.1155/2012/725237

Borowiec, L. & Salata, S. (2015). *Pheidole symbiotica* Wasmann, 1909, an enigmatic supposed social parasite, is a nematode-infested form of *Pheidole pallidula* (Nylander, 1849) (Hymenoptera: Formicidae: Myrmicinae). Sociobiology, 62: 181-186. doi: 10.13102/sociobiology.v62i2.181-186

Cole, R. & Viney, M. (2018). The population genetics of parasitic nematodes of wild animals. Parasites & Vectors, 11: article 590. doi: 10.1186/s13071-018-3137-5

Crawley, W.C. & Baylis, H.A. (1921). *Mermis* parasitic on ants of the genus *Lasius*. Journal of the Royal Microscopical Society, 257: 353-372.

Czechowski, W., Czechowska, W. & Radchenko, A. (2007a). Strikingly malformed host morphology: *Myrmica rugulosa* Nyl. and *Myrmica sabuleti* Mein. (Hymenoptera: Formicidae) parasitised by mermithid nematodes. Fragmenta Faunistica, 50: 139-148.

Czechowski, W., Radchenko, A. & Czechowska, W. (2007b). Mermithid infestation strikingly alters the morphology of *Myrmica rubra* (L.) (Hymenoptera : Formicidae): possible taxonomical involvements. Annales Zoologici, 57: 325-330.

Csősz, S. (2012). Nematode infection as significant source of unjustified taxonomic descriptions in ants (Hymenoptera: Formicidae). Myrmecological News, 17: 27-31.

Csősz, S. & Majoros, G. (2009). Ontogenetic origin of mermithogenic *Myrmica* phenotypes (Hymenoptera, Formicidae). Insectes Sociaux, 56: 70-76. doi: 0.1007/s00040-008-1040-3

de Bekker, C., Will, I., Das, B. & Adams, R.M.M. (2018). The ants (Hymenoptera: Formicidae) and their parasites: effects of parasitic manipulations and host responses on ant behavioral ecology. Myrmecological News, 28: 1-24. doi: 10.25849/myrmecol.news_028:001

Kaiser, H. (1986). Über Wechselbeziehungen zwischen Nematoden (Mermithidae) und Ameisen. Zoologischer Anzeiger, 217: 156-177.

Kloft, W. (1949). Über den Einfluß von Mermisparasitismus auf den Stoffwechsel und die Organbildung bei Ameisen. Zeitschrift für Parasitenkunde, 14: 390-422.

Köhler, G. (2018). Geradflügler (Insecta: Saltatoria, Blattoptera et Dermaptera) von Kurztransekten an der Autobahn bei Leutra/Thüringen. Thüringer Faunistische Abhandlungen, 23: 117-134.

Kubo, R., Ugajin, A. & Ono, M. (2016). Molecular phylogenetic analysis of mermithid nematodes (Mermithida: Mermithidae) discovered from Japanese bumblebee (Hymenoptera: Bombinae) and behavioral observation of an infected bumblebee. Applied Entomology and Zoology, 51: 549-554. doi: 10.1007/s13355-016-0430-7

Kumar, C.M.S., Kurian, J.T., Selvakaran, D., Vasudevan, H. & D'Silva, S. (2018). Mermithid parasitism of shoot borer (*Conogethes punctiferalis*) infesting ginger and turmeric and its biocontrol potential. Annals of Applied Biology, 173: 243-250. doi: 10.1111/aab.12457

Lachaud, J.P. & Perez-Lachaud, G. (2015). Ectaheteromorph ants also host highly diverse parasitic communities: a review of parasitoids of the Neotropical genus *Ectatomma*. Insectes Sociaux, 62: 121-132. doi: 10.1007/s00040-015-0390-x

Laciny, A. (2017). Evidence of mermithism in a gyne of *Lasius niger* (Linnaeus, 1758) (Hymenoptera: Formicidae) from Burgenland, Austria. Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen, 69: 131-138.

Laciny, A., Zettel, H., Metscher, B., Kamariah, A.S., Kopchinskiy, A., Pretzer, C. & Druzhinina, I.S. (2017). Morphological variation and mermithism in female castes of *Colobopsis* sp. nrSA, a Bornean "exploding ant" of the *Colobopsis cylindrica* group (Hymenoptera: Formicidae). Myrmecological News, 24: 91-106.

Maeyama, T., Terayama, M. & Matsumoto, T. (1994). The abnormal behavior of *Colobopsis* sp. (Hymenoptera: Formicidae) parasitized by *Mermis* (Nematoda) in Papua New Guinea. Sociobiology, 24: 115-119.

Malysheva, S.V., Efeykin, B.D. & Teterina, A.A. (2016). A new primer set for amplification of COI mtDNA in parasitic nematodes. Russian Journal of Nematology, 24: 73-75.

McInnes, D.A. & Tschinkel, W.R. (1996). Mermithid nematode parasitism of *Solenopsis* ants (Hymenoptera: Formicidae) of Northern Florida. Annals of the Entomological Society of America, 89: 231-237.

O'Grady, A. & Breen, J. (2011). Observations on mermithid parasitism (Nematoda: Mermithidae) in two species of *Lasius* ants (Hymenoptera: Formicidae). Journal of Natural History, 45: 2339-2345. doi: 10.1080/00222933.2011.596634

Park, J.-K., Sultana, T., Lee, S.-W., Kang, S., Kim, H.K., Min, G.-S., Eom, K.S. & Nadler, S.A. (2011). Monophyly of clade III nematodes is not supported by phylogenetic analysis of complete mitochondrial genome sequences. BMC Genomics, 12: article 392. doi: 10.1186/1471-2164-12-392

Poinar, G. Jr (2012). Nematode parasites and associates of

ants: past and present. Psyche, 2012: article 192017. doi: 10.1155/2012/192017

Poinar, G.O. Jr (1975). Entomogenous nematodes: a manual and host list of insect-nematode associations. E. Brill, Leiden, 317 pp.

Poinar, G.O. Jr & Gyrisco, G.G. (1962). Studies on the bionomics of *Hexamermis arvalis* Poinar and Gyrisco, a mermithid parasite of the alfalfa weevil, *Hypera postica* (Gyllenhal). Journal of Insect Pathology, 4: 469-483.

Poinar, G.O. Jr, Porter, S.D., Tang, S. & Hyman, B.C. (2007). *Allomermis solenopsi* n. sp. (Nematoda: Mermithidae) parasitising the fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in Argentina. Systematic Parasitology, 68: 115-128. doi: 10.1007/s11230-007-9102-x

Porazinska, D.L., Giblin-Davis, R.M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T.O., Tucker, A.E., Sung, W. & Thomas, W.K. (2009). Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. Molecular Ecology Resources, 9: 1439-1450. doi: 10.1111/j.1755-0998.2009.02611.x

Radchenko, A.G. & Elmes, G.W. (2010). *Myrmica* ants (Hymenoptera, Formicidae) of the Old World. Natura Optima Dux Foundation, Warszawa, 789 pp.

Sambrook, J., Maccallum, P. & Russel, D. (2001). Molecular cloning: a laboratory manual. Cold Springs Harbour Press, New York, NY, 2344 pp.

Seifert, B. (1982). Die Ameisenfauna einer Rasen-Wald-Catena im Leutratal bei Jena. Abhandlungen und Berichte des Naturkundemuseums Görlitz, 56: 1-18.

Seifert, B. (2018). The ants of Central and North Europe. Lutra Verlags- und Vertriebsgesellschaft, Tauer, 407 pp.

Seifert, B., Bagherian Yazdi, A. & Schultz, R. (2014). *Myrmica martini* sp. n. – a cryptic species of the *Myrmica scabrinodis* species complex (Hymenoptera: Formicidae) revealed by geometrical morphometrics and nest-centroid clustering. Myrmecological News, 19: 171-183.

Stubbins, F.L., Agudelo, P., Reay-Jones, F.P.F. & Greene, J.K. (2016). *Agamermis* (Nematoda: Mermithidae) infection in South Carolina agricultural pests. Journal of Nematology, 48: 290-296.

Tartally, A., Thomas, J.A., Anton, C., Balletto, E., Barbero, F., Bonelli, S., Bräu, M., Casacci, L.P., Csősz, S., Czekes, Z., Dolek, M., Dziekańska, I., Elmes, G., Fürst, M.A., Glinka, U., Hochberg, M.E., Höttinger, H., Hula, V., Maes, D., Munguira, M.L. Musche, M., Stadel Nielsen, P., Nowicki, P., Oliveira, P.S., Peregovits, L., Ritter, S., Schlick-Steiner, B.C., Settele, J., Sielezniew, M., Simcox, D.J., Stankiewicz, A.M., Steiner, F.M., Švitra, G., Ugelvig, L.V., Van Dyck, H., Varga, Z., Witek, M., Woyciechowski, M., Wynhoff, I. & Nash, D.R. (2019). Patterns of host use by brood-parasitic *Maculinea* butterflies across Europe. Philosophical Transactions of the Royal Society B-Biological Sciences, 374: article 20180202. doi: 10.1098/rstb.2018.0202

Tripodi, A.D. & Strange, J.P. (2018). Rarely reported, widely distributed, and unexpectedly diverse: molecular characterization of mermithid nematodes (Nematoda: Mermithidae) infecting bumble bees (Hymenoptera: Apidae: *Bombus*) in the USA. Parasitology, 145: 1558-1563. doi: 10.1017/S0031182018000410

Villemant, C., Zuccon, D., Rome, Q., Muller, F., Poinar, G.O. & Justine, J.L. (2015). Can parasites halt the invader? Mermithid nematodes parasitizing the yellow-legged Asian hornet in France. PeerJ, 3: article e947. doi: 10.7717/peerj.947

Wheeler, W.M. (1901). The parasitic origin of macroërgates among ants. The American Naturalist, 35: 877-886.

Wheeler, W.M. (1928). *Mermis* parasitism and intercastes among ants. Journal of Experimental Zoology, 50: 165-237.

Witek, M., Barbero, F. & Markó, B. (2014). *Myrmica* ants host highly diverse parasitic communities: from social parasites to microbes. Insectes Sociaux, 61: 307-323. doi: 10.1007/s00040-014-0362-6