A new nematode (Mermithidae) and spider (Araneae) associations, along with a molecular analysis of mermithids hosted in the spider

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Abstract

Mermithid parasitoids are well known to infect spiders, however, their impact on the hosts and their taxonomic identity are still poorly analyzed. We present the first record of a mermithid nematode infection in the spider genera Piratula (Lycosidae) and Coelotes (Agelenidae), and in the species Alopecosa pulverulenta and Pardosa paludicola (Lycosidae). We describe the interesting maldevelopment of the spiders' female genitalia induced by the parasitoid and summarize the data on the impact of nematode parasitoids on spider development and behaviour. Phylogenetic analysis, based on the 18S rRNA, showed that spider' parasitoids arose independently in different phylogenetic branches of the family Mermithidae.

Introduction

Mermithids (Nematoda: Mermithidae) are parasitoids of a wide range of invertebrates, including various insects e.g. bumblebees [1], hornets [2], hemipterans [3], moths [4] and beetles [5]. However, they parasitize other arthropods, such as spiders [6] and crustaceans [7], and they can parasitize other invertebrates, e.g. slugs [8]. Mermithid parasitism of spiders is widespread in the world and has been reported in different ecological groups of spiders [6]. The first report of a mermithid infecting spiders comes from as long ago as the end of the 18th century [9]. Since that time over 60 species of spiders have been reported as hosts of these parasitoids. The recorded hosts belong to 23 spider families and represent both ground hunters and web weavers [6]. Mermithids have been recorded in various habitats, although the parasitized spiders are usually more common in humid environments. This wide spectrum of spider hosts may be linked to the life history of mermithids, which includes two ways of infecting hosts [10,11]. In the direct life cycle, the pre-parasitic juvenile mermithid, which hatches from an egg in the environment, directly penetrates the host. In the case of the indirect life cycle, which is thought to be more common, a mermithid reaches a spider via a paratenic host (larvae of aquatic insects for example), in which the nematode remains inactive [10,12]. The mermithids feed parenterally on the spider hosts' tissues (mainly in the opisthosoma), which eventually leads to the death of the spider [13]. The mermithid parasitoids may also induce the deformation of the opisthosoma, or copulatory organs, and reduce the amount of guanine deposition [14]. Infection by mermithids can also lead to behavioral changes in the parasitized spiders, which may include a tendency of the hosts to move towards water [12].

Mermithids emerge from spiders, as postparasitic juveniles, by penetrating their cuticle. These juvenile nematodes molt twice in order to turn into adults. As immature specimens they are unidentifiable to species or even genus level, as the present classification is based on adult characteristics [12,15]. Thus, all immature specimens of mermithid recorded from spiders before 1986 are treated as *species/ genera inquirende* [15]. Presently, only a single extant genus of spider parasitoids from Mermithidae – *Aranimermis* Poinar & Benton, 1986 is recognized based on the features of adult specimens. It includes three species: *A. actereki* Gafurov & An 1987; *A. aptispicula* Poinar & Benton, 1986 and *A. giganteus* Poinar & Early, 1990 [12,15,16]. Therefore, we know extremely little about the taxonomy or phylogeny of mermithids parasitizing in spiders, especially considering the fact that these parasitoids are often and

widely recorded. Up to now, only five sequences of 18S rRNA, from mermithids emerged from two spider species, have been published [17,18], and only sequences of mermithids from *Tetragnatha* have been included in papers revealing a phylogenetic analysis of Mermithidae [1,7,17,19].

Our study reports new mermithid parasitoid-spider host associations, presents new data on the maldevelopment of spiders' female genitalia induced by the parasitoid and provides a molecular identity and phylogenetic analysis of the hitherto undescribed nematode of spiders.

Material and Methods

Field and laboratory studies

Spider specimens with mermithid parasitoids were recorded in three independent surveys (Table 1). Six spider specimens with mermithid parasitoids were sampled in the montane mires of the Izera Valley (SW Poland; Table 1). They were caught in pitfall traps (diameter – 75 mm, volume – 250 ml), with ethylene glycol. The traps were sampled on average every 19 days (Table 2). The material was stored in 75% denatured ethanol. The habitats in which the infected spiders lived were both minerotrophic (i.e. supplied with water from both ground and rain) and ombrotrophic mires (supplied with water from precipitation only; Fig. 1a). The two habitat types in the Izera Valley are usually situated close to each other, and they rely mainly on the presence of Sphagnum spp. mosses, thus they are constantly saturated with water. The minerotrophic mires were dominated by Carex rostrata Stokes or Eriophorum angustifolium Hunck., and the raised mires were covered partly by mountain pine (Pinus mugo Turr., growing here at ca. 800m a.s.l.) and dominated by different plants characteristic of peat bogs, i.e. Oxycoccos, Calluna vulgaris (L.) Hull, and Andromeda polifolia L. The climate of the Izera Valley is extremely severe for its altitude (ca. 800-900 m a.s.l.), and the valley hosts an array of unique habitats – different mires being among the most important. A further infected spider specimen was also found in a pitfall trap installed from 20 June to 4 July 2016 during a spider study in another humid habitat – alder mixed forest along the small river Horodnianka near Turczyn (NE Poland; Table 1).

The other spiders parasitized by mermithids were revealed during behavioral surveys on Pardosa paludicola (Clerck, 1757) (Lycosidae). They were collected from a population near Kruszyniany (NE Poland; Table 1), at the beginning of May in 2021 and 2022 (during the reproduction period). For this study, females of P. paludicola with swollen abdomens (which suggests that the female is just about to make the egg sac) were chosen. The habitat (Fig. 1b) was a wet grassland situated by a field drain, overgrown with low vegetation dominated by grasses (Poaceae), umbellifers (Apiaceae) and nettles (Urtica dioica L.). Spider females and a couple of males were collected by direct catch using plastic containers (60 ml). The females were kept alive in individual circular glass terrariums (10 cm diameter x 5 cm high), with soil from their habitat and vermiculite pellets, and were bred at 21 ± 1 °C, 56 ± 1% relative humidity under a light:dark (L:D), 14:10 h photoperiodic cycle. The spiders were fed once a day ad libitum with juvenile crickets (Acheta domesticus), and the substrate of the terrariums was sprinkled twice a day with water. All females were checked twice a day (in the morning and evening) to capture the moment of

making the egg sac and any changes related to their behaviour. Spiders that did not make egg sacs and died in the laboratory were dissected and examined for the presence of nematodes. Both parasitoids and spiders were preserved at -80 °C.

Table 1 Characteristics of localities in which the parasitized spiders were sampled, and sampling methods used.

Region	Coordinates (N/E)	Locality	Habitat type	Method
SW Poland, Sudetes, Izera Mts., Izera Valley	50.85278, 15.36008; 50.85222, 15.36111	"Młyńskie Bagno" peat bog	Ombrotrophic mire	Pitfall traps
	50.83651, 15.37371	"Kobyła" stream	Minerotrophic mires (periodically flooded)	
	50.86467, 15.31303	"Izerskie Bagno"		
NE Poland, Podlachia	53.183272, 23.793402	Kruszyniany	Wet meadow	Direct catch
	53.098312, 23.097947	Turczyn	Alder mixed forest	Pitfall traps

All the spider species were identified using [20], and the nomenclature follows the World Spider Catalog [21]. The epigynes of infested females were compared with the genitalia of females coming from the same or a closely situated location. The epigynes were dissected and their pictures were taken using an Olympus DSX 110 microscope. The interpretation of female genitalia structure follows Almquist [22] and Hepner & Milasowszky [23].

The voucher samples have been deposited at the Laboratory of Insect Evolutionary Biology and Ecology, Faculty of Biology, University of Bialystok.

Molecular analyses

We analyzed nematodes obtained from 13 spider individuals belonging to Alopecosa pulverulenta (N = 2), Coelotes sp. (N = 1), Pardosa paludicola (N = 7), Piratula hygrophila (N = 1) and Trochosa sp. (N = 3). The nematode DNA extraction was performed with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR amplification of 18S small subunit ribosomal RNA gene (18S rRNA) was carried out with a Labcycler Gradient (SensoQuest, Göttingen, Germany) in 5 µL volumes, and the reaction mixtures consisted of ~25 ng extracted genomic DNA as a template, 1.7 µL of Qiagen Multiplex PCR Master Mix (1x), 0.3 µL mix of primers (18S-F: 5'-CAA GGA CGA AAG TTA GAG GTTC-3'; 18S-R: 5'-GGA AAC CTT GTT ACG ACT TTTA-3') [24] and 1 µL of Qiagen nuclease-

free water. The reaction conditions were as follows: 15 min at 95 °C of an initial denaturation, 40 cycles with denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s, extension at 72 °C for 60 s, and final elongation for 30 min at 60 °C. The products of the amplification reaction were purified with the EPPiC Fast mixure (A&A Biotechnology, Gdańsk, Poland) contained two enzymes that effectively degrade dNTPs, and primer left-overs from previous PCR mixtures. Purification reaction was carried out in a thermal cycler following the manufacturer's protocols: 37 °C for 5 min, 80 °C for 1 min. Then the samples were processed for cycle sequencing PCR with a BigDye™ Terminator v.3.1 Cycle Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA) using primer forward (18S-F). Unincorporated dideoxynucleotides were then eliminated from the sequencing reaction using the ExTerminator Kit (A&A Biotechnology). In the last stage of laboratory analysis, the sequencing products of 18S rRNA were run on an automated capillary sequencer ABI 3130 (Applied Biosystems, Foster City, CA, USA).

Sequencing results were revised and aligned manually using BioEdit v.7.0.5.3 [25], and compared to the GenBank references (Supplementary Table S1) by BLAST (http://www.ncbi.nlm.nih.gov/, accessed on March 2023) to determine the nematode species. The sequences of the 18S rRNA gene obtained in this survey were submitted to the GenBank.

We calculated the number of polymorphic sites between the described haplotype using the software packages ARLEQUIN v.3.5.1.2 [26]. To test the phylogenetic relationships among the 18S rRNA haplotypes derived in this study and sequence downloaded from GenBank (Supplementary Table S1), we constructed phylogenetic tree using a maximum-likelihood (ML) algorithm in Mega v.6.06 [27] with 1,000 bootstrap replicates used to assess the support for tree nodes. In the phylogenetic analyses, the nucleotide substitution model GTR+I+G was determined under the Akaike information criterion [28] implemented in jModelTest v.0.1.1 [29].

Results

Rate of parasitism and host species

All of our observations come from fairly humid habitats. Mermithid nematodes were detected both in adult and juvenile spider females and males (Table 2). Six spiders from the Izera Mountains which were infested by mermithids belonged to the family Agelenidae (Coelotes cf. terrestris) and Lycosidae (Alopecosa pulverulenta (Clerck, 1757), Trochosa sp.). The parasitized female from the Turczyn forest was the lycosid spider Piratula hygrophila (Thorell, 1872). From Kruszyniany, eight parasitized Pardosa paludicola were recorded. The rate of parasitism in this population was 9.9% in 2021 (in total 81 collected specimens) and 1,5% in 2022 (66 collected specimens).

In all studied cases a single nematode emerged from each spider. Parasitoids appeared 21 to 29 days after collecting adult P. paludicola in the field, but in one case a mermithid was revealed only after dissection of a dead spider.

Table 2 Spider species and mermithid haplotypes (H1, H2), with sampling dates (for Izera Valley and Turczyn – pitfall trap exposure; in the case of Kruszyniany – sampling date and, in brackets, date of spider death). Number of specimens is indicated if more than one were observed). ns – sequence of 18S rRNA was not obtained, jv – juvenile.

Region	Locality	Dates	Spider genus or species	Spider (sex, stage)	Nematode 18S rRNA haplotype
Izera Valley	"Młyńskie Bagno" peat bog	10-30.8.2010	Trochosa sp.		H1
		22.7-10.8.2010	Trochosa sp.		
		24.07-12.8.2011	Trochosa sp.	jv	
	"Kobyła" stream	22.7-10.8.2010	Alopecosa pulverulenta		
	"Izerskie Bagno"	1-23.7.2010	Coelotes cf. terrestris	jv	ns
			Alopecosa pulverulenta		H1
Podlachia	Kruszyniany	9-12.5.2021 (7-28.6.2021) 2.5.2022 (7.6.2022)	Pardosa paludicola	7 , 1 jv	
	Turczyn	20.6-4.7.2021	Piratula hygrophila		H2

Morphological changes

Parasitized specimens of Pardosa paludicola showed no known signs induced by parasitoids. The opisthosomas of parasitized females looked just like those of fertilized ones. One female P. paludicola showed torsion of a pedicel (Fig. 2f). In the cases of spiders collected in pitfall traps, the presence of mermithids was noticeable during the sorting of material – parasitoids partly emerged from opisthosoma or were easily visible through a sometimes partly destroyed tegument. The nematodes were usually tightly coiled and occupied the entire opisthosoma (Figs 2a-c).

A few parasitized females had a deformed epigyne. This was strongly pronounced in the case of Piratula hygrophila (Fig. 3). The external appearance was distorted, namely the spermathaecae that are easily visible through the cuticule were almost completely absent, and the lateral parts were asymmetric. The inner structures were largely undeveloped. The anterior parts of the spermathaecae that are normally elliptical and the posterior, perpendicularly bent parts of the spermathecae were both completely missing. The overall proportions of the structure were also distorted and it could easily be mistaken for the epigyne of a completely different species. The only clear resemblance to the genital plate of an uninfected female was a thin scape in the posterior part of the epigyne. This structure might be also interpreted as a pre-epigyne of the species.

The genital structures in some parasitized Pardosa paludicola females (Fig. 4) seem to be slightly undeveloped. The usually strong and lightly curved spermathaecae are comparatively smaller, thinner and straighter. However, the typical structure of the epigyne seems to be preserved in all the specimens, and the apparent changes might have occurred only during the preparation. The other parts of the epigyne do not differ from those in unparasitized females.

The extent of distortion of the epigyne in Alopecosa pulverulenta was variable depending on the observed specimen. In one case the spermathaecae appeared to be smaller, the copulatory ducts weaker and less curved. In one case we observed an additional plate outside the epigyne, which might have been a pre-epigyne that did not fully detach itself during a moult. In other females the structure of the epigyne was similar to the one in unparasitized females. It must be, however, noted that the structure of epigyne in this species seems to be rather changeable [20].

As far as external structure is considered, the epigyne of Trochosa (most probably T. spinipalpis (F.O. Pickard-Cambridge, 1895) but also T. terricola (Thorell, 1856) should be considered) was not apparently modified. In all cases the internal organs were almost completely eaten out by the parasitoid; and one of the Trochosa specimens was internally covered with thick tissue, most probably developed as a defence against the nematode.

Behaviour of the parasitized spider

Most of the parasitized specimens of Pardosa paludicola kept in the laboratory showed no noticeable behavioural changes in relation to uninfected females. In these cases mermithids emerged entirely or partly from the host – they were found next to the hosts or in their opisthosoma. In P. paludicola a parasitoid emerged near the epigyne (Fig. 2d) and in Piratula hygrophila near the spinnerets (Fig. 2e). One parasitized female of P. paludicola turned upside down two days before death. After her death a torsion of the pedicel and the presence of a mermithid in this part of the body was revealed (Fig. 2f).

Eight specimens of P. paludicola were found dead after mermithid emergence, however one female lived for seven weeks after emergence of a parasitoid. She had not made any egg sac.

Molecular data

The obtained sequences of an 18S rRNA gene fragment yielded two new haplotypes of Mermithidae: haplotype H1 (766 bp; GenBank accession no. OQ836593) and haplotype H2 (760 bp; GenBank accession no. OQ836594), as defined by 39 polymorphic sites: 21 transitions and 18 transversions. Haplotype H1 was widely distributed, as it occurred in nematodes obtained from 12 spider individuals: Alopecosa pulverulenta (N = 2), Trochosa sp. (N = 3), and Pardosa paludicola (N = 7) from different parts of the country. On the other hand, haplotype H2 was found only in a nematode from Piratula hygrophila. We did not manage to obtain sequences of 18S rRNA gene fragments from a nematode from Coelotes sp.

The maximum-likelihood phylogenetic reconstructions produced a strong topology (Fig. 5). The ML tree revealed that our two 18S rRNA haplotypes and sequences downloaded from GenBank (Supplementary Table S1) joined in a phylogenetic branch together with haplotypes belonging to Agamermis changshaensis, A. xianyangensis, Hexamermis agrotis, H. popilliae and Mermithidae sp. Haplotype H1 showed almost 96% similarity with the haplotype of A. changshaensis (GenBank accession no. DQ628908) found in China, and the haplotype of Mermithidae sp. (GenBank accession no. AY284743) recorded from the Netherlands. The haplotype H2 shares 94% similarity with Hexamermis popilliae (MF040823), which was found in an invasive beetle Popillia japonica Newman, 1841 in Italy.

Discussion

This is the first time mermithids have been found in the genus *Coelotes* (Agelenidae) and *Piratula* (Lycosidae), and in the lycosid species *Alopecosa pulverulenta* and *Pardosa paludicola*. Concerning Agelenidae, which are typically funnel web weavers, the mermithid was only found in *Agelenopsis oregonensis* Chamberlin & Ivie, 1935 [10]. Our finding comes from the genus *Coelotes*, most probably *Coelotes terrestris* (Wider, 1834), which is very common in the area and was recorded at the same site from adult specimens. Spiders from this genus are ground dwellers and they are regularly sampled by pitfall traps. The representatives of Lycosidae, which are ground hunters, are most frequently recorded as hosts of mermithids. The nematodes have been recorded in 23 species belonging to 10 genera of Lycosidae, which constitute 37% of all known spider hosts. This applies in particular to the genus *Pardosa* (13 species, 20% known spider hosts) [6,30,31]. *Alopecosa* as mermithid hosts have only so far been known from *A. inquiline* (Clerck, 1757) and *A. trabalis* (Clerck, 1757) [10]. We add *A. pulverulenta* to the list, a very common spider species. Concerning the genus *Trochosa,* the nematode parasitoids were only found in *Trochosa robusta* (Simon, 1876) [32] and *Trochosa* cf. *terricola* [33]. Our record comes most probably from *Trochosa spinipalpis* or *T. terricola*, which prefers areas with high humidity. Females from the genus *Trochosa* in Central Europe are hard to identify to species level based on the structure of their genitalia, however it is not impossible [23]. The specimens we observed strongly resembled *T. spinipalpis*, nevertheless some doubts remained. Nevertheless, *T. terricola* was also present in the same peat bog, and the proportion of adult male numbers was 5.4 : 1 in favour of *T. spinipalpis*.

Mermithid parasitism of spiders is rather widespread, although rarely recorded [6,10]. Only in a few studies was estimation of the prevalence of nematodes possible. In our study the rate of parasitism in the *Pardosa paludicola* population was up to 10%. Similar results, indicating a high infection rate, were recorded in a population of a ground dwelling mygalomorph spider - *Atypoides riversi* O. P.-Cambridge 1883 in California [10] and in a lycosid *Pardosa milvina* in Illinois [34]. In the latter case, 8% of individuals were infected by mermithids. A lower prevalence of parasitoids was recorded in a population of *Pardosa glacialis* (Thorell 1872) in Canada, in which 0-5% individuals were parasitized, and the result depended on the changing humidity of the areas along the stream [10]. A similar level of infection rate (from 0 to more than 4%) was noted in long-jawed orb-weaver spiders *Tetragnatha* (*T. brevignatha* Gillespie, 1992; *T. quasimodo* Gillespie, 1992; *T. anuenue* Gillespie, 2002) in Hawai [17]. Mermithids are usually noted as they emerge from a spider and little is known about their development in spiders. A relatively complete life cycle is known only for *Aranimermis giganteus*, parasitoid of mygalomorph spiders [12]. In the case of this mermithid, however, it is common that more than a single individual develops in a host (up to seven specimens from a single host) [12], whereas in most of the Araneomorpha spiders only a single mermithid appears in each host (e.g. [6,15]). The only exceptions were observed in two specimens of *Pardosa pseudoannulata* (Bösenberg & Strand, 1906), from which two and ten parasitoids emerged [35].

Many authors pointed out that the indirect life cycle (involving a paratenic host) might be more common for mermithid recorded from spiders, especially taking into account that mermithids were recorded both from active hunters and web spiders [10,12,15,35]. On the other hand, the parasitized spiders are mainly recorded in wet habitats [31,35,36], which may be suitable for mermithid pre- and postparasitic stages. A large number of known spider hosts (about 41%) are ground dwellers [6], thus it seems possible that both life cycle types (direct and indirect) could be involved in the transmission of spider parasitoids belonging to Mermithidae [10].

In *Pardosa paludicola,* mermithids emerged near the epigyne, in the same way as in *Tenuiphantes* sp. [14], but in *Piratula hygrophila* – similarly to *Trochosa* sp. – the parasitoid came out near the spinnerets [33]. Before the emergence of a parasitoid most of *P. paludicola* showed no external signs of parasitism, with the exception of swollen opisthosoma, which was of a similar size to those of unparasitized females before making an egg sac. An enlarged opisthosoma was also observed in parasitized adult lycosid males of *Prolycosides amblygyna* (Mello-Leitão, 1942) and juveniles salticids (*Thiodina* sp., *Frigga* sp.) [31]. In infected females of *Pardosa pseudoannulata,* the opisthosoma was swollen and sometimes lopsided [35]. On the other hand, no external morphological change was observed in parasitized adult females of *Heriaeus spinipalpus* Loerbroks, 1983 [36] or *Caerostris sumatrana* Strand, 1915 [6]. The occurrence of swollen opisthosoma can be a result of the length of the mermithid and the body size of spiders [6,11], but also the placement of parasitoids in the host. In one of the parasitized females of *Pardosa paludicola,* a torsion of the pedicel was noticed after the spider's death. After the spider dissection it was revealed that the parasitoids had penetrated partly into the prosoma by the pedicel. This observation corresponds with the observation by Ranade & Prakash [37] that a mermithid was found in

the cephalothorax of *Heteropoda venatoria* (Linnaeus, 1767). Leech [13] showed that a parasitized *Pardosa glacialis* lacked some of the main prosomatic muscles, which may also suggest that mermithids in this species also resided in the prosoma.

In the opisthosoma, mermithids feed on the spider's tissues and haemolymph [38]. Leech [13] observed a lack of the entire digestive system, and body fat as well as various degrees of castration in infected males and females of *Pardosa glacialis*. The latter was also noted in an *Alopecosa inquiline* (Clerck, 1757) [39]. Castration can lead to anomalies in the copulatory organs. The maldevelopment of the epigyne, the external, sclerotized plate in spider females that is used for copulation and oviposition, may be caused by several factors [40] including teratologies [41,42], hybridization [43], development of gynandromorphic specimens [42,44], and damage of genitalia during mating [45], as well as parasitoids. Epigyne deformation caused by a mermithid was documented in *Pardosa furcifera* (Thorell, 1875) [11], *Pardosa glacialis* [13] and *Philodromus collinus* Koch, 1835 [18]. In the latter case this even led to the description of a new species, based on the undeveloped epigyne or pre-epigyne of infected specimens [18,46]. The undeveloped spermathaecae in parasitized *Piratula hygrophila* that we described could lead to a similar mistake. In general, the modifications of spider genitalia as a result of mermithid infection are extremely variable. We observed a whole continuum of changes, from a largely modified epigyne, which could easily be assigned to a completely different species, up to no visible modifications of external genitalia. The same rule applies to the data from the literature. Parasitoids do influence the maldevelopment of genitalia in some cases, however the extent of change might be a result of different, still unknown factors, e.g. time of infection, placement of a parasitoid, parasitoid species, and host organs that are first eaten out during the infection.

Different parasites and parasitoids may modify the behaviour of the spider [47]. In the case of mermithids there is a report that infected spiders move towards sources of water, where the parasitoids emerge from the bodies of the hosts to complete the life cycle [10,12]. Altered behaviour was shown by *Cantuaria* females infected by *Aranimermis giganteus*, which were found in a pan trap, whereas it is unusual for the female of this spider to leave or move far from its burrows [12]. In the case of our observations from the Izera Mountains, the spiders with nematodes were mostly found in pitfall traps that had been flooded (almost totally filled up with water). The peat bog in which parasitized *Trochosa* were observed was inhabited by two species from this wolf spider genus. Although all the mires were dominated by *Trochosa spinipalpis*, we cannot exclude that specimens of *Trochosa terricola*, which are common, and affiliated to drier habitats than *T. spinipalpis,* migrated more eagerly from the adjacent habitats due to changed behaviour. Only one parasitized *P. paludicola* kept in a laboratory showed behavior change. This female turned upside down two days before death, which could have been the result of torsion of the pedicel caused probably by the presence of a mermithid in this part of the body. In *Pardosa glacialis*, when the parasitoid was about to emerge, the spider crawled into a dark hole or corner [13].

As a parasitoid, the mermithid leads eventually to the death of the host. In eight out of nine cases of parasitized *Pardosa paludicola*, hosts were found dead during or after mermithid emergence. Because

spiders were checked twice a day, it is possible that infected spiders died similarly as in most other cases, for example *Pardosa glacialis* died 30-60 minutes before a mermithid emerged [13], *Pardosa pseudoannulata* was usually dead at the time of nematode emergence [35], *Caerostris sumatrana* Strand, 1915 died immediately [6] and *Heteropoda venatoria* (Linnaeus, 1767) within an hour after parasitoid emergence [37]. It was a huge surprise that one female of *P. paludicola* lived for seven weeks after the emergence of the parasitoids. Two salticid spiders (*Thiodina* sp. and *Frigga* sp.) [31] and individuals of *Pardosa milvina* [34], also remained alive, but only for a few days or 24 hours after parasitoid emergence, respectively.

Up to now, only five sequences of 18S rRNA from mermithids emerged from spiders have been known – three from *Tetragnatha* sp. (Tetragnathidae) from Hawaii [17] and two from *Philodromus collinus* (Philodromidae) from Germany [18]. Unfortunately none of them create a phylogenetic branch together with the haplotypes of studied specimens. All known haplotypes of mermithids obtained from spiders form clusters with haplotypes belonging to specimens found in insects, or even crustaceans (Fig. 5). Our results show that different mermithid taxa (species and genera) infest spiders, and that the mermethid parasitoid of spiders arose independently in different phylogenetic branches of Mermithidae.

Declarations

Data availability

The datasets generated during the current study are deposited in GenBank under accession number OQ836593 and OQ836594, and included in this published article and its supplementary information file.

Authors contribution

A.K.-A., U.J., K.W. and J.K. carried out the field work; U.J., A.K.-A. and M.Ś. conducted the laboratory work with input from K.W. and J.K.; A.K.-A., K.W. and M.Ś. wrote the manuscript with input from U.J. All the co-authors gave final approval for publication.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

The habitats where parasitizedspiders were collected: (a) ombrotrophic mires “Młyńskie Bagno” of the Izera Valley (SW Poland), (b) wet grassland near Kruszyniany (NE Poland).

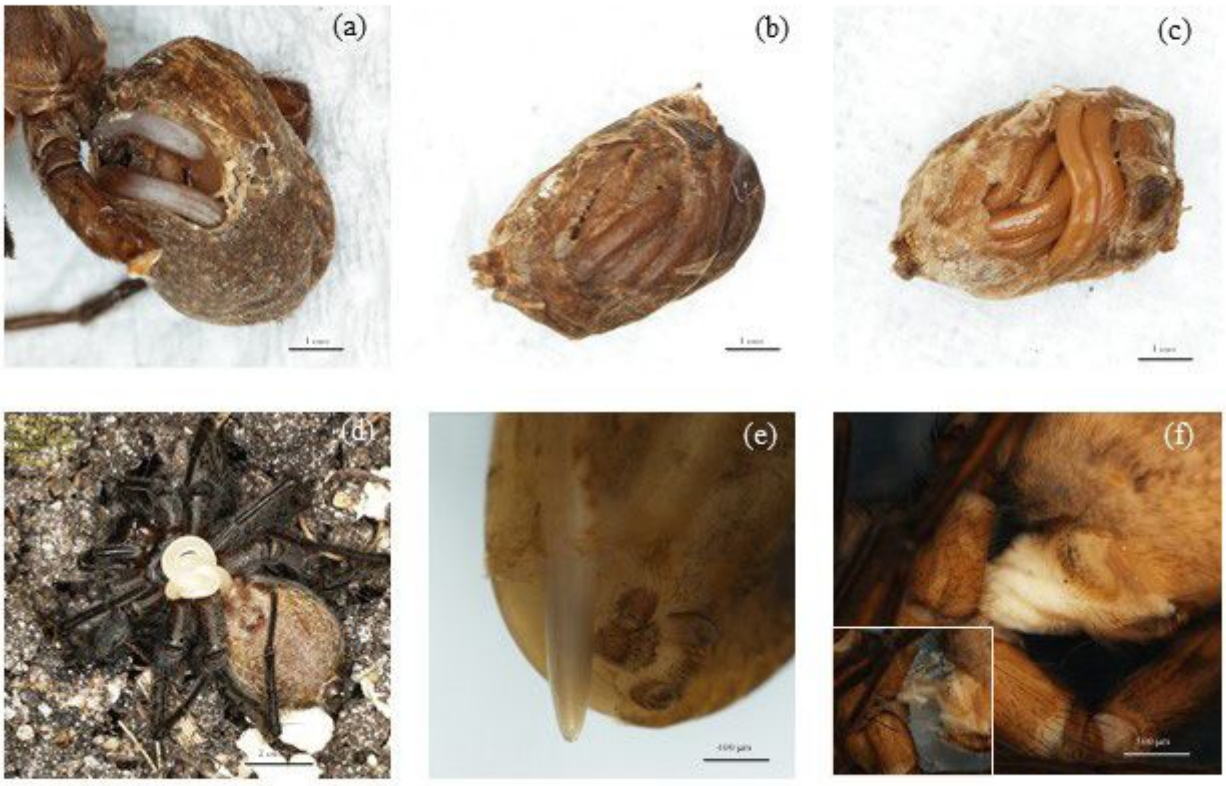

Figure 2

Mermithids in: (a) *Trochosa* sp., (b-c) *Alopecosa pulverulenta*, (d, f) *Pardosa paludicola*; (d) emergence from opisthosoma and (f) presence in pedicel, (e) *Piratula hygrophila*, emergence from opisthosoma.

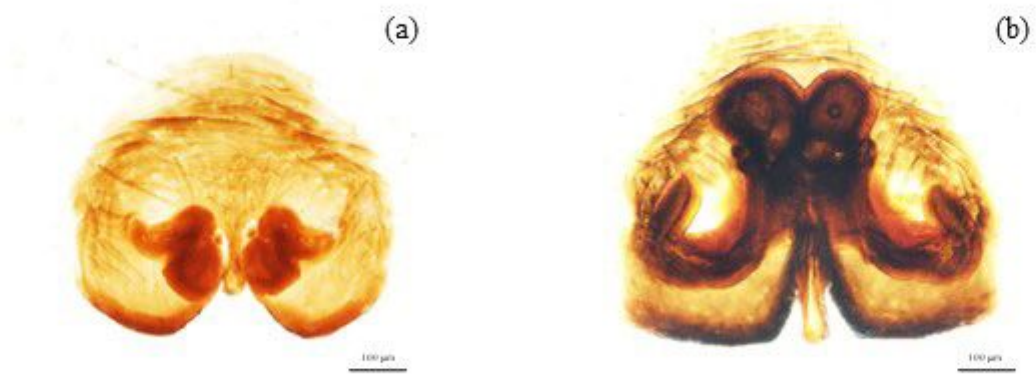

Figure 3

Epigyne of *Piratula hygrophila* in: (a) female parasitized by a mermithid, (b) unparasitizedfemale.

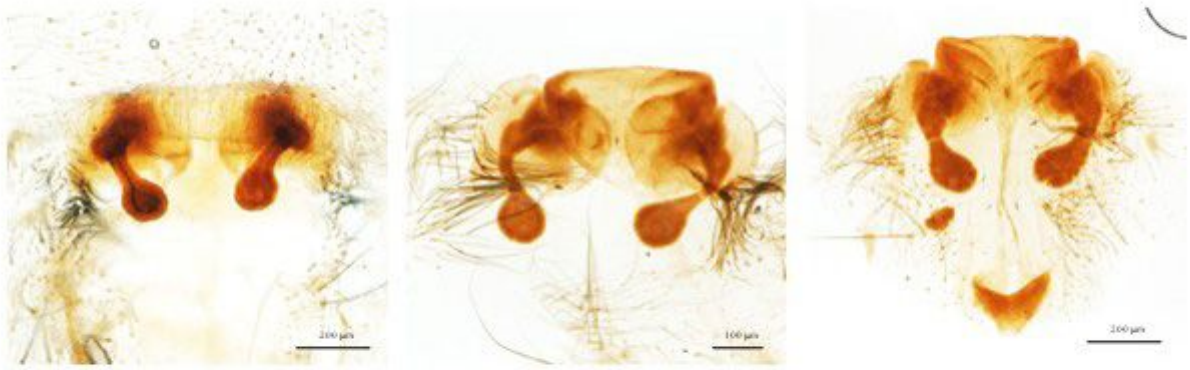

Figure 4

Epigyne of *Pardosa paludicola* in: (a-b) female (‘46’ and ‘53’, respectively) parasitized by a mermithid, (c) unparasitized female.

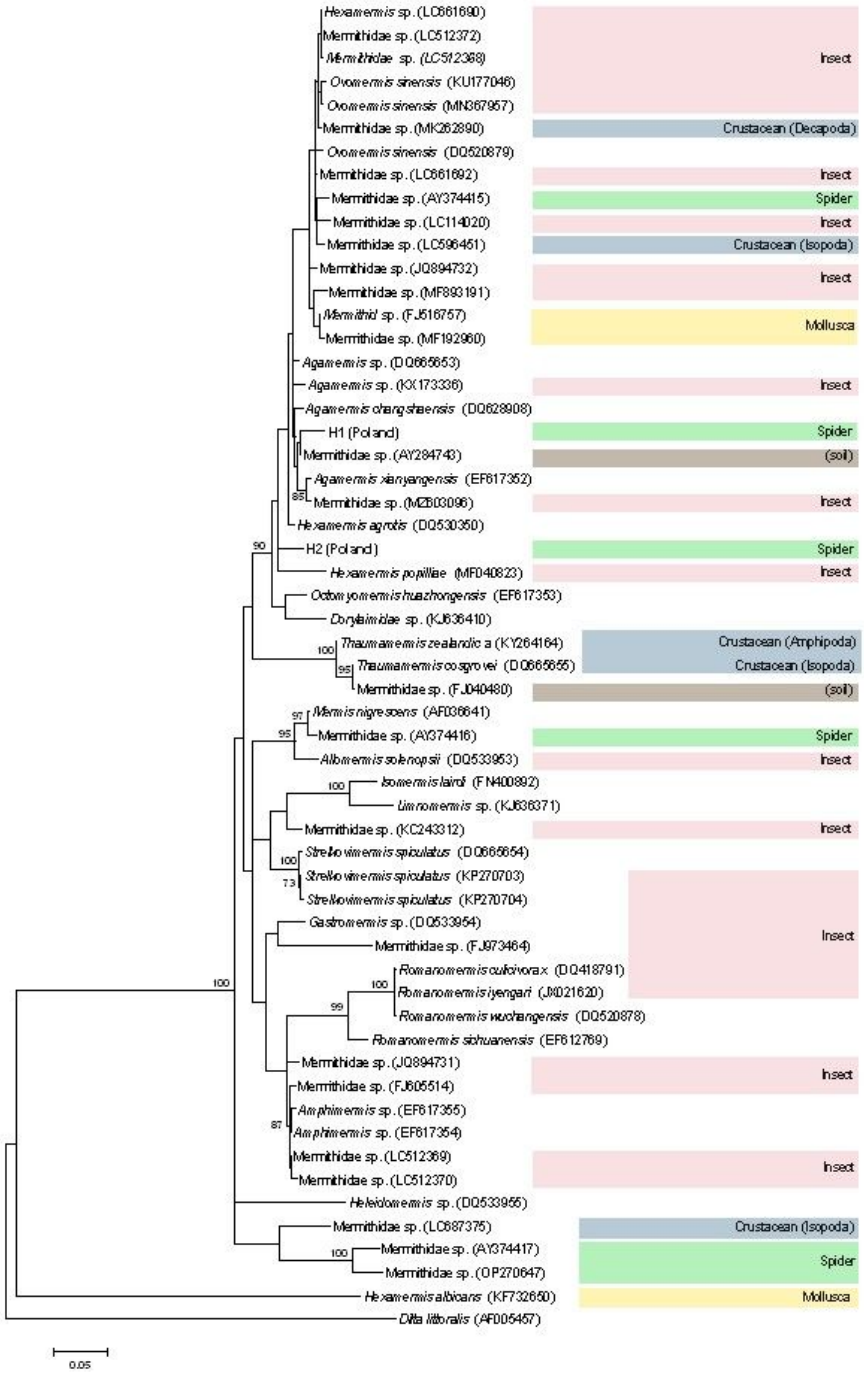

Figure 5

Maximum-likelihood topology computed with the GTR+I+G model of substitution evolution, representing the phylogenetic relationships among the sequences of the 18S small subunit ribosomal RNA gene in nematode parasites. Numbers listed at the nodes represent the percentage support for the node from 1000 bootstrap replicates. The ML tree has been rooted with sequences of *Hexamermis albicans* and *Dilta littoralis*. The haplotypes obtained in this study are marked in bold.

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