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Original papers

Tarantobelus arachnicida (Nematoda: Rhabditida) invasion of exotic spiders in Poland

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ABSTRACT. Nematodes of the Brevibuccidae family were stated among spiders of the Theraphosidae family (the South American species), which were bred in Poland. The first sign of infection was anorexia which led to gradually increasing lethargy progressed to a huddled posture. Additionally, a white discharge with nematodes between mouth and chelicerae was noted. All of the derived nematodes were morphologically identified and determined to the species *Tarantobelus arachnicida*. A molecular analysis covered amplification and sequencing of small subunit ribosomal RNA (18S rRNA). A post mortem examination demonstrated the presence of nematodes not only near the chelicerae, but also inside the intestine, hence the source of infection might be insects used as a food. The research showed that such kind of infection is an important disease, which poses a serious risk to the breeding spiders. To date there is no effective treatment, however, we demonstrated that usage of the Lugol's solution seems to be promising.

Key words: nematodes, parasites, Brevibuccidae, *Tarantobelus arachnicida*, Theraphosidae, diagnosis

Introduction

Spiders of the Theraphosidae family (Arthropoda: Araneae), known as tarantulas are one of the most common terrarium invertebrates [1]. Different factors that make those spiders so widespread can be listed, e.g. a high tolerance for breeders mistakes or usually problem-free reproduction. Nevertheless, spiders breeding is not always a long-lasting period. The most frequent reasons of its fatal descent are: dehydration, injury, mould or problems with moulting. Regrettably, many invasions of exotic spiders have been recorded over the years. Some of them were pure parasitic infections or enhanced with an additional bacterial invasion. However, the parasite species identification was difficult and therefore predominantly ambiguous. Recently, parasitic invasions of exotic invertebrates have

Fig. 1. Discharge around the chelicerae of the theraphosid spider

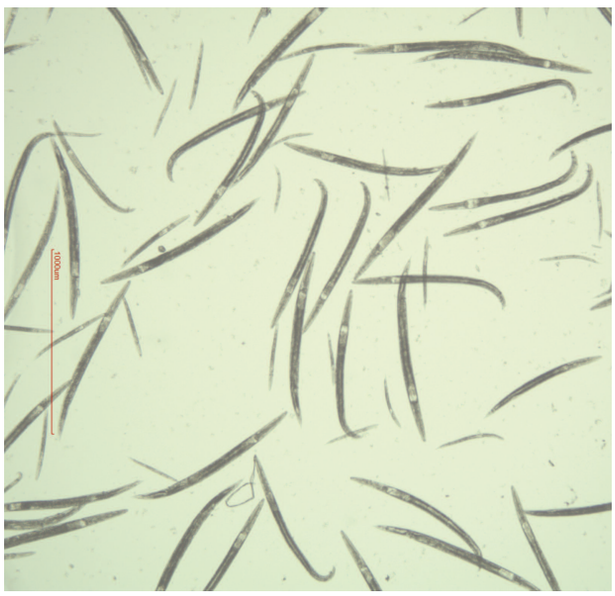

Fig. 2. Nematodes derived from the discharge

become a serious issue that requires special attention.

In our research we dealt with parasitic invasion among spiders of the Theraphosidae family.

Materials and Methods

The study focused on 16 spiders (juvenile and adults) belonging to the Theraphosidae family (the South American species from Ecuador hatched in Poland). Although each spider was kept in a separate container, the same worrying symptoms occurred among all of them. First of all, spiders stopped to eat, hence the first sign was anorexia. We also observed a gradually increasing lethargy and a huddled posture with shrunken legs under the abdomen. All spiders did not react to any stimulus. Furthermore, unusual sweet smell wafted from the containers. Nevertheless, the most important and distinctive symptom was white and sticky discharge near the chelicerae (Fig. 1), recognized as a fluid. This strange secretion included a huge amount of small, white and motile worms.

The microscopic analysis of the collected discharge revealed the presence of the nematodes (Fig. 2). The post-mortem examination was carried out in order to find out if these specimens were also present inside the digestive tract (note: all spiders died of the infection). The inner part of the cephalothorax was flushed with physiological saline solution and then the obtained solution was analyzed under the microscope. The nematodes were found in the initial section of the intestine, as well as in the excrement samples. We flushed the area between mouth and chelicerae (n=8) with small dose of the Lugol's solution in order to eradicate the nematodes in still living spiders.

Derived nematodes were harvested in liquid paraffin for further molecular analysis. Genomic DNA was extracted from pooled nematodes using Sherlock AX (A&A Biotechnology, Poland), according to the manufacturer's instructions. The standard Polymerase Chain Reaction (PCR) was performed with the primers targeted to the small subunit ribosomal RNA (18S rRNA) [2] (StartWarm HS-PCR Mix, A&A Biotechnology). The purified PCR products were sequenced (Genomed, Poland) and then compared with the sequences available in GenBank database using MrBayes Software [3] – *Zeldia punctata* Thorne, 1937 (ZPU61760) was used as the outgroup. Since there were no sequences available for *Tarantobelus arachnicida* Abolafia and Peña-Santiago, 2017 before proper species description we choose another marker (i.e. 18S rRNA) to gather further data on the biodiversity of the nematode.

Results

Based on the morphological features of specimens of both sexes the collected nematodes were identified as *T. arachnicida* – recently described species parasitizing tarantula spiders [4].

The newly obtained sequence (923bp) was submitted to the GenBank database under accession number MG669658. The Bayesian tree based on sequences of the 18S rRNA region (Fig. 3) do not show as clear systematic overview as that presented by Abolafia and Peña-Santiago [4], however, provides additional data for further studies.

Usage of the Lugol's solution not only eradicated nematodes, but also succeeded in elongating the spiders survival (for up to three months unlike the untreated ones that survived only two weeks), but the infection was fatal for all of analyzed spiders due to the permanent paralysis of chelicerae.

Discussion

Firstly, observed nematodes were distinguished as a some exotic parasitic species due to the spiders origin (e.g. nematodes of the *Halicephalobus* genus that can also pose a serious zoonotic risk to human). Nevertheless, within species of the genus *Halicephalobus* the occurrence of males has not been

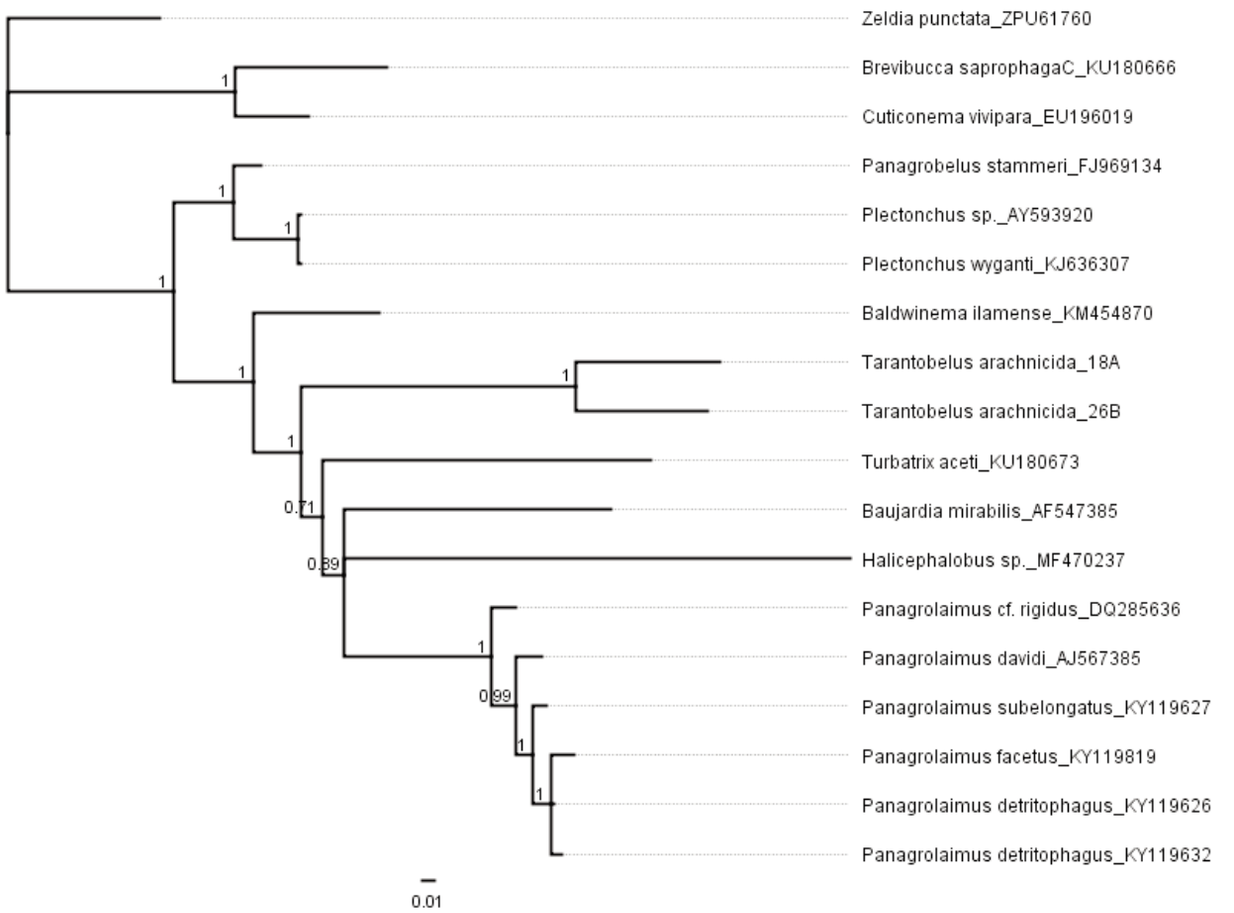

Fig. 3. The Bayesian tree inferred from sequences of the 18S rRNA region of *Tarantobelus arachnicida* and other sequences available in GenBank

demonstrated [5]. Hence, the presence of *Halicephalobus* sp. in our research had to be excluded. The collected specimens were initially classified as members of the *Panagrolaimus* genus (Panagrolaimidae family, soil nematodes), however, further microscopical analysis confirmed the presence of *T. arachnicida* [4].

Until now, infection with *T. arachnicida* was reported once in subadult male of the tarantula *Chromatopelma cyaneopubescens* (Strand, 1907) (Theraphosidae), originating from Venezuela, and bred in captivity in Poland [4]. Our study provides a second report, however, a difference important from a veterinary point of view should be pointed out. During the research we used nematodes obtained from spiders hatched in Poland, not directly from imported ones. Therefore, we can hypothesize that the potential source of the infection were insects used as a food (e.g. crickets). The life cycle and exact way of transmission are so far unknown, however, it is highly likely that spiders with no clinical symptoms, as well as a fodder originating from spiders farms may play a significant role. In-depth investigation of the life cycle and a role of other invertebrates in parasite spread are essential.

Many similar invasions of exotic spiders have been recorded over the years. The majority of them was stated in North America, the United Kingdom and other European countries [1] among numerous captive-bred and wild-caught terrestrial, as well as arboreal theraphosid genera (including *Brachypelma*, *Grammostola*, *Theraphosa*, *Poecilotheria*, from the Americas, Asia and Africa). These issues were described as panagrolaimid invasion, however, species identification was regarded to be doubtful.

Pizzi et al. [1] have demonstrated secondary or possibly symbiotic bacterial (i.e. genus *Bacilllus* and *Proteus*) infection of the surrounding tissues by means of the histopathology analyzes of euthanized spiders' mouth parts. Such infection in the studied case should not be excluded. We suppose that this kind of parasitic nematode in association with these bacteria caused the aforementioned paralysis of chelicerae. Moreover, we assume that arising immobilization may be the result of the mechanical impairment of chelicerae or some toxic compounds

excreted by bacteria, which cause damages in spiders' nervous system. Since it is not so obvious, the further histopathological studies are required.

Euthanasia of all affected spiders is strongly advised (note: affected spiders should not be used as a food for insects) due to the unconfirmed lack of potential zoonotic risk and unsuccessful therapies in similar invasions [6]. The quarantine period for newly acquired spiders should also be implemented, to avoid the spread of possible infection [1].

Since in our study flushing with Lugol's solution has eliminated nematodes – spraying containers, terrariums and insects used as a food with iodine-containing compounds seems to be recommended as a prevention method. It is essential to determine the appropriate dose so as not to harm spiders. Finding a method that may cause a renewed food intake by infected spiders is also worth the effort.

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