Infection with mermithid nematodes causes the depriesteri morphology in Philodromus collinus (Araneae: Philodromidae)

Authors: Wegewitz, Viktoria, Muster, Christoph, and Astrin, Jonas J.

Source: Arachnologische Mitteilungen: Arachnology Letters, 64(1) : 25-28

Published By: Arachnologische Gesellschaft e.V.

URL: https://doi.org/10.30963/aramit6403

Infection with mermithid nematodes causes the *depriesteri* morphology in *Philodromus collinus* (Araneae: Philodromidae)

Viktoria Wegewitz, Christoph Muster & Jonas J. Astrin

doi: 10.30963/aramit6403

Abstract. Two female *Philodromus* specimens were found with genital structures similar to those described for *Philodromus depriesteri* Braun, 1965, currently considered a nomen dubium. Molecular analysis revealed that the two specimens belong to the species *Philodromus collinus* C. L. Koch, 1835. The opisthosomas of both spiders each contained a parasitoid, and one of the worms was identified as a member of the nematode family Mermithidae. It is very likely that the parasitoids interfered with the development of the female genital organs, resulting in the characteristic *depriesteri* vulva structure. Consequently, we propose *Philodromus depriesteri* Braun, 1965 = *Philodromus collinus* C. L. Koch, 1835 **syn. nov.**, thus releasing *P. depriesteri* from being considered a nomen dubium.

Keywords: DNA barcoding, new synonym, nomen dubium, parasitoid, *Philodromus depriesteri*, spider

Zusammenfassung. Die Infektion mit Mermithidae (Nematoda) verursacht das *depriesteri*-Erscheinungsbild bei *Philodromus collinus* (Araneae: Philodromidae). Zwei weibliche *Philodromus*-Exemplare wurden gefunden, deren Genitalstrukturen *Philodromus depriesteri* Braun, 1965 ähneln, ein Name, der aktuell als nomen dubium gilt. Eine molekulare Analyse ergab, dass die Exemplare zur Art *Philodromus collinus* C. L. Koch, 1835 gehören. Die Opisthosomata beider Spinnen enthielten je einen parasitoidischen Wurm. Die Analyse ergab, dass einer von diesen der Nematoden-Familie Mermithidae angehört. Es wird angenommen, dass diese Parasitoiden die Entwicklung der Vulven verändert und dabei das charakteristische *depriesteri*-Erscheinungsbild hervorgerufen haben. Folglich synonymisieren wir *Philodromus depriesteri* Braun, 1965 mit *Philodromus collinus* C. L. Koch, 1835 (**syn. nov.**), so dass *P. depriesteri* nicht mehr als nomen dubium betrachtet werden muss.

To identify spiders at the species level, genital morphological characteristics are usually used. These are relatively constant morphologically (Huber 2004). Aberrant appearances of the genital organs are rare (Jocqué 2002) and mostly due to gynandromorphism and intersexuality (Holm 1941, Kaston 1961). The latter are mostly asymmetrical. Symmetrical aberrations are extremely rare (Jocqué 2002). Infestation with parasitoids has been discussed as a possible cause for such alterations (Martin 2013). Outside the Araneae, the phenomenon of sexually abnormal individuals that coincide with the presence of a parasitoid, namely Mermithidae, occurs in the fly *Simulium trangense* (Ya´cob et al. 2021).

Mermithidae are a family of nematodes. They are very long and thin worms that mainly parasitize insects such as mosquitoes, grasshoppers, butterflies, damselflies or beetles, but also in other invertebrates such as crustaceans, leeches and spiders. Often the mermithid hosts are unknown (Nickle 1972). The occurrence of these nematodes in spiders seems to be a frequent phenomenon (Penney & Bennet 2006, Košulič & Mašová 2019) and could have an impact on their general morphological appearance, including the appearance of the genitalia. It is interesting to note that all parasitized spider species found generally feed on adult insects that have an aquatic larval stage, e.g. Chironomidae, Culicidae, Trichoptera (Poinar 1985). Eggs of mermithids laid in or near aquatic habitats are ingested and hatch in the gut of the paratenic host, which may be one of various invertebrates (insect larvae, turbellarians, annelids). The pre-parasitic larvae invade the tissue and enter a state of dormancy, which is only broken when the paratenic host is eaten by the developmental host (e.g. a spider). There, the parasite completes its development into a post-parasitic juvenile. Such an infestation is thus indirect (Poinar 1985).

The indirect cycle includes two additional variables compared to the direct form. The paratenic host must fall prey to the correct predator and the parasitized host must return to the aquatic environment with the nematode when the latter is ready to emerge (Poinar 1987). The direct cycle involves a single developmental host. The pre-parasitic juvenile hatching from the egg laid in the environment enters a receptive host, develops there and hatches as a post-parasitic juvenile. This stage returns to the environment to mature, mate and lay eggs (Poinar 1987). The indirect cycle, when mermithid parasitoids use insects as a paratenic host, which then get consumed by, e.g. spiders, may explain the parasitism of spider species associated with different habitats (Poinar & Benton 1986). A potential explanation for how infestation works is that juvenile mermithids are quite small and live in the prey haemolymph, not encysted in tissues, and thus could be ingested along with the liquid diet of the spider (Poinar & Early 1990). Whether the mermithids exhibit defences that protect them against digestive enzymes is unknown. Also unknown is how they penetrate the gut to enter the cavity of the spider body (Carl N. Keiser, pers. comm.). Parasitized spiders were found in a variety of habitats (on plants, in webs and on the ground). They were observed going into water where the nematodes hatched from the bodies of the hosts (Poinar 1987). It is likely that a number of different mermithid genera and species infest spiders and that some of them may also have a direct cycle (Poinar 1987).

Except for Mermithidae, well-known parasites of spiders include Diptera (Schlinger 1987) and Nematomorpha (Poinar 1987). The latter bear a superficial resemblance to Mermithidae and can best be distinguished by colour, when only morphological examination is possible. Mermithid nematodes are usually whitish or cream-coloured with shades of pink, yellow and green, while nematomorph gordiids are mostly brown or black (Poinar 1987). Overall, it can be said

Viktoria WEGEWITZ, Popitzweg 10 B, 13627 Berlin, Germany;
E-mail: viktoria.wegewitz@pm.me
Christoph MUSTER, Neukamp 29, 18581 Putbus, Germany;
E-mail: cm@christoph-muster.de
Jonas J. ASTRIN, Leibniz Institute for the Analysis of Biodiversity Change, Museum Koenig, Adenauerallee 127, 53113 Bonn, Germany; E-mail: j.astrin@leibniz-lib.de

Academic Editor: Theo Blick

Submitted 20.6.2022, accepted 25.11.2022, online 28.12.2022

Tab. 1: Vouchers deposited at LIB Museum Koenig and GenBank/BOLD IDs

	DNA voucher ID	morphological/tissue ID	ID of genital preparation	GenBank Acc #	BOLD Process ID
Spider 1	ZFMK-DNA-FD14330503	ZFMK-TIS-53920	ZFMK_Ar23967	OP270649	LIBBB002-22
Spider 2	ZFMK-DNA-FD14331659	ZFMK-TIS-56486	ZFMK_Ar23968	OP270648	LIBBB001-22
Worm 2	ZFMK-DNA-FD14331675	ZFMK-TIS-53919		OP270647	LIBBB003-22
Holotype* *P. depriesteri*	ZFMK-DNA-FD15676217				

*: DNA of the type specimen of *P. depriesteri* (Senckenberg Museum Frankfurt) was too degraded to produce any PCR product, but has been deposited at LIB Biobank for potential future non-Sanger based approaches

that despite the high diversity and ecological impact of both parasites and spiders, the understanding of parasitoid-spider interactions is very limited compared to similar fields, e.g., parasites or parasitoids of insects (Durkin et al. 2021).

As already mentioned, infestations with mermithids can affect the development of the vulva or other sex-specific traits (Leech 1966, van den Berg & Dippenaar-Schoemann 2009). Consequently, an altered genital morphology could lead to the description of new species, as this an important diagnostic character in spider taxonomy (Breitling et al. 2015). Some of these cases are now considered nomina dubia, and in this study the status of such a name, *Philodromus depriesteri* Braun, 1965, is investigated and discussed. This species was first described by Braun (1965) based on two specimens found in the early 1950s, one in Krimml (Austria, Oberpinzgau in Salzburg) near the great waterfalls and a second in Geisenheim (Germany: Rheingau in Hesse). Both sites are approximately 600 km apart. Despite this large distance and generally increased sampling activity since this time, it took more than 50 years until the next specimens were recorded in 2012 in the Allgäu region in Bavaria (Breitling et al. 2015). Due to the high similarity of the non-genital characters, *P. depriesteri* was considered closely related to *P. collinus* by Braun (1965). When the last specimen of *P. depriesteri* was found in 2012, a parasitic worm was also discovered in the opisthosoma, which already led to the consideration that the aberrant form of the *P. depriesteri* vulva was induced by this nematode (Breitling et al. 2015). However, Breitling et al. (2015) did not synonymize *P. depriesteri* and *P. collinus* because of the similarity of females in the *aureolus* group. In the present study, new arguments supporting this synonymy based on gene sequence (cytochrome c oxidase subunit I) data are presented.

Material and methods

Material examined. GERMANY: Bavaria: Bayerischer Wald, Steinklamm Spiegelau, 48.90276°N, 13.37237°E, 2 ♀♀ beating from pine, 22. Jul. 2020. Sample availability see below.

Morphological examination. The examination of the spiders and the further preparation of the epigynes and the worms was carried out using an Optika SZM-2 stereoscope. Epigynes were macerated with lactic acid at room temperature and were subsequently fixed in Euparal. Genital images were taken with a Zeiss Standard 15 microscope and a Canon EOS 80d. Stacks were taken in RAW format using Helicon Remote software and merged using Helicon Focus 7 software. The resulting TIFF images were lightly edited in Adobe Photoshop and saved in JPEG format (photos: Viktoria Wegewitz).

Molecular analysis. Total genomic DNA was extracted from ethanol-preserved tissue (spiders: legs; worm 2: body fragments, worm 1: whole specimen) using silica membrane columns of the Blood and Tissue kit by Qiagen (Hilden, Germany), following the manufacturer's specifications. We amplified 658 bp of spider DNA from the 5'-end of the COI (cytochrome c oxidase subunit I) gene with primers HCO2198-JJ and LCO1490-JJ (Astrin & Stüben 2008) in reaction volumes of 20 µl, including 2.0 µl of DNA template, and using the "Multiplex PCR Master Mix" (Qiagen). PCR conditions were as follows: first cycle set (15 repeats): 35 s denaturation at 94°C, 90 s annealing at 55°C (–1°C per cycle) and 90 s extension at 72°C. Second cycle set (25 repeats): 35 s denaturation at 94°C, 90 s annealing at 45°C, and 90 s extension at 72°C. PCR products were sent for bidirectional Sanger sequencing to BGI (Hong Kong, China). For the nematodes, the ribosomal SSU (18S) Hyper Variable Region I (HVR I) was amplified as described by Zhou et al. (2019). The primers SSU18A and SSU26R were used for PCR and SSU9R for sequencing. These primers are suitable for a wide range of nematodes (Floyd et al. 2002, Blaxter et al. 1998).

DNA extracts and tissue samples (except for worm 1, which was processed destructively elsewhere) are available from the LIB Biobank at Museum Koenig, Bonn under the voucher IDs specified in Tab. 1.

Results

Morphological analysis

Figure 1 shows the dorsal view of a normally developed vulva from *Philodromus collinus*, beaten from a pine in the Harz Mountains on 30. Jul. 2019 (51.52505°N, 11.09100°E). The two dissected female reproductive organs of specimen 1 (Fig. 2, body size 5.0 mm) and specimen 2 (Fig. 3, body size 5.8 mm) from Spiegelau show incomplete vulva structures, in particular the receptacula seminis are barely developed. The epigyne/vulva of specimen 1 is somewhat more developed than in specimen 2.

The structure of their epigyne/vulva is characterized by the following points:

Missing fully developed receptacula seminis and overall underdeveloped appearance.

The structure of vulva 2 corresponds to the holotype of *P. depriesteri* (fig. 93 in Braun 1965) and the structure of vulva 1 corresponds to that of the paratype of *P. depriesteri* (fig. 94 in Braun 1965).

The sclerotized structures of the vulvae correspond to "Rezeptakularöhren" (which translates to receptacula tubes) by Braun (1965) and "copulatory ducts" by Muster & Thaler (2004), respectively.

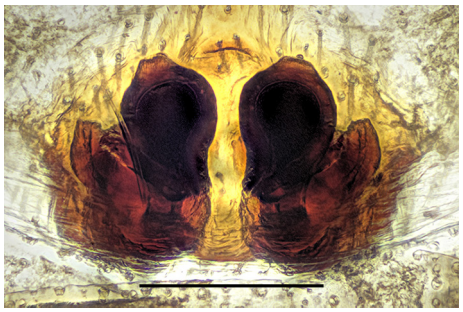

Fig. 1: *Philodromus collinus*, vulva, dorsal view, typical morphology; scale bar 0.2 mm

Fig. 4: Specimen 2 with nematode

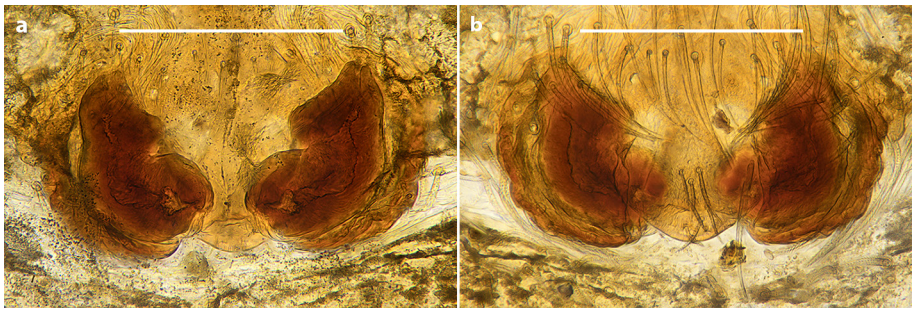

Fig. 2: Specimen 1 from Spiegelau. **a.** vulva, dorsal view; **b.** vulva, ventral view; scale bars 0.2 mm

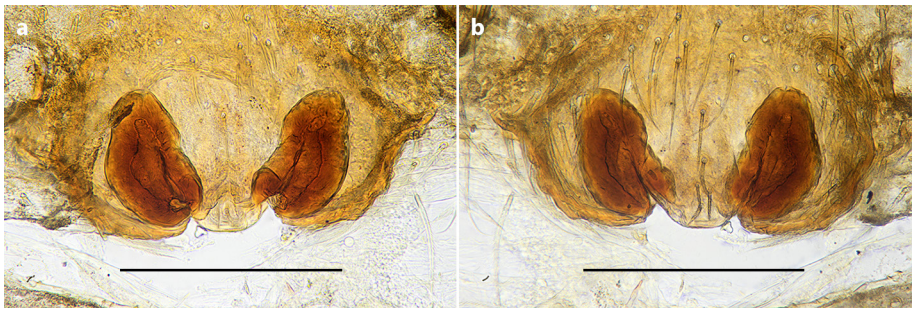

Fig. 3: Specimen 2 from Spiegelau. **a.** vulva, dorsal view; **b.** vulva, ventral view; scale bars 0.2 mm

In both specimens the opisthosoma was filled with a worm (Fig. 4). The worm of specimen 2 had a cream colour immediately after dissection. Gradually it turned brown, probably due to contact with oxygen. The worm of specimen 1 was darker (picture not shown). In general appearance, the infected spiders resembled specimens with the typical morphology of the *Philodromus aureolus* group, with specimen 1 looking slightly damaged.

Molecular analysis of spiders

Both sequences of specimens 1 and 2 from Spiegelau could be unambiguously assigned to the species *Philodromus collinus*. In addition to BLAST searches in GenBank and BOLD, the sequences were also compared with the *Philodromus* specimens sequenced in the German Barcode of Life (GBOL) data release (Astrin et al. 2016). Embedded in this dataset, the sequences of the two spider specimens (which differed from each other in one base) had pairwise genetic distances (uncorrected p-distances) of 0.2% to 1.1% with respect to *Philodromus collinus* sequences. The attempt to sequence one leg of the holotype of *Philodromus depriesteri* failed.

Molecular analysis of nematodes

Primer pair SSU HVR1 is the standard for amplification of the18s rDNA hypervariable region. Both worms yielded an identical band in the expected range. Unfortunately, the band from worm 1 turned out to be host derived and also in several rounds of new PCR with different conditions and different primers, we were unable to amplify the worm sequence, presumably due to high contamination with host derived DNA. The sequence of worm 2 was very clear. In a BLAST search (on 3. May 2021 and repeated on 13. Jun. 2022) with the sequence of worm 2 as bait, the 10 top hits were all sequences from various mermithids with identities between 94.17% and 97.51% and e-values of 0.0. The fragment considered for the BLAST search corresponds to positions 51 to 531 in AB647219.1 (unclassified Mermithidae), which is the best hit. From this, we conclude that worm 2 (and presumably also worm 1) is a molecularly not yet characterized species of Mermithidae.

Discussion

The status and identity of the mysterious *Philodromus depriesteri* has caused long-lasting confusion and discussion. When Braun described *P. depriesteri* as the sister species to *P. collinus* in 1965, he was hesitant because the epigyne had an aberrant appearance. The *depriesteri* vulva with its reduced receptacula would be in the *aureolus* group, where voluminous receptacula are otherwise present (Breitling et al. 2015). In later personal conversations between Rudolf Braun und Peter Jäger, Braun assumed that he had described a juvenile specimen, most probably belonging to *P. collinus*, based on preepigynes of juvenile specimens (P. Jäger, pers. comm.). However, this can be excluded, since *Philodromus* preepigynes show a weakly developed septum without sclerotizations (S. Indzhov, pers. comm.). Expressed in detail, the preepigyne occupies about

half the distance between the epigastral fold and the petiolus. It is not sclerotised and looks like the surrounding cuticula. A weakly developed septum is defined by two shallow, semicircular furrows (S. Indzhov, pers. comm.). Instead, the repeated observation of this aberrant vulva phenotype in specimens that carry parasitic nematodes in the opisthosoma suggests a causal relationship. Most probably, the absence of fully developed receptacula is caused by a general developmental delay due to the mermithid infestation. Other explanations, e.g. that the parasites consumed the initially developing receptacula or that the receptacula broke off are less likely, since the receptacula are missing on both sides and the specimens appeared otherwise uninjured. Currently we do not know whether the "*depriesteri* phenomenon" may also occur in related *Philodromus* species as consequences of worm infections. However, the structure of the copulatory ducts in the holotype of *P. depriesteri* is highly specific for *P. collinus*. Thus, regardless of the fact that the sequencing of the holotype failed, we propose the new synonymy *P. depriesteri* Braun, 1965 = *Philodromus collinus* C. L. Koch, 1835 **syn. nov.**, instead of considering it a nomen dubium. Most probably the holotype also contains a worm in its opisthosoma. Thus, it is evident that an unidentified nematode of the family Mermithidae induces a degenerate appearance of the vulva in *P. collinus* which falsely led to *P. depriesteri* being described as a new species.

Acknowledgements

We would like to thank Lenka Kubcová, Sylvain Lecigne and Dmitri Logunov for reviewing the manuscript. We thank Adrian Streit for his help in sequencing the nematodes and carefully reading the manuscript. We thank Florian Frankenberger for assistance with field work and Carl N. Keiser for answering our questions on parasitoids. Infinite gratitude goes to Simeon Indzhov for support from the beginning of the project. We thank Laura von der Mark for lab work in Bonn. The authors express their gratitude to Peter Jäger for providing the leg from the holotype of *Philodromus depriesteri*.

References

Astrin JJ, Höfer H, Spelda J, Holstein J, Bayer S, Hendrich L, Huber B, Kielhorn KH, Krammer HJ, Lemme M, Monje JC, Morinière J, Rulik B, Petersen M, Janßen H & Muster C 2016 Towards a DNA barcode reference database for spiders and harvestmen of Germany. – PLoS ONE 11 (0162624): 1-24 – doi: 10.1371/journal.pone.0162624

Astrin JJ & Stüben PE 2008 Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). – Invertebrate Systematics 22: 503-522 – doi: 10.1071/IS07057

Berg A van den & Dippenaar-Schoemann A 2009 First report on nematodes parasitizing spiders in South Africa. – SANSA Newsletter 10: 15

Blaxter ML, Ley P De, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT & Thomas WK 1998 A molecular evolutionary framework for the phylum Nematoda. – Nature 392: 71-75 – doi: 10.1038/32160

Braun R 1965 Beitrag zu einer Revision der paläarktischen Arten der *Philodromus aureolus*-Gruppe (Arach., Araneae). I. Morphologisch-systematischer Teil. – Senckenbergiana Biologica 46: 369-428

Breitling R, Lemke M, Bauer T, Hohner M, Grabolle A & Blick T 2015 Phantom spiders: notes on dubious spider species from Europe. – Arachnologische Mitteilungen 50: 65-80 – doi: 10.5431/aramit5010

Durkin ES, Cassidy ST, Gilbert R, Richardson EA, Roth AM, Shablin S & Keiser CN 2021 Parasites of spiders: Their impacts on host behavior and ecology. – Journal of Arachnology 49: 281-298 – doi: 10.1636/JoA-S-20-087

Floyd R, Abebe E, Papert A & Blaxter M 2002 Molecular barcodes for soil nematode identification. – Molecular Ecology 11: 839-850 – doi: 10.1046/j.1365-294x.2002.01485.x

Holm Å 1941 Über Gynandromorphismus und Intersexualität bei Spinnen. – Zoologiska Bidrag från Uppsala 20: 397-415

Huber BA 2004 The significance of copulatory structures in spider systematics. In: Schult J (ed.) Biosemiotik – praktische Anwendung und Konsequenzen für die Einzelwissenschaften. VBW (Verlag für Wissenschaft und Bildung), Berlin. pp. 89-100

Jocqué R 2002 Genitalic polymorphism – a challenge for taxonomy. – Journal of Arachnology 30: 298-306 – doi: 10.1636/0161-8202-(2002)030[0298:GPACFT]2.0.CO;2

Kaston BJ 1961 Spider gynandromorphs and intersexes. – Journal of the New York Entomological Society 69: 177-190

Košulič O & Mašová Š 2019 First report on mermithid parasitism (Enoplea: Mermithidae) in a Southeast Asian spider (Araneae: Araneidae). – Helminthologia 56: 157-167 – doi: 10.2478/helm20190012

Leech RE 1966 The spiders (Araneida) of Hazen Camp 81°49'N, 71°18'W. – Quaestiones Entomologicae 2: 153-208

Martin D 2013 Aberrante Epigynenbildungen bei der Wolfspinne *Pardosa palustris* (Araneae, Lycosidae). – Arachnologische Mitteilungen 46: 1-5 – doi: 10.5431/aramit4601

Muster C & Thaler K 2004 New species and records of Mediterranean Philodromidae (Arachnida, Araneae): I. *Philodromus aureolus* group. – Denisia 12: 305-326

Nickle WR 1972 A contribution to our knowledge of the Mermithidae (Nematoda). – Journal of Nematology 4: 113-146

Penney D & Bennet SP 2006 First unequivocal mermithid-linyphiid (Araneae) parasite-host association. – Journal of Arachnology 34: 273-278 – doi: 10.1636/S04-92.1

Poinar GO 1985 Mermithid (Nematoda) parasites of spiders and harvestmen. – Journal of Arachnology 13: 121-128

Poinar GO 1987 Nematode parasites of spiders. In: Nentwig W (ed.) Ecophysiology of spiders. Springer, Berlin, Heidelberg. pp. 299-308 – doi: 10.1007/978-3-642-71552-5_22

Poinar GO & Benton CLB 1986 *Aranimermis aptispicula* n. g., n. sp. (Mermithidae: Nematoda), a parasite of spiders (Arachnida: Araneida). – Systematic Parasitology 8: 33-38 – doi: 10.1007/BF00010307

Poinar GO & Early JW 1990 *Aranimermis giganteus* n. sp. (Mermithidae: Nematoda), a parasite of New Zealand mygalomorph spiders (Araneae: Arachnida). – Revue de Nématologie 13: 403-410

Schlinger EI 1987 The biology of Acroceridae (Diptera): True endoparasitoids of Spiders. In: Nentwig W (ed.) Ecophysiology of spiders. Springer, Berlin, Heidelberg. pp 319-327 – doi: 10.1007/978-3-642-71552-5_24

Ya'cob Z, Low L van, Tan TK, Noor-Izwan A, Lourdes EY, Ramli R, Takaoka H & Adler PH 2021 Sexually anomalous individuals of the black fly *Simulium trangense* (Diptera: Simuliidae) infected with mermithid parasites (Nematoda: Mermithidae). – Parasitology Reseach 120: 1555-1561 – doi: 10.1007/s00436-021-07087-x

Zhou S, Harbecke D & Streit A 2019 From the feces to the genome: a guideline for the isolation and preservation of *Strongyloides stercoralis* in the field for genetic and genomic analysis of individual worms. – Parasites & Vectors 12 (496): 1-11 – doi: 10.1186/s13071-019-3748-5