# Evidence of mermithism in a gyne of *Lasius niger* (LINNAEUS, 1758) (Hymenoptera: Formicidae) from Burgenland, Austria

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## Abstract

This paper presents a new record of mermithid infestation in a gyne of the ant species *Lasius niger* (LINNAEUS, 1758) from Burgenland, Austria. The aberrant anatomy of the parasitized specimen is described and illustrated. The taxonomic placement of host and parasite is corroborated by molecular methods. In addition, comparisons of morphometric characters to those of healthy individuals and to a previously published dataset are conducted. The observed morphological aberrations of the mermithogyne include reduced gyne-specific characters and elongated appendages. These results, as well as caste identity, parasitic load and ecological conditions are in accordance with the current state of knowledge on comparable host-parasite systems.

K e y   w o r d s : Hymenoptera, Formicidae, *Lasius*, Mermithidae, parasitism, morphometry.

## Zusammenfassung

In dieser Arbeit wird ein neuer Beleg einer mit einem parasitischen Fadenwurm (Mermithidae) befallenen Gyne der Ameisenart *Lasius niger* (LINNAEUS, 1758) aus dem Burgenland vorgestellt. Die abweichende Anatomie des parasitierten Exemplars wird beschrieben und illustriert. Die taxonomische Stellung von Wirt und Parasit wird durch molekulare Methoden gestützt. Zusätzlich werden morphometrische Merkmale mit jenen gesunder Individuen sowie mit einem zuvor publizierten Datensatz verglichen. Die Mermithogyne zeichnet sich morphologisch u. a. durch reduzierte Gynenmerkmale und verlängerte Extremitäten aus. Diese Ergebnisse, sowie Kastenzugehörigkeit, Parasitenlast und ökologische Gegebenheiten decken sich mit dem aktuellen Wissensstand über vergleichbare Wirt-Parasiten-Systeme.

## Introduction

The earliest published scientific record of ants in general – and *Lasius* FABRICIUS, 1804 in particular – infested by mermithid nematodes dates back to GOULD (1747), who noted long white worms in "large and small ant-flies" (i.e., female and male sexuals). Later CRAWLEY & BAYLIS (1921) only reported mermithid infections of *Lasius* gynes.

To date, seven genera of the family Mermithidae are known to parasitize ants, with three species of the genus *Pheromermis* POINAR, LANE & THOMAS, 1967 described from *Lasius* spp. (POINAR 2012). In all studied cases, infection takes place when an intermediate host containing the juvenile nematodes is fed to the larvae of the host ant as a protein source (KAISER 1986, POINAR 2012). Intermediate hosts of *Pheromermis* spp. are oligochaete worms (KAISER 1986) or aquatic insects, such as caddisflies (POINAR 1981). To complete their life cycle, mermithid parasites alter their hosts' behavior, eventually leading to the infested ants

becoming restless, seeking a body of water or damp earth to release the mature nematode and subsequently dying (Crawley & Baylis 1921, Kaiser 1986, Maeyama et al. 1994).

Mermithid infestations are usually accompanied by morphological changes in the hosts, possibly brought about by hormonal perturbations during development (O'Grady & Breen 2011). Such aberrant morphologies of parasitized individuals are known from several subfamilies of ants (Wheeler 1928, Csősz 2012, Maeyama et al. 1994, Laciny et al. 2017). Parasitized specimens are often morphologically intermediate between castes, resulting in so-called intercaste phenotypes (Wheeler 1928). Characteristic changes include reduced body size, elongated extremities, enlarged gaster (due to distension by the nematode), reduced size of the head, deviations in color, pilosity and sculpture, as well as reduction of all sexual characters, such as wings, thoracic sclerites, ovaries, and ocelli (Wheeler 1928, Kaiser 1986, Czechowski et al. 2007, O'Grady & Breen 2011, Poinar 2012, Laciny et al. 2017). Phenotypes produced by mermithid infestation can vary considerably, lying anywhere on a range from almost normal worker morphology ("mermithergates") to gyne-like specimens with only slightly reduced features ("mermithogynes") (Csősz & Majoros 2009). Studies on *Lasius* (Kaiser 1986, O'Grady & Breen 2011) and *Myrmica* Latreille, 1804 (Csősz & Majoros 2009) have revealed that all known mermithogenic phenotypes develop from larvae destined to be sexuals, i.e., gynes or males, and no confirmed infestation of workers has been documented for these taxa.

### Material and methods

Sampling conditions: During an excursion to Neusiedl am See (Burgenland) on July 18th 2017, a living alate gyne with an enlarged gaster and short wings was found crawling on a log, seemingly unable to fly (M. Madl, pers. comm.). It was caught alive and kept under laboratory conditions for the purpose of breeding experiments: The specimen was placed in a glass petri dish containing a small paper tube for shelter and a glass vial with cotton wool soaked in sugar-water for nutrition. It survived in captivity for approximately one week and was then found dead with an – also already deceased – nematode protruding ventrolaterally from its ruptured gaster (see Fig. 3).

The animal was dry-mounted on a cardboard point for determination, morphometry and photography. It was identified as a gyne of *Lasius niger* with the help of Seifert (2007). To assess morphological aberrations, it was compared to an alate *L. niger* gyne from the NHMW Hymenoptera collection (species identity confirmed with Seifert 2007), as well as illustrations of a conspecific specimen provided by AntWeb.org. Based on previously published records of host-parasite-relations (Poinar 2012), the parasitic nematode was presumed to belong to the genus *Pheromermis* sp., but a more exact determination was not feasible. Three legs from the right side of the mermithogyne as well as the parasitic nematode were subsequently removed for molecular analysis.

Molecular methods: For DNA barcoding, genetic material was extracted from three detached legs of the mermithogyne, as well as half of the removed nematode. Fragments of cytochrome c oxidase subunit I and II (*coI*, *coII*) were amplified and sequenced. For detailed methods and primers used, see Laciny et al. (2017). The resulting sequences were compared to previously deposited sequences in NCBI GenBank using BLAST (Benson et al. 2005). Sequences are deposited in NCBI GenBank under accession numbers MF993322 and MF993323 for *coI* and *coII* of the mermithogyne, respectively, and MF993321 for *coI* of the nematode.

Measurements and indices:

HW     Head width. Maximum width of head in full-face view (including eyes).

HL       Head length. Maximum length of head in full-face view, excluding mandibles, measured from anteriormost point of clypeus to posterior-most point of head vertex, parallel to midline.

EL       Eye length. Maximum diameter of compound eye, measured in lateral view.

FeL     Femur length. Maximum length of profemur, measured from base to apex.

FWL   Forewing length. Length of forewing, measured from tegula to distal tip.

ML     Mesosoma length (Weber's length). Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.

MSW  Mesoscutum width. Maximum diameter of mesoscutum, measured dorsally.

OcW   Ocellus width. Maximum diameter of median ocellus.

PSL    Palp segment length. Combined lengths of maxillary palp segments 5 and 6.

SL       Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.

TL       Total length. The added lengths of head (excluding mandibles), mesosoma, petiole, and gaster.

NL       Nematode length. Maximum length of longest parasitic nematode found within mermithogynes.

CI       Cephalic index. HW / HL × 100

EI       Eye index. EL / HW × 100

FeI     Femur index. FeL / HW × 100

PSI    Palp segment index. PSL / HW × 100

SI       Scape index. SL / HW × 100

WI     Wing index. FWL/TL × 100

Examination and morphometric analysis of specimens was carried out on a Nikon SMZ1500 binocular microscope with an ocular micrometer, at magnifications of up to 256×.

Stacked digital photos were taken with a Leica DFC camera attached to a Leica MZ16 binocular microscope with the help of Leica Application Suite V3, stacked with Zerene-Stacker 64-bit, and processed with Adobe Photoshop 7.0. Diagrams and calculations were made with MS Excel 2016.

Depositories:

CASC    California Academy of Sciences, San Francisco, CA, USA
NHMW  Natural History Museum Vienna, Austria

Material examined:

Specimens examined: 1 mermithogyne (NHMW), Austria, Burgenland, Neusiedl am See, bike path (47.945555° N, 16.833358° E), 118 m a.s.l., 18.VII.2017, leg. M. Madl. 1 alate gyne (NHMW), Spain, Pontevedra env., leg. H. Franz (no collection date).

Illustrations examined: CASENT0178773, 1 alate gyne (CASC), Netherlands, Bergen (52.67694° N, 4.69944° E), 5.VII.2007, leg. P. Boer.

Data examined: Biometric data of 20 mermithogynes and 19 alate gynes, Ireland, Clare Island, (53.8028° N, 9.9545° W), 20.VIII.2005, leg. A. O'Grady & J. Breen (see O'Grady & Breen 2011).

Figs. 1–3: *Lasius niger* mermithogyne, habitus. (1) Frontal, (2) dorsal, (3) lateral (note parasitic nematode protruding through gastral wall).

## Results

M e a s u r e m e n t s  (in mm): Mermithogyne: HW 1.61, HL 1.46, EL 0.44, FeL 1.47, FWL 6.66, ML 3.06, MSW 1.86, OcW 0.07, PSL 0.53, SL 1.32, TL 10.59, NL 35; CI 110, EI 27, FeI 91, PSI 33, SI 82, WI 63.

Alate gyne: HW 1.67, HL 1.54, EL 0.54, FeL 1.30, FWL 9.38, ML 3.25, MSW 1.83, OcW 0.13, PSL 0.38, SL 1.30, TL 9.66; CI 108, EI 27, FeI 78, PSI 23, SI 78, WI 97.

A b e r r a n t  m o r p h o l o g i c a l  c h a r a c t e r s  o f  m e r m i t h o g y n e  (Figs. 1–3): In comparison to healthy specimens, the examined mermithogenic phenotype exhibited con-

Tab. 1: Comparison of four biometric characters between alate gynes (AG) and mermithogynes (MG) from three localities (AUT Burgenland, Austria; IRL Clare Island, Ireland; ESP Pontevedra, Spain). IRL data are means of 20 mermithogynes and 19 alate gynes, respectively.

|  | HW (mm) | FeL1 (mm) | FeI | NL (mm) |
|---|---|---|---|---|
| **MG AUT** | 1.61 | 1.47 | 91 | 35 |
| **MG IRL (mean)** | 1.40 | 1.31 | 93 | 41 |
| **AG ESP** | 1.67 | 1.30 | 78 | – |
| **AG IRL (mean)** | 1.49 | 1.20 | 79 | – |

spicuously elongated legs (FeI 91 vs. 78, see Fig. 4) and maxillary palps (PSI 33 vs. 23, see Fig. 3), and somewhat elongated scapes (SI 82 vs. 78). Gyne-specific sexual characters were reduced in the parasitized specimen: The size of all ocelli was reduced, with the diameter of the median ocellus measuring only 50 % of that in the similarly sized uninfected gyne (Fig. 1). The wings were completely developed regarding their shape and venation, but the forewings were considerably reduced in size, reaching only 63 % of the total body length, while they were almost exactly as long as the body in the measured healthy specimen as well as the gyne imaged by AntWeb.org (WI 63 vs. 97, see Figs. 2, 3). The length of the mesosoma was somewhat reduced, while its width was unaffected. The total body length was greater than in healthy specimens, which was at least partly due to the distension of the gaster by the parasitic nematode. Characters of head size (HW and HL) were marginally smaller in the mermithized specimen. The proportions of the compound eyes, as well as pubescence, coloration and cuticular sculpture showed no detectable aberrations.

Comparison to sample of O'Grady & Breen (2011) (Tab. 1, Fig. 4): The two specimens measured for this study were compared to selected morphometric characters taken from the dataset of Irish specimens treated in O'Grady & Breen (2011). Only sexuals (gynes and males) of *L. niger* and *L. flavus* were found to be parasitized. As in the individual described herein, mermithogynes were readily recognizable in the field by their short wings and distended gaster. Although the Irish specimens were consistently somewhat smaller than those measured for this study, similarities in allometry were apparent (Tab. 1, Fig. 4): Regarding the size of the observed parasitic nematodes, the authors reported lengths of 27 – 68 mm for parasites found in *L. niger* gynes. The parasite discovered in the Austrian specimen falls well within this range at 35 mm. As in the specimen from Burgenland, most infested gynes only had one parasitic nematode; an occurrence of multiple parasites was only recorded in two cases. Irish specimens also showed significant differences in femur length and head width between healthy and parasitized individuals, with the mermithogenic phenotypes possessing longer legs (mean FeI 93 vs. 79) and smaller heads (see Tab. 1). The radial cells (a size indicator of the forewing) of mermithogynes were significantly smaller, but the wings showed no aberrant proportions. No effect of parasitism on overall variation of morphological characters was detected.

Molecular analysis: The amplification and sequencing of both *coI* and *coII* was successful for the mermithogyne of *L. niger*. Comparisons to *coI* sequences deposited in NCBI GenBank yielded similarities of 100 % to *L. niger* (GQ503247). Along with the morphological examination, this confirms the species identity of the parasitized specimen.

For the parasitic nematode, only *coI* was successfully amplified. Due to a lack of comparable sequences deposited in GenBank, no conclusive result regarding species identity
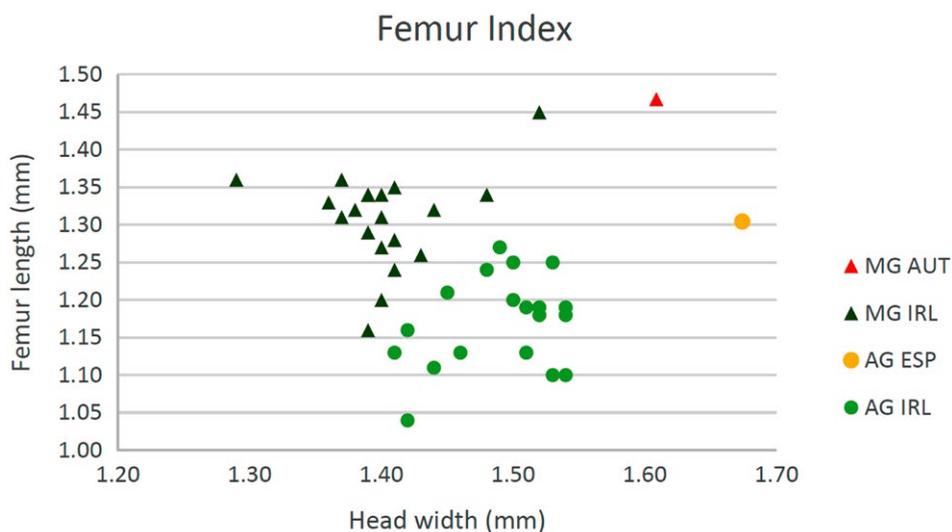
Fig. 4: Compared Femur index of alate gynes (AG) and mermithogynes (MG) from three localities (AUT Burgenland, Austria; IRL Clare Island, Ireland; ESP Pontevedra, Spain).

can be drawn here. The most similar sequences – with 74 %, 72 %, and 71 % similarity, respectively – were those of other entomopathogenic mermithid nematodes: *Hexamermis agrotis* (EF368011), which is parasitic on noctuid moths (Li & Xiong 2005), as well as *Romanomermis nielseni* (EF175763) and *Strelkovimermis spiculatus* (DQ520860), both parasitic on mosquitos (Finney & Mokry 1980, Poinar & Camino 1986).

Discussion

This paper represents the first published record of mermithid parasitism on *Lasius* for the Austrian province of Burgenland. Other Austrian records come from Kaiser (1986), who reported *L. niger* and *L. flavus* from Styria parasitized by *Pheromermis villosa* Kaiser, 1986.

The species identity of the examined mermithogyne is supported by morphological as well as molecular data. The purely morphological identification of parasitogenic phenotypes can prove difficult, as traditional keys rely heavily on characters such as pubescence, sculpture and morphometric indices (e.g., Seifert 2007). All these characters can be altered by parasitic influence, which has led to cases of erroneous species descriptions in the past (Czechowski et al. 2007, Csösz 2012).

The results obtained within this study correspond well with previously published records of mermithogenic phenotypes and parasitic loads in this genus (e.g., Crawley & Baylis 1921, Kaiser 1986, O'Grady & Breen 2011). In all of these cases, parasitized gynes were infested with one to three individual nematodes of one to seven centimeters in length. The described mermithogynes all possessed a distended gaster, and a smaller head and mesosoma. All mermithogynes were brachypterous to some extent, but the wings were always present and fully formed, apart from their size. While more extremely altered mermithogenic phenotypes have been documented for species of *Myrmica* (Czechowski et al. 2007, Csösz & Majoros 2009) and *Colobopsis* (Laciny et al. 2017), I have found

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no case of entirely apterous or worker-like *Lasius* mermithogynes ("mermithergates") in the literature. It is possible that the development of certain host species is more resilient to parasitic stress or that the reason lies in the different species of parasitic nematodes.

A further interesting result was the consistently increased length of extremities in mermithogynes across different genera and subfamilies: Studies have found mermithogynes to possess elongated metafemora (in *Colobopsis* sp., LACINY et al. 2017), profemora (in *Lasius* spp., O'GRADY & BREEN 2011, this study), metatibiae (in *Myrmica* spp., CZECHOWSKI et al. 2007), maxillary palps (in *Colobopsis* sp., LACINY et al. 2017, in *Lasius niger*, this study), and antennal scapes (in *Colobopsis* sp., LACINY et al. 2017, in *Lasius niger*, this study, and in *Myrmica* spp., CZECHOWSKI et al. 2007, CSŐSZ & MAJOROS 2009). The reason for these particular aberrations is currently unknown. Presumably, the developmental perturbation by the parasite causes a shift from gyne-like to more worker-like growth programs (sensu MOLET et al. 2012), resulting in animals that are as large as gynes but more closely resemble the morphological proportions of workers.

Judging by the high incidence of mermithism in sexuals of European *Lasius* species (CRAWLEY & BAYLIS 1921, O'GRADY & BREEN 2011), it seems likely that the intermediate hosts (e.g., oligochaetes) of the parasitic nematodes constitute a significant part of the diet fed to queen- and male-destined larvae. Especially more damp environments seem to favor the development of both intermediate hosts and parasites (KAISER 1986). The sampling locality of the Austrian specimen treated in this study also fits this scheme: The mermithogyne was discovered close to a small channel only 2 km from the shore of Lake Neusiedl (M. Madl, pers. comm.).

The role of mermithogynes in *Lasius* colonies is hitherto unknown. According to previous publications (CRAWLEY & BAYLIS 1921, WHEELER 1928, O'GRADY & BREEN 2011), brachypterous mermithogynes are usually flightless with reduced ovaries and thus cannot take part in mating flights and reproduction. They seem to be generally accepted by their nestmates and have been observed to aid in brood care and beg workers for food (CRAWLEY & BAYLIS 1921). Mermithized specimens will stay with their native colony until compelled by the mature parasite to seek a suitable spot for its release (CRAWLEY & BAYLIS 1921, KAISER 1986, MAEYAMA et al. 1994). Recent models suggest that the survival of intercaste phenotypes may be facilitated by mechanisms of colonial buffering (MOLET et al. 2012). Building on these hypotheses, the study of morphological changes brought about by mermithid parasitism could provide valuable insight into phenotypic plasticity, developmental modularity, and the evolution of novel castes (MOLET et al. 2012, LONDE et al. 2015, LACINY et al. 2017).

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